ISSN 0030-9885

Coden: PSIRAA 46(6) 395-477 (2003)

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Vol. 46, No.6

November - December 2003

Physical Sciences. Pages 395-438
Biological Sciences. Pages 439-472
Technology. Pages 473-477

Published bimonthly by
Scientific Information Centre
PAKISTAN COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
Karachi

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

DR. ANWAR-UL-HAQ
Chief Editor

DR. KHURSHID ZAMAN
Executive Editor

Editorial Board

Field Editors

Production

Riazuddin Ahmed

Composing

Irshad Hussain

Editorial Address

Executive Editor, Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Karachi-75280, Pakistan.

Tel: 92 - 021 - 8151739, 8151741 - 43, Fax: 92 - 021 - 8151738, E-mail: pcsirsys@super.net.pk and pcsir-sic@cyber.net.pk

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Vol.46, No.6 **CONTENTS** November - December 2003

PHYSICAL SCIENCES

SHORT COMMUNICATIONS

BIOLOGICAL SCIENCES

SHORT COMMUNICATION

TECHNOLOGY

Printed: December 2003

SUBSCRIPTION RATES

LOCAL	**US$***	**Rupees***
For Organization / Institution Subscriptions		
Per Volume	—	2000
Per Issue	—	350
For Academic Subscriptions		
Per Volume	—	1800
Per Issue	—	325
For Personal Subscriptions		
Per Volume	—	1500
Per Issue	—	300
FOREIGN		
For Organization / Institution Subscriptions		
Per Volume	400	—
Per Issue	70	—
For Academic Subscriptions		
Per Volume	350	—
Per Issue	60	—
For Personal Subscriptions		
Per Volume	325	—
Per Issue	50	—
PHOTOCOPYING OF THE BACK ISSUES		
Local (Normal Registered Mail)	—	25
(Courier)		115
Foreign (Normal Registered Mail)	10	—
(Courier)	25	—

*Variation in rates will depend on the current local & foreign currency exchange rates and will vary accordingly.

AIMS & SCOPE

Pakistan Journal of Scientific and Industrial Research is a bimonthly Journal aims to publish research articles, current reviews and short communications from varied key scientific disciplines. It covers all relevant topics of fundamental, technical and applied aspects of significant industrial importance. Each bimonthly issue is reviewed by the eminent International experts and contributions are acquired from scientists and industrially related academics and researchers.

The scope of the Journal is broad and provides widest coverage in the fields of Technology, Organic Chemistry, Inorganic Chemistry, Physical Chemistry including Natural Products and Synthesis, Biology, Agriculture, Physics, Mathematics and Geology.

This Journal is indexed/abstracted

Biological Abstracts and Biological Abstracts Reports, Chemical Abstracts, Geo Abstracts, CAB International, Bio Science Information Service, Zoological Record, BIOSIS, NISC, NSDP, Current Contents, CCAB, Rapra Polymer Abstracts, Reviews and Meetings and their
CD-ROM counterparts etc.

Subscription Rates

Local: Rs.1000/-per vol.; Single issue Rs.170/-; Postage is free.
Foreign: US$ 168/- per vol.; Single issue US$ 30/- including air mail charges.

Electronic format

Electronic format of this journal is available with: *Bell & Howell Information and Learning, 300 North Zeeb Road, P.O. 1346, Ann Arbor, Michigan 48106, U.S.A;* Fax No.: 313 - 677 - 0108; http://www.umi.com.

Photocopies

Photocopies of back issues can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 10.00 per page per copy within Pakistan. Rest of the World, US$ 2.00 per page per copy (US$5.00 per page per copy by courier service).

Copyrights

Published by

Scientific Information Centre, PCSIR Laboratories Campus, Karachi - 75280, Pakistan.

Printed by

Printed by Saad Publications, Karachi, Pakistan.

ACKNOWLEDGEMENT

Scientific Information Centre, Pakistan Council of Scientific and Industrial Research, Karachi, Pakistan extend utmost gratitude to the following eminent Scientists/Subject experts for their thorough review and valuable feedback for the articles appearing in November - December 2003 issue of Pakistan Journal of Scientific and Industrial Research.

EXECUTIVE EDITOR

Afroz, H.	Pakistan
Akhtar, H.	Canada
Ali, M.	Bangladesh
Angers, D.	Canada
Angus, R.	USA
Clarke, J. M.	Canada
Denysenko, S.	USA
El-Marhomy, A. A.	Egypt
Gilani, A.-ul-H.	Pakistan
Haque, I. ul	Bangladesh
Hossain, I.	Pakistan
Irfanullah	Pakistan
Jabbar, A.	Pakistan
Jafri, S. I. H.	Pakistan
Khalil, M. I.	Bangladesh
Khan, M. A.	Pakistan
Laakso, J.	Finland
Milburn, P. H.	Canada
Minhas, N. M.	Pakistan
Mujib, B.	Pakistan
Narine, S.	Canada
Nasreen, Z.	Pakistan
Qazi, I.	Pakistan
Qureshi, J.	Pakistan
Rahim, T.	Pakistan
Rathod, S.	India
Samed, A. K. M. A	Egypt
Sarfaraz, B.	Pakistan
Shahzad, S.	Pakistan
Sharkovsky, V.	Russia
Smith, F.	Australia
Yasmin, A.	Pakistan

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 395 - 398*

HEAVY METAL IONS CONCENTRATION IN WHEAT PLANT (*TRITICUM AESTIVUM* L.) IRRIGATED WITH CITY EFFLUENT

Sajid Farid

NFC Institute of Engineering and Fertilizer Research (IEFR), Faisalabad, Pakistan

(Received December 14, 2001; accepted July 4, 2002)

Pakistan lies under arid and semi arid zones. There is shortage of water for irrigation. Farmers near being cities raise crops by diverting the city effluent towards their fields. It contains heavy toxic metal ions like cadmium, chromium, cobalt and nickel, which may accumulate in the edible portion of crops and cause clinical problems to human being. The concentration of metal ions in the effluent and effluent irrigated wheat (*Triticum aestivum* L.) was determined by Atomic Absorption Spectrophotometer. Heavy metal ions (Cd, Cr and Co) mean concentrations were found above the permissible limits recommended for irrigation water. In the grains of wheat plant concentration of Cd, Cr and Co was found above the permissible levels recommended by World Health Organization (WHO) for foodstuff.

Key words: Wheat plant, *Triticum aestivum* L, City effluent, Toxic metal, Atomic absorption spectrophotometer.

Introduction

The climate of Pakistan is mainly subtropical arid to semiarid in more than 90% of the total geographical area. Annual rainfall is variable, with less than 10 cm in some parts of the country and more than 50 cm near the foothills of the Himalayas. Average annual rainfall in the arid and semiarid areas is around 20 cm, which is not sufficient for growing any crops of economic importance. In order to overcome this situation, city effluent is used for raising crops around big cities (Khan *et al* 1994).

City effluent contains heavy metals like cadmium, chromium, cobalt and nickel, along with a source of irrigation and nutrients (Ghafoor *et al* 1994). These heavy metals may accumulate in the edible portion of the crops and enter the human food chain causing different clinical problems. This all is due to effluents coming from various industries situated in the urban areas. Usually, a few industries are equipped with satisfactory operating treatment plants (Nabi *et al* 2001). City effluent, which carries heavy metals when used for raising crops, may cause environmental threat.

Many industries dispose off effluent via the open and covered routes into the main channels, which also carry domestic water. Farmer's fields near these channels are irrigated with this polluted effluent for raising crops (Ghafoor *et al* 1994). The object of study was to know the level of heavy metals in liquid effluents being used as an irrigation source. By the study awareness among the people would be raised, involved in discharging city and industrial effluents.

Presently, much work has not been done in Pakistan for the metal ion contamination of crop raised by utilizing city effluent for irrigation. Study was carried out in order to evaluate the metal ion concentration and its suitability for the irrigation purposes. Level of metal ions in the crop grown was also evaluated for its suitability for human consumption.

Materials and Methods

The city effluent samples were taken from open channel flowing alongwith Satiana Road out of Faisalabad city for analysis. Four localities were selected where farmers grow wheat (*Triticum aestivum* L.) by irrigating fields with city effluent from more than 15 years due to shortage of canal water and poor quality of under ground water (i.e. they mixed city effluent with canal water if available or cyclic use one irrigation with city effluent and other with canal water but from more than 5 years they are mostly depending on city effluent for irrigation). Mean pHs (Saturated paste pH) from all four sites was 7.80, 7.90 and 8.03 at 0-15 cm, 15-30 cm and 30-60 cm depth, respectively. The selected fields were located in the vicinity of Gandakhue, Mulkhanwala, Awanwala and Kanuwala areas. The effluent being used for irrigation at a particular site was sampled on weekly basis for six weeks. The effluent samples were analysed for toxic metal ions namely Cd, Cr, Co and Ni on Varian AA-1445 series Atomic Absorption Spectrophotometer (AOAC 1984).

On maturity stage of crop grain, straw were separated in wheat (*Triticum aestivum* L.) plant. Samples were digested in di-acid mixture (10 ml concentrated HNO_3 + 5 ml of $HClO_4$). Concentrations of above mentioned heavy metals were determined by a Varian AA-1445 series Atomic Absorption Spectrophotometer (AOAC 1984).

Table 1

Heavy metal ions concentration (ppm) in effluent

	Cd		Cr		Co		Ni	
Area	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Gandakhu	0.01 - 0.04	0.02	0.30 - 0.54	0.41	0.06 - 0.21	0.12	0.07 - 0.21	0.14
Malkhanwala	0.01 - 0.04	0.02	0.30 - 2.14	1.00	0.08 - 0.21	0.13	0.02 - 0.25	0.14
Awanwala	0.01 - 0.05	0.02	0.07 - 0.88	0.38	0.09 - 0.23	0.14	0.07 - 0.26	0.15
Khanuwala	0.01 - 0.03	0.02	0.16 - 1.29	0.60	0.08 - 0.24	0.15	0.03 - 0.26	0.16

Table 2

Heavy metal ions concentration (ppm) in wheat (*Triticum aestivum* L.) plant by effluent irrigation

	Cd		Cr		Co		Ni	
Area	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Gandakhu	0.50	0.50	13.50	32.00	2.50	5.00	8.00	9.50
Malkhanwala	1.00	1.00	13.50	32.00	2.50	2.50	6.00	16.50
Awanwala	0.50	1.00	9.00	36.50	4.00	3.00	5.00	9.50
Khanuwala	0.50	0.50	9.00	30.00	2.50	2.00	8.00	5.00

Results and Discussion

Cadmium. Major sources of Cd in effluents are industries related to electroplating, pigments for plastics and paints, plastic stabilizer and batteries (Brady 1996).

Cadmium mean concentration in effluent was 0.02 ppm (Table 1). As shown in Fig 1, in all samples Cd concentration was at or above the critical level of 0.01 ppm for irrigation water suggested by FAO (Ayres and Westcot 1985).

In the case of wheat plants, Cd concentration was found at same level in both straw and grains except in the case of wheat plant sampled from one site where higher concentration (1.00 ppm) was accumulated in grain as compared to straw (0.50) ppm) as shown in Table 2. In the grains, concentration was found above the critical level of 0.01 ppm for foodstuff (WHO 1996).

It was observed from the results that concentration of Cd was higher in the crop irrigated by city effluent. Similarly, Cd concentration in foodstuff was sufficiently high to cause clinical problems like severe nausea, salivation vomiting, diarrhoea, abdominal pain and neuralgia (Prasad 1978; WHO 1996).

Chromium. Major sources as in the city effluent are from the tanning industry, manufacture of catalyst, pigments/paints, fungicides, ceramics, glass, photography chrome alloy/metal production/plating and corrosion control (WHO 1996).

The mean concentration of Cr in effluent samples was in the range of 0.38 to 1.00 ppm (Table 1). As illustrated in Fig 2, almost samples had Cr concentration above critical level of 0.10 ppm recommended for irrigation by FAO (Ayres and Westcot 1985).

Chromium concentration in straw was in the range of 30.00 to 36.50 ppm, while in case of grains it was in range of 9.00 to 13.50 ppm taken from all four sites (Table 2). Higher concentration was accumulated in the leaves. Its mobility from leaves to grain was low. In the grains, its concentration was found higher than permissible level of 1.30 ppm in food stuff (WHO 1996).

In general, Cr (VI) salts are more soluble than those of Cr (III) making Cr (VI) relatively mobile. This salt causes different diseases like lung cancer, gastrointestinal upsets, hepatitis, ulcer, edema when comes into human food in excessive quantity (Prasad 1978; WHO 1996).

Cobalt. The dominant form of cobalt in water is Co^{+2}. Cobalt compounds are mostly used in industries related to ceramics, glass, varnishes, ink, pigments, fabrics, paints and electroplating (Kirk - Othmer 1964).

Cobalt means concentration in effluent samples was 0.12, 0.13, 0.14 and 0.15 ppm from four respective sites (Table 1). Cobalt concentration in all samples as illustrated in Fig 3 was found higher than critical level of 0.05 ppm given by FAO (Ayres and Westcot 1985).

Cobalt concentration in straw taken from all four sites was in the range of 2.00 to 5.00 ppm while in case of grains it was in order of 2.50 to 4.00 ppm (Table 2). It was found above critical level of 0.01 ppm suggested for foodstuff (WHO 1996).

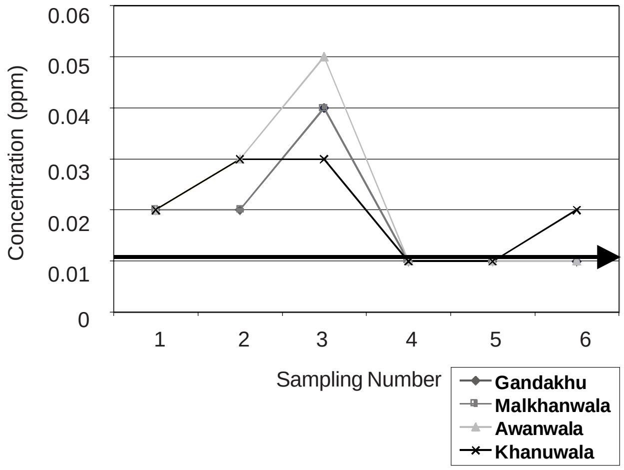


Fig 1. Trend of cadmium concentration in effluents.

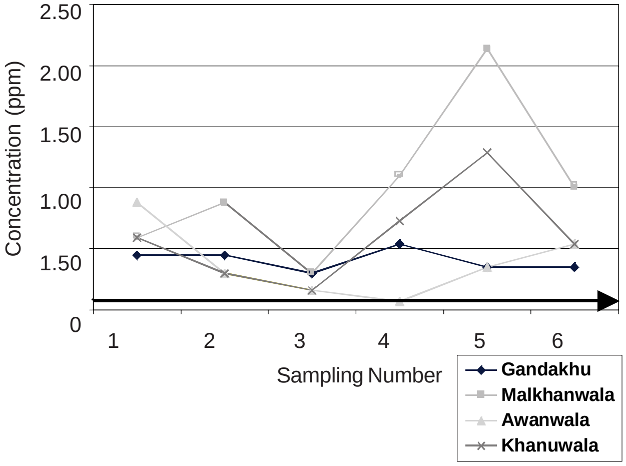


Fig 2. Trend of chromium concentration in effluents.

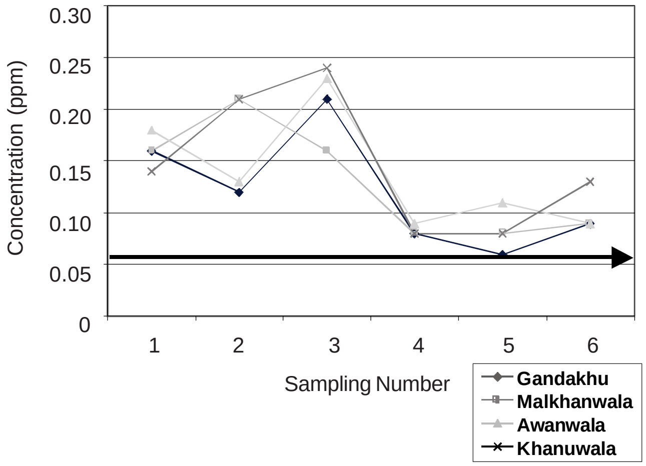


Fig 3. Trend of cobalt concentration in effluents.

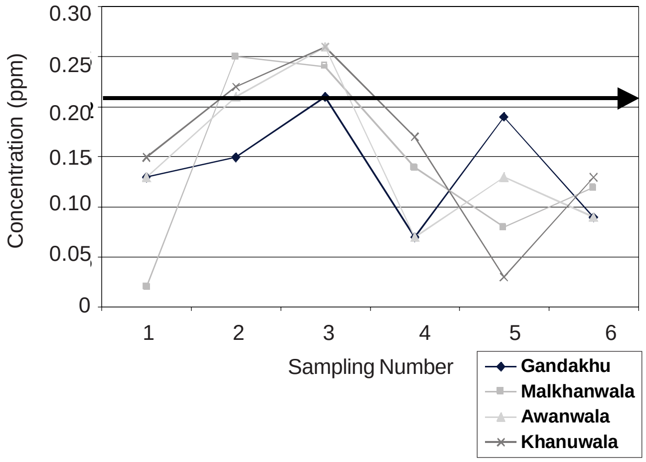


Fig 4. Trend of nickel concentration in effluents.

It causes different diseases like vomiting diarrhoea, blood pressure, giddiness and damage to nerves when comes into human food in excessive amount (Asthana and Asthana 2001).

Nickel. Major sources of Ni are combustion of coal, gasoline was well as industries related to oil, alloy manufacturing, electroplating and batteries (Brady 1996).

Concentration of nickel in effluent was 0.14, 0.14, 0.15 and 0.16 ppm from four sites, respectively (Table 1). Most of the effluent samples have concentration below critical level (0.20 ppm) as shown in Fig 4 suggested by FAO (Ayreas and Westcot 1985).

Nickel concentration in the wheat samples was in the range of 5.00 to 16.50 ppm in straw and 5.00 to 8.00 ppm in the grain sampled from four sites (Table 2). Nickel was found below the permissible level of 10.00 ppm given for food-stuff (WHO 1996).

It can cause different diseases like nausea, vomiting, abdominal discomfort, diarrhoea, giddiness, headache, cough and shortness of breath if come into human food chain in excessive concentration (Prasad 1978; WHO 1996).

Conclusion

City effluent is not suitable for raising crops because it is heavily loaded with metal ions, which through food cause different disease. Unsuitability of city effluent is due to the industrial water, which is drained out in the domestic sewage water without treatment. Industrial water should be treated before disposed off in the domestic sewage channels and along with this zero-effluent system should be adopted in industries.

References

AOAC 1984 *Official Methods of Analysis of the Association of Official Analytical Chemists.* AOAC Inc., Virginia, USA.

Asthana D K, Asthana M 2001 *Environmental: Problems and Solutions.* Publishers S Chand and Company Ltd, New Delhi, India, p 174.

Ayres R S, Westcot D W 1985 Water quality for Agriculture. *FAO Irri and Drain Paper* **29** 95 - 97.

Brady N C 1996 The Nature and Properties of Soil. Macmillian Publishing Company, New York, USA, 10th ed, p 529.

Ghafoor A, Rauf A, Arif M, Muzaffar W 1994 Chemical composition of effluents from different industries of the Faisalabad city. *Pak J Agric Sci* **31** 367 - 369.

Khan A, Ibrahim M, Ahmad N, Anwar S A 1994 Accumulation of heavy metals in soil receiving sewage effluent. *J Agric Res* **32** 525 - 533.

Kirk - Othmer 1964 Encyclopedia of Chemical Technology. John Wiley and Sons, Inc. New York, USA, 2nd ed, **5** pp 716 - 748.

Nabi G, Arshad M, Aslam M R 2001 Heavy metal contamination of agriculture soils irrigated with industrial effluents. *Sci Tec & Development* **20** 32 - 36.

Prasad A S 1978 *Trace Elements and Iron in Human Metabolism*. Plenum Publishing Corporation 227 West 17th Street, New York, USA, p 20.

World Health Organization (WHO) 1996 *Guidelines for Drinking Water Quality*. Health criteria and other supporting information 94/9960 - Mastercom/Wiener Verlag - 800 Australia.

Pak. J. Sci. Ind. Res. 2003 **46**(6) 399 - 405

ENVIRONMENTAL IMPACT ASSESSMENT OF AIR POLLUTION IN DIFFERENT AREAS OF KARACHI

Durdana Rais Hashmi and Muhammad Ishaq Qaim Khani*

PCSIR Laboratories Complex, Karachi-75280, Pakistan

(Received February 19, 2002; accepted October 29, 2002)

Measurements of major ambient air pollution components such as O_3, SO_2, CO, NO, and NOx were carried out to obtain baseline data for some selected areas in Karachi. These areas have been categorized on the basis of traffic congestion. Total average concentration of O_3 in Zone - A was 20.80 ppb. In Zone - B 20.36 ppb and in Zone - C 19.10 ppb. Concentration of SO_2 in Zone - A was determined to be 7.30 ppb, in Zone - B 11.60 ppb and in Zone - C 44.30 ppb. Similarly, concentration of CO in Zone - A was 0.96 ppm, in Zone - B 2.50 ppm and in Zone - C 3.49 ppm. Whereas, average concentration of NO and NOx was 13.00 ppb and 23.50 ppb in Zone - A, 2.73 ppb and 5.70 ppb in Zone - B, 69.90 ppb and 83.50 ppb in Zone C. The main contributors of pollutants in these areas are vehicular traffic and industries. A survey of local hospitals was also conducted to correlate the prevailing diseases with air pollution levels. The survey showed that 70% of the patients were suffering from air pollution related diseases, like chronic bronchitis, pulmonary edema and pulmonary emphysema. The data further reveals that the ratio of male to female patients is 2:1.

***Key words*:** Ambient air, Impact of pollutants , Health effect.

Introduction

The proportion of the world's population living in the large town or cities has grown from around 5% to 50% over the past two centuries, Demographers estimate that by the year 2030 approximately two third of the world population will live in large town or cities (Anon 2000).

The high rise of urbanization has created a number of environmental problems such as inadequacy of water supply and sewerage system, over congestion, inadequate transport, slums, haphazard and unplanned development, particularly for the metropolitan areas such as Karachi.

The main environmental problems of Karachi are water pollution, marine pollution, disposal of solid waste and air pollution. Among these environmental degradation, air pollution is a major concern, which is affecting the urban areas of Karachi. The pollutants are being discharged in to the atmosphere from a number of sources but the vehicular traffic and industries are the major contributors.

A few decades ago traffic did not play an important role in air pollution. Today it is the main source of contaminant in the developed and industrialized countries. With an improved standard of living and increased demand on the transport sector, automobile related pollution is fast growing into a problem of serious dimension in our cities. This is caused not only by rapid rise in number of automobiles but also due to narrow roads, slow moving traffic, unfavorable driving cycles, poor enforcement of the laws relating to vehicles road worthiness and poor emission control measures etc.

Traffic introduces dust, soot, carbon dioxide, carbon monoxide, sulphur dioxide, oxides of nitrogen and hydrocarbons in to the air. There are more than one million different types of registered motor vehicles consisting of three wheelers (autorickshaws), cars, buses, motor bikes, etc. plying on the roads of Karachi and discharging toxic gases into the atmosphere.

In USA, about 140 to 150 million tons of pollutants are given off to the air every year. Industries account for 20 to 30 million tons, space heating 10 to 15 million tons, refuse disposal 5 to 10 million tons and motor vehicles 90 million tons or more (Mehboobani 1991). Absence of legislation, lack of public awareness towards conservation of nature and control of pollution has created such a situation, which demands stringent control over pollution emitting sources.

Main object of this study was to assess the existing environmental impact of air pollution components in different areas of Karachi. The generated data could be used for implementation of appropriate measures against hazardous effects of air pollution.

Experimental

Monitoring of ambient air pollution component was carried out for some selected areas to measure the impact of air

---

*Author for correspondence

pollutants in Karachi. The areas that have been categorized are as follow:

1. Moderately populated area with low vehicular traffic (Zone - A).
2. Densely populated area with heavy vehicular traffic (Zone - B).
3. Industrial area with different types of industries (Zone - C).

The ambient air quality measurements were performed by an Air Pollution Monitoring Mobile Laboratory design and fabricated by environmental S.A. France. This Mobile Laboratory is fully equipped with ambient air and particulate monitors designed to measure low concentration of gases, such as O_3, SO_2, NO, NOx, CO, and inhalable particulate in suspension SPM (PM10). It is also equipped with meteorological sensors mounted on a telescopic mast. These advanced technology instruments are microprocessor regulated and define a homogenous and coherent range. An intelligent data logger SAM32 records spot concentrations every second and accumulates these to provide 15-min averages. The logger also monitors instrument alarm and diagnostic functions and controls daily instrument zero/span response checks. Calibrations were made by NO_2 / SO_2 permeation tube oven and zero gas generator. Ozone analyzer $O_3$41M has its own ozone generator for span gas. CO11M was calibrated by standard CO span gas supplied and certified by M/s. Alphagaz, France. A SCANAIR software was used for acquisition, editing and recording logical and analogical data from SAM 32.

Continuous measurement of major ambient air pollution components such as O_3, SO_2, CO, NO, NOx were carried out in the month of February during the year 1998. Fifteen minutes average data of selected areas from Zone - A, Zone - B and Zone - C are presented in the form of Graph I, II, III, IV and V.

A survey of hospitals located in the study area Zone - C was carried out and data was obtained regarding the patients suffering from air pollution related diseases like chronic bronchitis, pulmonary emphysema, pulmonary edema etc. Data for heart diseases was also obtained to search for a relationship with the nature of air pollution to that of heart ailment. Results are provided in Table 1.

Results and Discussion

The subtropical city of Karachi is located in a semi arid zone. It is the biggest industrial and commercial center in Pakistan. According to 1998 census, Karachi has a population of 9.2 million, whereas at the time of the independence in 1947 it was only 0.3 million (Anon 1998). Karachi has also been declared as megacities among 20 megacities of the world (Zarski 1993) the majority of the world's megacities are facing environmental problems. Growing number of urban population, level of industrialization and traffic congestion are the main causes of air pollution in Karachi. Therefore, pollution measurements were carried out to obtain baseline data for some selected areas in Karachi. These areas have been categorized on the basis of traffic congestion.

Table 1

Number of patients suffering from air pollution related diseases in study areas hospital

No. of Hospital	Diseases	No. of Cases			Male & Female Ratio
		Male	Female	Total	
1	T.B.	3735	1701	5436	2.2:1
	Air pollution related diseases	9452	4876	14328	1.9:1
	Chest cancer	372	232	604	1.6:1
	Heart diseases	8114	4206	12320	1.9:1
2	T.B.	680	340	1020	1.2:1
	Air pollution related diseases	1265	625	1950	1.8:1
	Chest cancer	316	149	465	2.1:1
	Heart diseases	708	392	1100	1.8:1

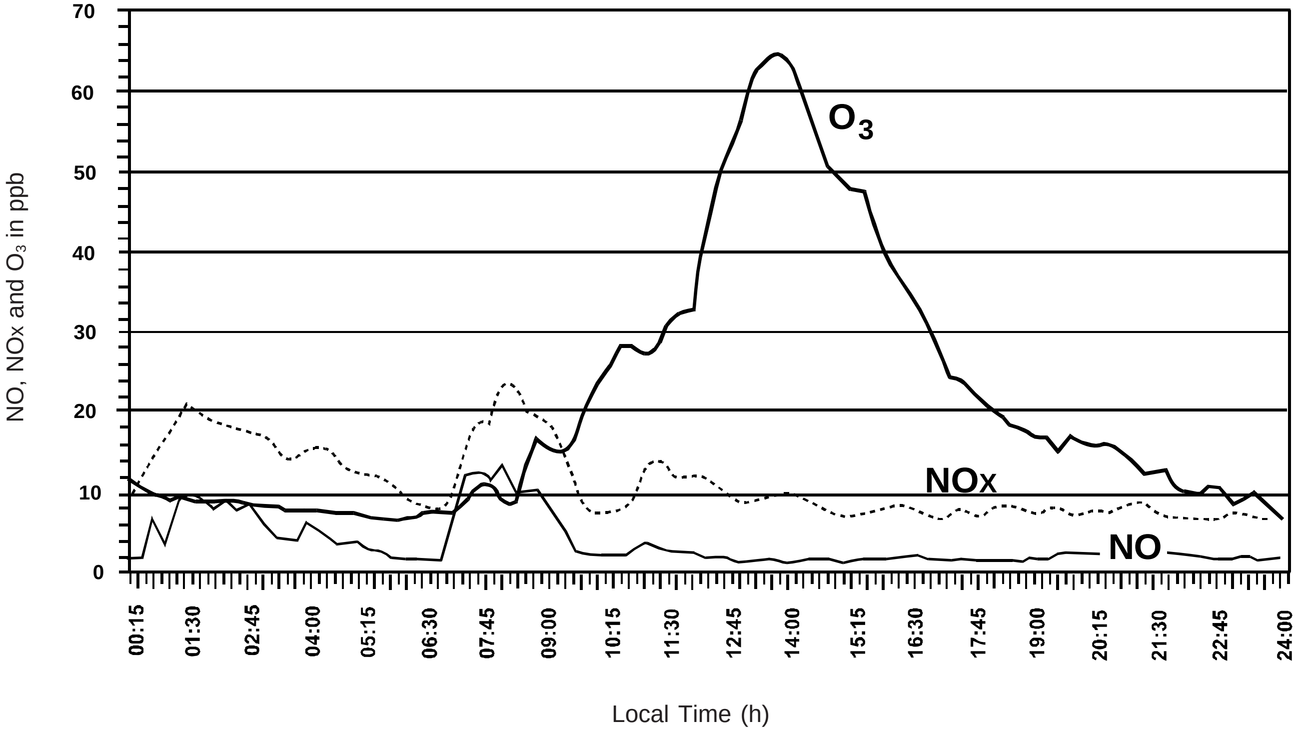
A Scanair software was used for acquistion, editing and recording logical and analogical data from data logger. Continuous measurements of major ambient air pollution components such as O_3, SO_2, CO, NO and NOx were carried out for eight days in the month of February 1998. Fifteen minutes average concentration of ambient pollutants at Zone - A, B and C are presented in the form of Graphs I to V.

The data obtained through this study indicates that almost all the pollutants are well with in WHO limits but a serious situation of air quality degradation is developing in Karachi. There is an urgent need to monitor the air quality over the whole city and adopt suitable control strategies.

Zone - A: Urban background site with moderately populated area having low vehicular traffic density. This sampling site is located at latitude 24°71′ and longitude 67°08′. The site is 390 km away from the main super highway. The area around the sampling site is very sparsely populated. At this sampling site Zone - A, during measurement period, the average wind speed was 1.5 m / sec, wind direction 200.7 degrees, humidity 75.1 %, temperature 19.7°C and barometric pressure 1014.5 m. Bars and solar flux was 196.1 W/m^2.

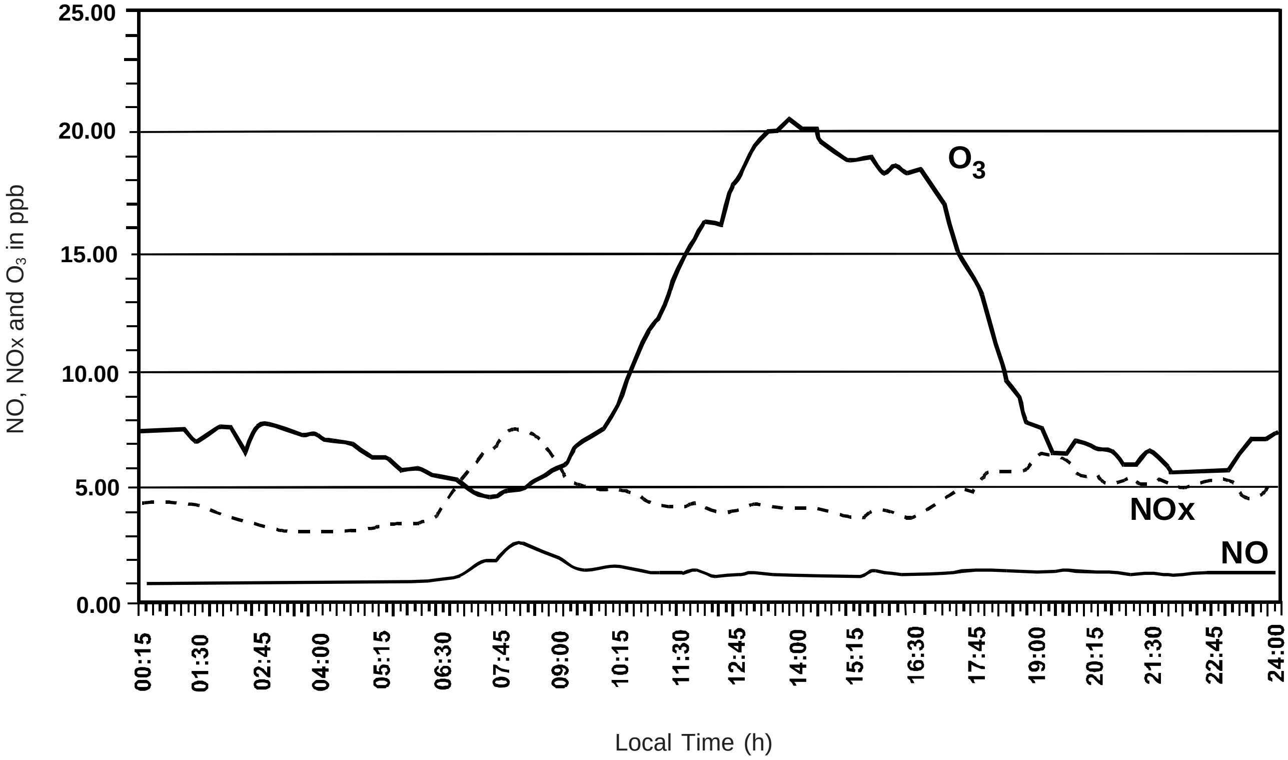
Graph - I

Weekly average concentration of photochemical oxidants in urban background site Zone - A



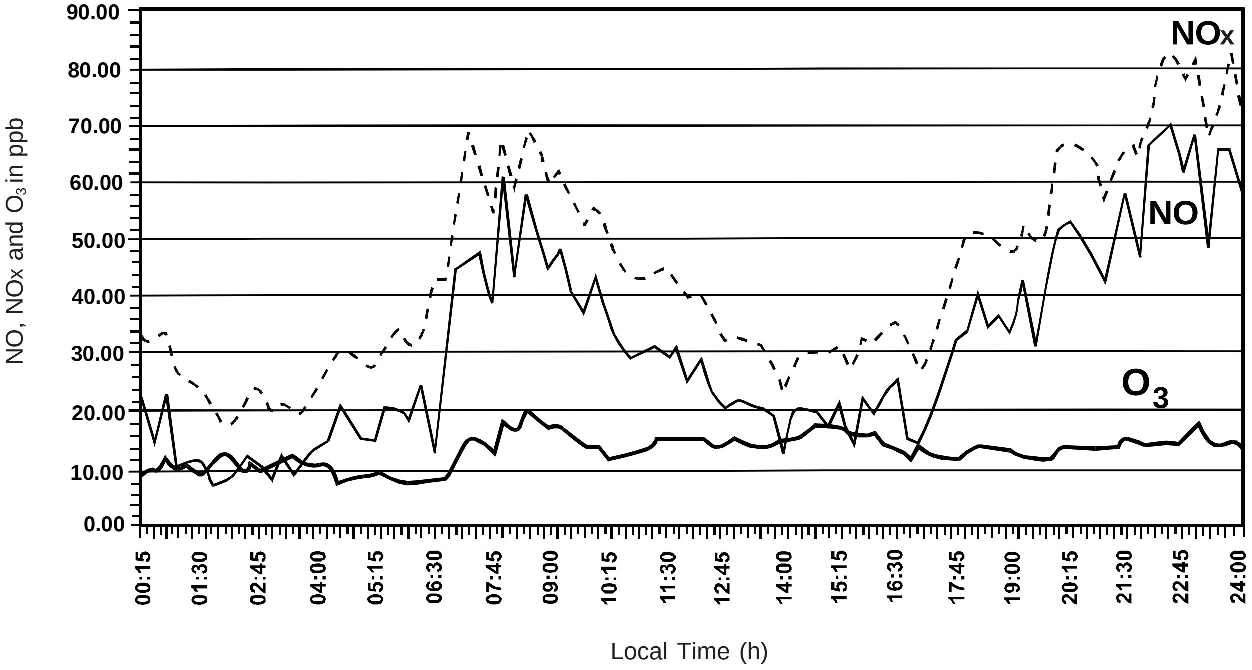
Graph - II

Weekly average concentration of photochemical oxidants in densely populated area Zone - B



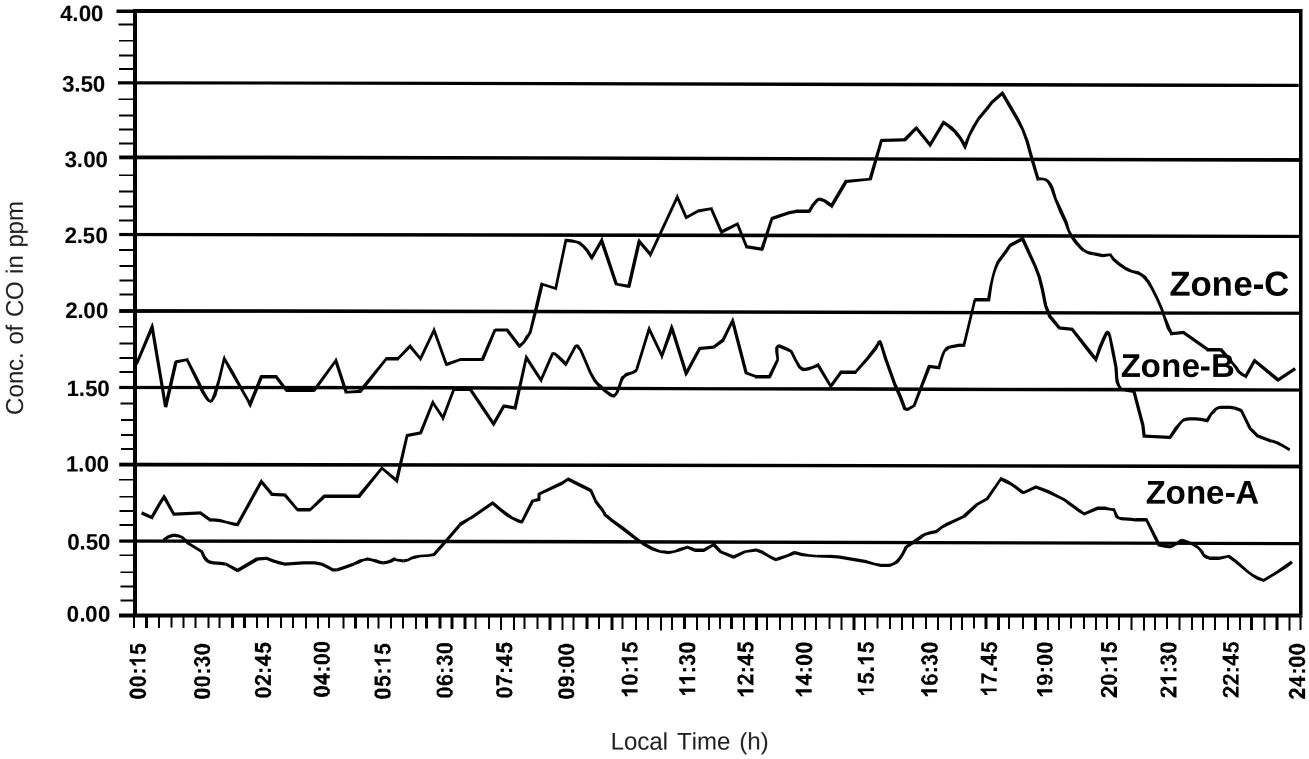
Graph - III

Weekly average concentration of photochemical oxidants in industrial area Zone - C



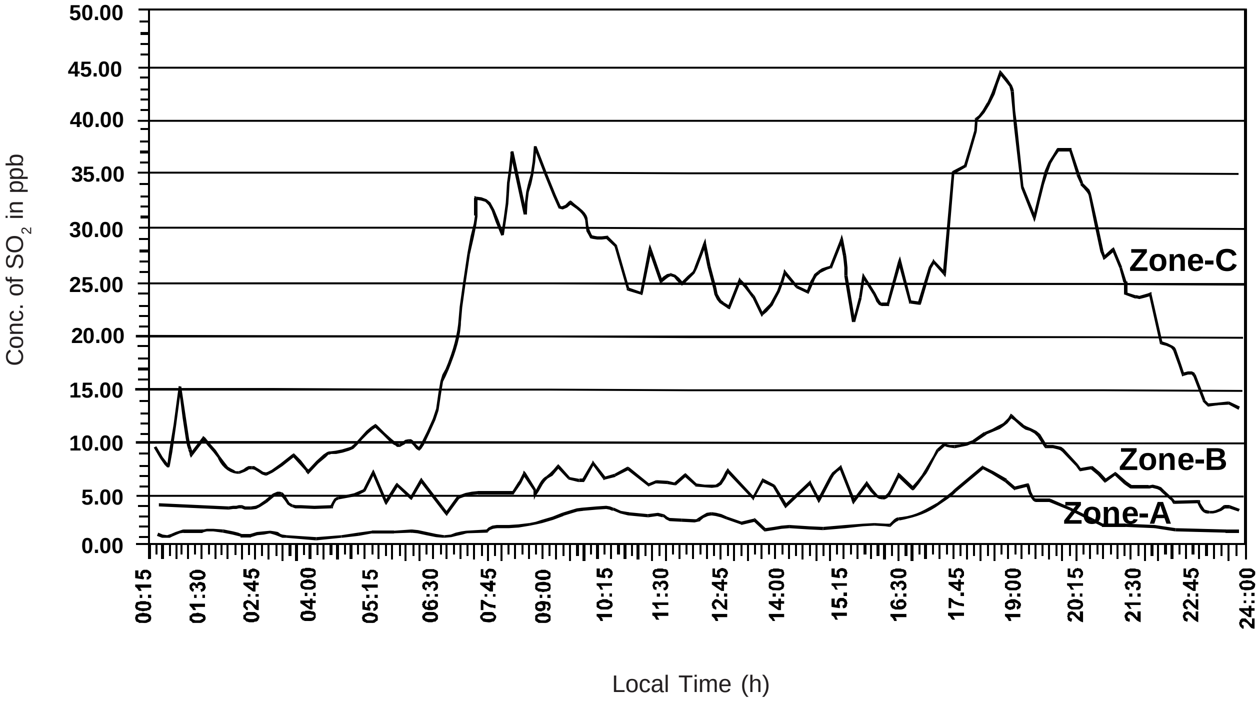
Graph - IV

Weekly average concentration of CO in Zone - A, B & C



Graph - V

Weekly average concentration of SO_2 in Zone - A, B & C



Zone - B: Sub urban site with densely populated area having high traffic density. This sampling site is located at latitude 24°53′ and longitude 67°06′. The site is relatively open place and is surrounded by the residential area. In 320° NW to 240° SW there is a main university road about 1 km away from sampling site having traffic density of 323245 vehicles per day (Anon 1993). The population living around the site belongs to the middle and high-income group. During measurement period, in Zone - B, the average wind speed was 2.75 m / sec. Wind direction 194.6 degrees, humidity 63.71%, temperature 24.1°C and barometric pressure 100.4 m. Bars and solar flux was 228.8 W/m^2.

Zone - C: Industrial area having different types of industries. This sampling site is located at latitude 24°54′ and longitude 67°10′ in south district. The site has nearly 2000 different types of industries. Approximately 60 percent of these industries are textile mills, while others involve pharmaceuticals, chemicals, detergents, iron and steel sulphur refining, vegetable oil, beverages and food products. The daily average traffic density at this sampling site was 39743 vehicles per day (Anon 1993). The average wind speed in this zone during the period of measurement was 2.2 m/sec, wind direction 169.6 degrees, humidity 45.2 %, temperature 22.6°C and barometric pressure 1014.4 m. Bars and solar flux was 215.0 W/m^2.

Graph-I shows the weekly average concentration of photochemical oxidants at urban background site (Zone - A). Maximum average concentration of NO was 13.0 ppb and NOx was 23.5 ppb was found to be at 8:15 h local time. Whereas, maximum average con-centration of O_3 was found to be 64.5 ppb at 13.45 h local time.

It can be seen from the Graph - I that the balance among NO, NO, and O_3 is shifted in the favour of net ozone production. The formation of ozone is evident during day time and highest concentration of ozone was found when solar radiation was also high. The sampling site is located 20 km down wind from the city center and diurnal pattern was clearly observed. The masses were coming from the university road. The main contributor of photochemical oxidants at this location may be due to motor vehicles.

Graph-II shows the weekly average concentration of photochemical oxidants at densely populated area (Zone - B). Maximum average concentration of NO 2.73 ppb and NOx 7.5 ppb was found at 08:00 h local time. Whereas, the maximum average concentration of O_3 was found to be 20.36 ppb at 13:45 h local time. It can also be seen from the Graph - II that the balance between NO, NOx and ozone shift in favour of net ozone production due to photochemical dissociation of NO_2, resulting in the maximum concentration of ozone in the mid afternoon. The main contributor of photochemical oxidants at this location is also main road that has very high traffic density. A some what photo stationary state may exist at this location.

Graph-III shows the weekly average concentration of photochemical oxidants at industrial area Zone - C. Maximum average concentration of NO was found to be 69.9 ppb and NOx was 83.5 ppb at 22:15 h local time, whereas, maximum average concentration of O_3 was found to be 19.9 ppb at 8:45 h local time. It can be seen from the graph that ozone concentration is less than NO and NOx concentration. It has been reported that at typical ambient air and NO concentration, the reaction of photochemical oxidants has a time scale of one to a few minutes (Clark 1988). A power generation plant and boiler of pharmaceutical industry was located only 50 - 75 meters away from the receptor. It shows that most of NO and NOx were coming from combustion sources. Graph-III also shows that in recently emitted plume, the reaction of NO with O_3 is even more rapid having a time scale of only few seconds. So, the chemical reaction between two mixing species was not completed due to time lag and thus low concentration of ozone was observed at this site.

The incomplete burning of carbon containing fuels produce carbon monoxide. It is almost entirely a man made pollutant. Carbon monoxide is most hazardous to human at concentration of 100 ppm or more if experienced over a period of several hours (Bassow 1989). It is estimated that motor vehicles contribute to more than 80 % man made global carbon monoxide emission, with a smaller amount resulting from other combustion processes (Baig 1993).

Graph-IV shows the concentration of carbon monoxide in zone A, B and C. The maximum average concentration of carbon monoxide in Zone - A, (urban background site) was found to be 0.96 ppm at 18.00 h local time, in Zone - B (densely background site) was 2.50 ppm at 18:30 h local time whereas, in Zone - C (Industrial Area) the maximum average concentration of carbon monoxide was 3.49 ppm at 18:00 h local time. In the morning hours, the movement of traffic is towards down town and is the reverse in the evening. The variation in the concentration of carbon monoxide shows that the concentration gradually increases till 9:00 h and then comes down at 13:00 h and again increase around 18:00 h, the rush hours. In Zone - A and B the air pollution being generated by vehicular traffic. The study further shows that the level of carbon monoxide in industrial area (Zone - C) is relatively higher than densely populated area Zone - B. The pollution in industrial area is mainly due to industrial processes.

Graph-V shows the concentration of SO_2 in the selected zones A, B and C. The major sources of SO_2 are combustion of fossil fuels, coke ovens, metal smelting, wood and pulp production, petroleum refining and brick manufacture. The estimated background concentration of SO_2 is 0.2 ppb and calculated atmospheric residence time is 4 days (Kenneth and Cecil 1976). Short term high level of SO_2 may increase respiratory diseases, lung function disturbance and mortality in adult and children (Wieslaw 1995). The maximum average concentration of SO_2 at urban background site (Zone - A) was found to be 7.30 ppb at 18:15 h local time, at densely populated area (Zone - B) was 12.60 ppb at 19:00 h local time while at industrial area (Zone - C) was found to be 44.3 ppb at 18:45 h local time.

The variation in the concentration of SO_2 indicates the same pattern as carbon monoxide concentration in Zone - A, B and C, whereas the concentration of SO_2 in zone C is higher than Zone - A and B due to the combustion process in industries. The average concentration of SO_2 in all the selected areas are well with in WHO limits ($40 - 60\,\mu g / m^3$) (WHO 1987). The low level of SO_2 may be due to the fact that the use of coal in Karachi is negligible and almost 99 percent of the population and factories use natural gas (Sui gas) as a fuel, which is sulphur free.

Hospital survey. A hospital survey was carried out to assess the impact of pollution on human health (Table 1). This survey revealed that a total 6456 cases of tuberculosis were reported during last two-year, out of which 4415 were males and 2041 were females.

A total number of 16078 patients were suffering from air pollution related diseases consisting of 10577 males and 5501 females.

A total of chest cancer cases 1069 attributed to air pollution, out of which 688 were males and 381 were female patients. The hospital data indicates the trend of cancer shifting from old age group of middle age group, which is an indicator of deteriorating air environment.

The heart ailment cases of 13420 were reported during the same period, 8822 were males and 4598 were females.

The degrading effects on human health can also be seen from the increasing number of patients in the hospitals suffering from air pollution related diseases. Air pollution has become a world wide public health problem, particularly in large cities of the developing countries. An estimated 130,000 premature deaths and 50 - 70 million incidents of respiratory illness occur each year due to episodes of urban air pollution in developing countries, half of them in East Asia (Maddison 1997).

Air pollution increases the risk of chronic obstructive pulmonary diseases and acute respiratory infections in childhood, lung and chest cancer, tuberculosis, prenatal out comes including low birth weight and eye diseases.

Survey of hospitals show that the number of patients suffering from air pollution related diseases to that of tuberculosis is about 3:1. The number of male cases as compared to female regarding air pollution related chest diseases, are in the ratio of 2.1:1. This may be due to an extensive exposure of males to the polluted ambient air and professional hazards as compared to females who are housewives and remain indoor.

Few decades ago, only tobacco smoke was considered as an important risk for lung cancer but now a days polluted air is the most important factor for lung cancer. People in developing countries are commonly exposed to very high levels of pollution for 3 - 7 h daily over many years (Engel and Hartodo 1998). The number of lung cancer cases by air pollution are also on the increase and mostly male cases due to their exposure to air. The worst effected age group is between 50 - 60 years but now this is reducing up to 45 - 60 years. This is mainly because of increasing air pollution level but some other factors are also involved like personal hygiene, social activity, socio-economic condition, mental worries and smoking etc.

The cases of heart diseases are also on the increase. This is mainly due to the increase of ambient air pollution. The male and female ratio of heart diseases is approximately 2.1:1, indicating that men suffer more than women due to exposure in society. The worst effected age group of heart patients is between 40 - 50 years, which can be attributed to the exposure. Effect of air pollution on human health varies according to both the intensity and duration of exposure and health status of exposed population.

Conclusion

The baseline data for ambient air pollutants in selected areas of Karachi reveals that the average concentration of O_3, SO_2, CO, NO, and NOx are well with in WHO limits, But the variation indicates a rising trend due to multiple factors like growth in population, motor vehicles and industries etc. The observed values of NO_2 and NOx during the survey indicate that these pollutants originate from the combustion of fuel in motor vehicle power generation plant and boiler of industries. It was also observed that O_3, SO_2 and CO are mainly emitted from motor vehicles and from Industrial processes. The generated data has the potential to lay the foundation for implementation of appropriate ambient air quality standards.

References

Anon 1993 *Traffic Survey Programme for DKA, Karachi*. Traffic Engineering Bureau Report No. 926. Traffic Engineering Bureau Karachi.

Anon 1998 *Pakistan In Figure*. Federal Bureau of Statistics, Statistical Division, Government of Pakistan.

Anon 2000 "Environment and Health", *Bulletin of WHO*. **78**(9) pp 1117-1126.

Baig M A A 1993 *International Seminar on Environmental Pollution.* Pak. Association of Scientist and Scientific Profession (PASSP), 29th April, 1993.

Bassow H 1989 *Air Pollution Chemistry*, An experimenter's source book. Hyden Book Company. Inc. Rochella Park, New Jersey, USA. pp 37.

Clark P A 1988 Mixing models for simulation of plume interaction with ambient air. "*Atmospheric Environment*" **22** 1097 - 1106.

Engel P, Hartodo E, Ruel M 1998 Smoke exposure of women and young children in highland Guatemala, *Predications Recall Accuracy, and Human Organization*. **54** 408 - 417.

Kenneth W, Cecil F 1976 *Air pollution, Its Origin & Control*. Harper & Row Publishers. NewYork. pp 103.

Maddison D 1997 A meta analysis of air pollution epidemiological studies. London Centre for Social and Economic Research on the Globle Environment, University College London.

Mehboobani A K 1991 *Automobile Pollution Vehicle Emission and Pollution Control*. Ashish Publishing House, New Dehli, 110026, ASBN 81 - 7024 - 414 - 5, pp 41.

Wieslaw J 1995 Review of recent studies from Central and Eastern Europe. Associating of Respiratory health effect with high level of exposure to traditional air pollutants. *Environmental Health Perspective.* **103** (suppl. 2) pp 15.

WHO 1987 Global Pollution and Health. Results of Health Relating Environmental Monitoring WHO & UNEP Publication, Global Environment Programme. *Environmental Data Report*. pp 10,17 & 24.

Zarski L 1993 Urban air pollution in megacities of the world. *World Environment.* **36**(2) 4.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 406 - 408*

Synthesis of Hetero-Bicyclic Compounds
Part-X. Formation of 2H,4H,5H 2,2-Diphenyl-4, 5-Dioxopyrido [4, 3-d] 1,3 Dioxin

Abdul Salam and Ausaf Akhtar*

PCSIR Laboratories Complex, Karachi-75280, Pakistan

(Received May 24, 2002; accepted December 14, 2002)

Aminopyranodioxin derived from benzophenone isomerize to yield 6 substituted 1, 2-dihydropyridodioxins **(III)**,whose structures were determined by chemical conversions and spectroscopic studies.

Key words: Pyranodioxin, Pyridodioxin, Hetero-bicyclic compound.

Introduction

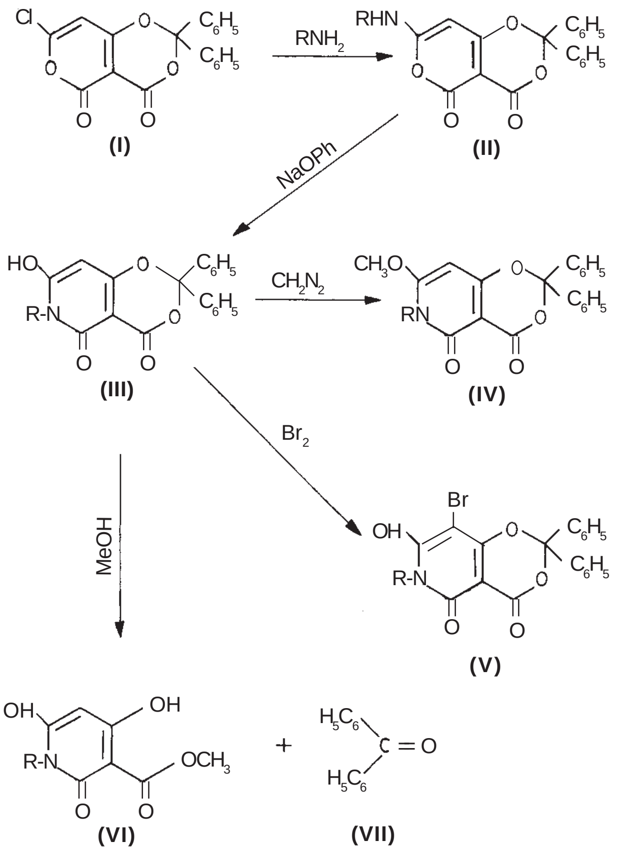
The reaction of acetone with malonyl chloride yields chloropyranodioxins (Davis and Elvidge 1952). These chloropyranodioxins react with amines to produce aminopyranodioxins (Butt *et al* 1992). The aminopyranodioxins isomerize to the corresponding pyridodioxins in the presence of sodium phenoxide (Butt and Akhtar 1965). This study was extended to the reaction of ketones other than acetone with malonyl chloride and the subsequent reaction with amines followed by isomerization to yield pyridodioxins (Butt *et al* 1997). Benzophenone yields similar chloro product with malonyl chloride which reacted with aromatic amines and isomerized then gives the corresponding 2,2-diphenyl 4,5-dioxopyrido (4, 3-d) (1,3) dioxins (Butt *et al* 1990). In the present study, 2,2-diphenyl chloropyranodioxin has been reacted with aliphatic amines to yield the amino compound, which undergo rearrangement under the action of sodium phenoxide to the corresponding aminopyridodioxins. The title compound was characterized by elemental analysis supported by degradations to known product, formation of derivatives and spectroscopic studies.

Materials and Methods

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. UV Spectra were recorded on Perkin Elmer UV visible spectrophotometer λ 4C.

7-Chloro-2,2-diphenyl-4,5-dioxopyrano[4,3-d]1,3 dioxin ***(I)***. The title compound **(I)** was prepared by heating benzophenone (0.1 mole, 3.7 g) and malonyl chloride (0.2 moles, 4.0 ml) on a water bath until the mass is solidified. Trituration of the product with ether gave 7-chloro-2,2-diphenyl-4,5-dioxopyrano [4, 3-d] 1,3 dixoin **(I)**, which crystallized from benzene, m.p 179°C. Found: C, 64.1; H, 3.3; Cl, 9.8% For $C_{19}H_{11}O_5Cl$ requires: C, 64.3; H, 3.1; Cl, 10.0%.

7-Ethylamino-2,2-diphenyl-4,5-dioxopyrano [4,3-d]-1,3 dioxin ***(II)*** *R=ethyl.* To a solution of **(I)** (5.0g, 0.02 mole) in chloroform (10 ml), ethylamine (2.3 ml, 0.04 mole) in 10



Scheme 1

---

*Author for correspondence

ml chloroform was added with constant stirring. The solid product obtained was washed with water and dried. 7-ethylamino-2, 2-diphenyl-4, 5-dioxopyrano [4, 3-d] 1, 3-dioxin (4.2g) was crystallized from methanol, m.p. 162°C. Found: C, 69.2; H, 4.4; N, 3.6; $C_{21}H_{17}O_5N$ requires: C, 69.4; H, 4.6; N, 3.8%. Other 7-amino 2, 2-diphenyl-4, 5-dioxopyrano [4, 3-d] 1, 3 dioxins **(II)** prepared as above are listed in Table 1.

Reaction of 4,5 dioxo-2,2-diphenyl 7-ethylamino [4,3-d]1,3 dioxin with sodium phenoxide in phenol. 4, 5 dioxo-2, 2-diphenyl 7-ethylamino [4, 3-d] 1, 3 dioxin (2.5 g, 0.01 mole) was added to a solution of sodium (0.7 g.) in phenol (20 ml) and the mixture was heated at 120°C for two minutes. The solution was cooled, diluted with water and extracted with ether to recover excess of phenol. The ethereal layer was again extracted with water and the combined aqueous extracts (150 ml) were acidified with 2N HCl. The solid product obtained 4, 5-dioxo-2, 2-diphenyl-6-ethyl-7-hydroxy pyrido [4, 3-d] 1, 3 dioxin **(III)** R = ethyl, 2.1g was crystallized from methanol, m.p. 198°C. It produced reddish brown colour with aq. $FeCl_3$ and gave effervescence with aq. sodium bicarbonate. Found: C, 69.3; H, 4.6; N, 3.7% for $C_{21}H_{17}O_5N$ requires: C, 69.4; H, 4.6; N, 3.8%.

Table 1

7-Amino-2, 2-diphenyl-4, 5-diphenyl-4, 5-dioxopyrano [4, 3-d]-1, 3-dioxins **(1)**

S. No.	Primary amine	Quantity ml	7-Chloro-2-2-diphenyl, 4, 5-dioxopyrano-[4,3-d] 1,3 dioxing	Product IR	Yield %	M.P °C	Solvent for crystallization	Molecular formula	Analysis Found			Analysis Requires			UV Light absorption in methanol	
									C	H	N	C	H	N	λ_{Max}	Log
1	Methyl amine	3.90	5	Methyl	82.0	165°C	$CH_3OH+CHCl_3$	$C_{10}H_{15}O_5N$	68.8,	4.1,	3.8	68.7,	4.2,	4.0	305	4.57
2	Ammonia	1.00	5	Hydrogen	53.0	270°C	CH_3OH	$C_{19}H_{13}O_5N$	67.9,	3.7,	4.3	68.0,	3.8,	4.1	314	4.51
3	Ethyl amine	2.27	5	Ethyl	68.0	162°C	$CHCl_3+ CH_3OH$	$C_{21}H_{17}O_5N$	69.2,	4.4,	3.6	69.4,	4.6,	3.8	302	4.54
4	Propyl amine	2.29	5	Propyl	66.0	148°C	CH_3OH	$C_{12}H_{19}O_5N$	69.4,	4.8,	3.3	70.0,	5.0,	3.7	325	4.54
5	*n*-Butyl amine	2.80	5	*n*-Butyl	71.0	164°C	CH_3OH	$C_{23}H_{21}O_5N$	70.8,	5.3,	3.5	70.5,	5.3,	3.5	305	4.57
6	Benzyl amine	3.00	5	Benzyl	58.3	170°C	$CHCl_3$	$C_{26}H_{19}O_5N$	73.9,	4.3,	3.15	73.4,	4.4,	3.2	315	4.56

Table 2

N-Substituted 4, 5-dioxo, 2-2-diphenyl-7-hydroxy-6-pyrido [4, 3-d] 1, 3 dioxins **(III)**

S. No.	7-Amino pyrano (1,3) dioxin	Quantity (g)	Sodium/ phenol	Pyridino (4, 3-d) 1, 3-dioxin **(III)**	Yield %	MP °C	Molecular formula	Analysis Found			Analysis Requires		
								C	H	N	C	H	N
1.	Methyl amino	4.0	0.65g/ 3.2 ml	4, 5-dioxo 2,2-diphenyl hydroxy 6-methyl	50	201°C	$C_{20}H_{15}O_5N$	68.9	4.3	3.9	68.7	4.2	4.0
2.	Ammonia	2.5	0.70g/ 20 ml	6-amine 4, 5 dioxo 2, 2-diphenyl 7-hydroxy	48	210°C	$C_{19}H_{13}O_5N$	67.9	3.7	3.9	68.0	3.8	4.1
3.	Ethyl amino	2.5	0.70g/ 20 ml	4, 5-dioxo 2, 2-diphenyl 6-ethyl 7-hydroxy	61	280°C	$C_{21}H_{17}O_5N$	69.3	4.6	3.7	69.4	4.6	3.8
4.	*n*-Propyl amino	2.5	0.60g/ 18 ml	4, 5-dioxo 2, 2-diphenyl 7-hydroxy 6-propyl	70	228°C	$C_{22}H_{19}O_5N$	69.4	5.1	3.9	70.0	5.0	3.7
5.	*n*-Butyl amino	2.0	0.70g 21 ml	6-butyl 4, 5 dioxo 2, 2-diphenyl 7-hydroxy	45	214°C	$C_{23}H_{21}O_5N$	70.1	5.0	3.2	70.7	5.1	3.5
6.	Benzyl amino	4.0	0.86g/ 25.8 ml	6-benzyl 4, 5 dioxo 2, 2-diphenyl 7-hydroxy	60	198°C	$C_{26}H_{19}O_5N$	73.1	4.3	3.1	73.4	4.4	3.2

Other alkylamino pyranodioxins **(II)** were reacted similarly with sodium phenoxide in phenol and the products obtained by formula **(III)** are listed in Table 2.

Both 4,5-dioxo-2,2-diphenyl 6-ethylamino 7-methoxy pyrido [4,3-d] 1,3 dioxin (IV). To 0.5g **(III)** R = ethyl in ether (10 ml), a solution of diazomethane in ether was added in portions until yellow colour persisted. The solution was kept overnight in a refrigerator and the excess solvent was removed. The residue upon trituration with ether yielded a neutral product, which showed no colouration with aq $FeCl_3$ **(IV)** 0.3 g obtained was crystallized from methanol, m.p 183°C Found: C, 69.9; H, 4.9; N, 3.8% for $C_{22}H_{19}NO_5$ requires: C, 70.0; H, 5.0; N, 3.7%.

8-Bromo 4,5-dioxo 2,2-diphenyl-6-ethylamino-pyrido [4,3-d]-1,3 dioxin (V) R = ethyl. The compound **(III)** R = ethyl (0.5 g) was dissolved in chloroform (20 ml) and bromine in chloroform was added dropwise till an orange colour persisted. The reaction mixture was kept at room temperature for 1 h and subsequently, the solvent was removed. The solid bromo product (0.5 g, 75%) **(V)** R = ethyl was re-crystallized from methanol, m.p. 189°C. Found: C, 57.0; H, 3.6; N, 3.1% requires: C, 56.8; H, 3.8; N, 3.1% for $C_{21}H_{16}O_5N\,Br$.

Degradation of (III) with methanol. The compound **(III)** R = ethyl (0.05g) was refluxed with methanol (25 ml) for 6 h. The solution upon concentration in vacuum yielded **(VI)** 0.3 g which was crystallized from MeOH, m.p 221°C. Found: C, 50.5; H, 5.3; N, 6.3% requires: C, 50.7; H, 5.1; N, 6.5 %. From the filtrate benzophenone was isolated and characterized as 2,4 dinitrophenyl - hydrazone derivative for $C_9H_{11}NO_5$.

Results and Discussion

Isomerization of 7-alkylamino 4, 5-dioxopyrano 2, 2-diphenyl [4, 3-d] 1, 3 dioxins **(II)** under the influence of sodium phenoxide to the corresponding alkyl substituted pyridodioxins **(III)** has been studied. For instance, 7-ethylamino 4, 5 dioxo 2, 2-diphenyl 6-ethylamino pyrano [4, 3-d] 1, 3 dioxin **(II)** on reacting with phenoxide in phenol produced $C_{21}H_{17}O_5N$ **(III)** R-C_2H_5, m.p. 198°C (Scheme 1). This product is enolic in nature ($FeCl_3$ test) dissolves in aq. sodium bicarbonate solution and is isomeric with the starting material. It is moderately stable towards alcohol and is decomposed on boiling. The other alkylamino pyranodioxins yield similar isomeric products upon treatment with sodium phenoxide in phenol. These products **(III)** absorb in the UV region 310-315 mμ. Table 3 closely resembling pyridodioxins.

The OH group at position 7, was methylated into the product **(IV)** R = ethyl, λ_{max} 300 log ∈ 4.0 (λ_{max} 275, log ∈ 4.2). Similarly, bromo derivative **(V)** R = ethyl had (λ_{max} 300 log ∈ 4.87). Finally, the structure **(III)** for these new products was confirmed by boiling it **(III)** R = ethyl in methanol to form pyridine methyl ester **(VI)** R = ethyl and benzophenone **(VII)**.

References

Butt M A, Akhtar I A 1965 Synthesis of hetero-bicyclic compound. *Part-I.* Synthesis of pyridino-(1-3) dioxins. *Tetrahedron* **21** 1917-1922.

Butt M A, Kemal R, Salam A, Akhter A 1990 Synthesis of hetero-bicyclic compounds. *Part-VII*: Formation of 2, 2-diphenyl-4, 5-dioxopyridino (4, 3-d)(1,3) dioxins. *Pak J Sci Ind Res* **33** (1-2) 27-29.

Butt M A, Kemal R, Salam A, Mumtaz G 1992 Synthesis of hetero-bicyclic compounds. *Part-VIII:* Formation of 6-alkyl-2, 2-dimethyl, 4, 5-dioxo-7-hydroxy pyridino (4, 3-d) (1,3). *Pak J Sci Ind Res* **35**(9) 325-327.

Butt M A, Kemal R, Salam A, Akhter A 1997 Synthesis of hetero-bicyclic compounds. *Part-IX*: Formartion of 2, 2-disubstuted 4, 5-dioxo-pyridino (4, 3-d) (1,3) dioxin. *Pak J Sci Ind Res* **40**(5-12) 75-78.

Davis S J, Elvidge J A 1952 Heterocyclic synthesis with malonyl chloride. *Part I*: Pyrano-1, 3 dioxins from ketones. *J Chem Soc* 4109-4114.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 409 - 413*

TERNARY LIQUID EQUILIBRIA OF ETHANOL-WATER-OLEYL ALCOHOL AND ETHANOL-WATER-OLEIC ACID SYSTEMS

M S Rahman*, M A Rahman and M N Nabi

Department of Applied Chemistry and Chemical Technology, University of Rajshahi, Rajshahi, Bangladesh

(Received Feburary 14, 2002; accepted January 2, 2003)

The ternary equilibrium data are presented for the ethanol -water - oleyl alcohol and ethanol- water - oleic acid systems at 30°C. The binodal curves, tie lines, plait points, distribution coefficients and separation factors have been determined to extract ethanol from the aqueous solution. Hand's method has been used to correlate tie lines and to calculate coordinates of plait points. Tie line data were satisfactorily correlated by the Othmer - Tobias method on a mass fraction basis.

Keywords: Ternary equilibrium data, Tie line, Ethanol-water-oleyl alcohol.

Introduction

The production of anhydrous alcohol from lower concentration of aqueous solutions, requires almost complete removal of water. This operation is often complicated by the formation of azeotropes. Typically, azeotropic or extractive distillations are used for such separations. These traditional technology for the separation of alcohol from aqueous solutions are energy intensive and expensive because of the high reflux ratio and large number of stages required for nearly complete separation.

Liquid - liquid extraction is one of the separation process in chemical industries and it requires a reliable knowledge of the liquid -liquid equilibria for the system to be separated. The extraction of alcohol from dilute solutions resulting from fermentation processes and many solvents have been tried to improve such recovery by means of liquid-liquid extraction (Munsan and King 1984; Botto *et al* 1989; Letcher *et al* 1991; Arda and Sayar 1992; Briones *et al* 1994; Maeda *et al* 1997; Gomez Marigliano *et al* 1998; Rahman *et al* 2001). For the design of an extracting device, quantitative representation is required of the liquid - liquid equilibria of the appropriate ternaries.

The purpose of this study is to determine precise binodal curves, tie lines and plait points for ethanol-water -oleyl alcohol and ethanol-water-oleic acid systems at 30°C. The distribution coefficients and separation factors have to be evaluated to investigate the extracting capabilities of the selected solvents.

Experimental

Materials. Ethanol (Merck KGaA, Germany, 99-100%, d = 0.79 g/cm^3), oleyl alcohol (BDH, England, d = 0.83 g/cm^3) and oleic acid (BDH, England, 92%, d=0.888 g/cm^3) were used without further purification. Distilled water was used throughout this work.

Solubility data. The solubility data for ethanol-water-oleyl alcohol and ethanol-water-oleic acid systems were determined by the titration method (Feki *et al* 1994). 10 cm^3 of water was measured into a 125cm^3 closed Erlenmeyer flask and solvent was added from a burette and agitated till the solution started to appear turbid. The amount of solvent added was recorded as the maximum solubility of the solvent in the water and gave the first point of the binodal curve on the base line. The appearance of turbidity indicated the beginning of formation of the second phase, the solvent layer. Therefore, further addition of a small amount of solvent gave a heterogeneous mixture. Then ethanol was added from a burette until the first appearance of distinct clear homogeneity. This gave another point of binodal curve on the aqueous side. Same procedure was applied starting with an initially measured quantity of solvent to construct the binodal curve on the solvent side. The refractive index of each mixture indicated as a point on the binodal curve which was measured by using an "Atago Precision Abbe Refractometer."

Equilibrium data. Equilibrium data were determined for these systems at 30°C. Aliquots of 20 cm^3 each of water and solvent were taken in five different 250 cm^3 closed Erlenmeyer flask and then various amounts of ethanol were added until the formation of single phases were noticed. These flasks were vigorously shaken by an electric shaker for 30 min and were permitted to settle for 60 min. After settling, two coexisting phases were formed. 1-2 Drops of each equilibrated phase were removed by pipette, and their refractive index was carefully measured. Compositions of the phases were determined from the solubility data using calibration curves for refracto-

---

*Author for correspondence

Table 1
Solubility data of the ethanol-water-oleyl alcohol system at 30°C.

	Composition, wt%		
	Water	oleyl alcohol	ethanol
Water-rich phase	100.0	0.0	0.0
	40.4	1.3	58.3
	37.5	3.1	59.4
	34.0	5.6	60.4
	31.6	7.9	60.5
	28.6	12.1	59.3
	26.6	15.5	57.9
Oleyl alcohol-rich phase	0.0	100.0	0.0
	3.0	68.1	28.9
	7.0	58.5	34.5
	10.5	50.0	39.5
	13.9	42.4	43.7
	18.2	32.0	49.8
	21.3	25.3	53.4
	24.0	19.6	56.4
Plait point	18.2	32.0	49.8

Table 2
Solubility data of the ethanol-water-oleic acid system at 30°C.

	Composition, wt%		
	Water	oleic acid	ethanol
Water-rich phase	100.0	0.0	0.0
	44.4	1.7	54.3
	41.0	4.0	55.0
	38.2	9.6	54.9
	36.2	9.6	54.2
	32.5	14.5	53.0
	29.7	18.4	51.9
Oleyl alcohol-rich phase	0.0	100.0	0.0
	3.4	78.6	18.0
	6.0	67.4	26.6
	10.3	53.7	36.0
	14.5	44.0	41.5
	19.0	35.5	45.5
	22.1	30.0	48.9
	26.4	23.3	50.3
Plait point	22.5	28.7	48.8

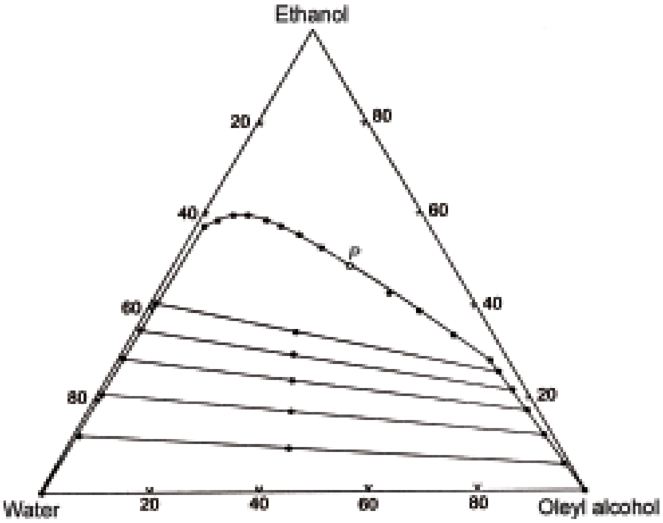


Fig 1. Binodal curve for the ethanol - water - oleyl alcohol system at 30°C

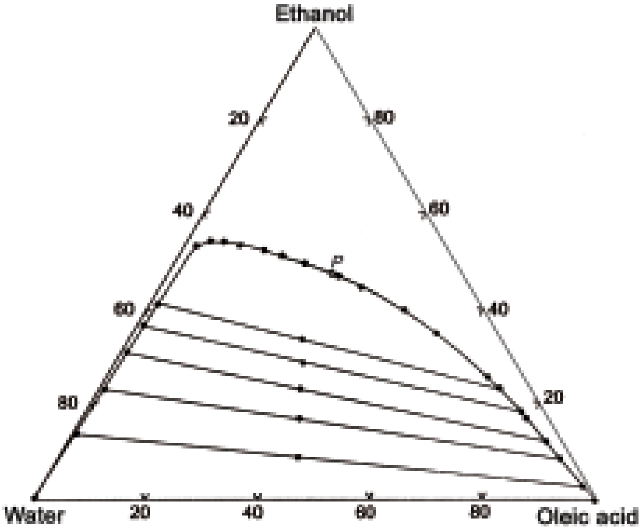


Fig 2. Binodal curve for the ethanol - water - oleic acid system at 30°C.

metric measurements (Ananthanarayanan and Rao 1968; Hegazi and Salem 1983).

Results and Discussion

The composition to points of binodal curves for ethanol-water-oleyl alcohol and ethanol-water-oleic acid systems have been experimentally determined at 30°C. Binodal data are given in Table 1 and 2 and the ternary diagrams are plotted in Fig. 1 and 2. It is seen that the binodal region of ethanol-water-oleyl alcohol system is slightly broader than that of ethanol-water-oleic acid system. It is also found that the binary systems of water-oleyl alcohol and water-oleic acid are immiscible.

Experimental data on compositions of coexisting phases are presented in Table 3 and distribution coefficients and separation factors between the coexisting liquid phases have been calculated. These data allowed to draw the corresponding equilibrium distribution curves in Fig 3 and equilibrium tie lines in Fig 1 and 2. Fig 3 shows that the concentration of ethanol in organic phase increases with increasing concentration of ethanol in aqueous phase. Ethanol containing one methyl group (- CH_3) and one methylene group (= CH_2) in the

Table 3

Composition of co-existing phases in the ethanol- water - oleyl alcohol/oleic acid systems at 30˚C.

Composition of initial mixtures, wt%			Composition of organic phase, wt%			Composition of aqueous phase, wt%			K_D	α
Water	Oleyl alcohol	Ethanol	Water	Oleyl alcohol	Ethanol	Water	Oleyl alcohol	Ethanol		
49.3	40.9	9.8	0.8	92.5	6.7	87.2	0.2	12.6	0.532	57.82
44.9	37.3	17.8	1.2	86.0	12.8	78.2	0.3	21.5	0.595	39.67
41.3	34.3	24.4	1.6	80.4	18.0	70.1	0.4	29.5	0.610	26.52
38.2	31.7	30.1	2.1	75.5	22.4	63.9	0.6	35.5	0.631	19.12
35.5	29.5	35.0	2.6	71.0	26.4	58.2	0.8	41.0	0.644	14.31
Water	Oleic acid	Ethanol	Water	Oleic acid	Ethanol	Water	Oleic acid	Ethanol		
47.9	42.6	9.5	0.8	95.9	3.3	85.5	0.4	14.1	0.234	26.00
43.8	38.9	17.3	1.7	89.0	9.3	75.8	0.7	23.5	0.396	18.00
40.3	35.8	23.9	2.3	84.8	12.9	67.7	1.0	31.3	0.412	12.12
37.3	33.2	29.5	3.8	77.0	19.2	61.7	1.1	37.2	0.516	8.32
34.8	30.9	34.3	5.2	70.6	24.2	56.9	1.2	41.9	0.578	6.35

K_D, Distribution coefficient of ethanol; α, Separation factor,

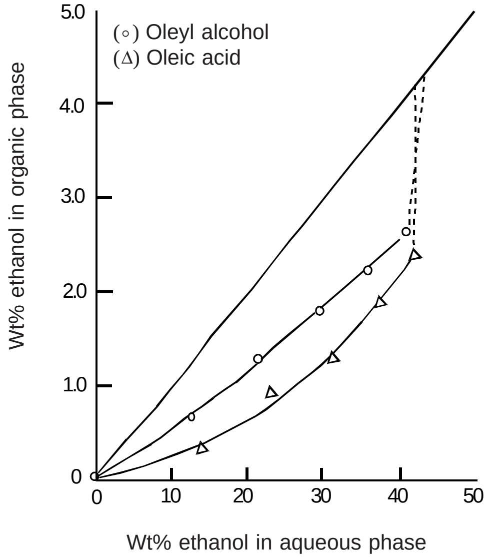


Fig 3. Equilibrium distribution curve for the ethanol - water - solvent systems.

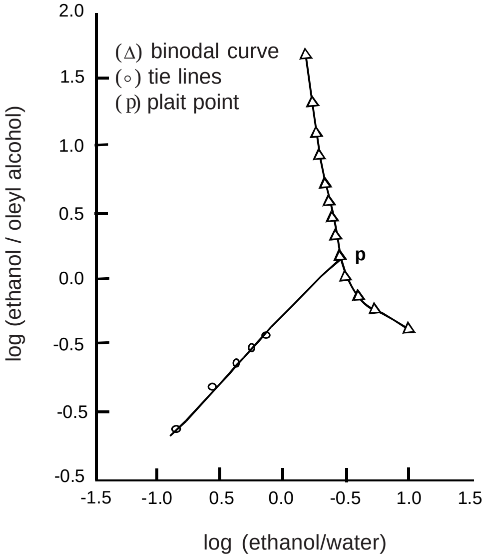


Fig 4. Hand type ternary diagram for plait point determination of the ethanol - water - oleyl alcohol system.

molecule, with a ratio of (OH:C) 1:2, has for stronger polarity (Katayama *et al* 1998) than oleyl alcohol and oleic acid. Oleyl alcohol has a considerably higher dielectric constant (Weast and Astle 1982-1983) that can both donate and accept hydrogen bonds (Loudon 1995), it is a better polar molecule than oleic acid. Fig 1 and 2 show that the concentration of ethanol in oleyl alcohol or oleic acid-richer phase is lower than that in water-richer phase; water has stronger affinity for ethanol than oleyl alcohol and oleic acid.

The separation factor (α) is determined numerically from the tie line data because, it is the ratio of distribution coefficient of ethanol to the distribution coefficient of water. The distribution coefficient of ethanol (K_D) is the ratio of concentration of ethanol in organic and aqueous phases, respectively. Similarly, the distribution coefficient of water is the ratio of concentration of water in organic and aqueous phases, respectively. Table 3 shows value of distribution coefficient (K_D) and separation factor (α) have been measured for extraction of ethanol with weight percent feed (EtOH-H_2O) concentration. It is seen from Table 3 that oleyl alcohol gives

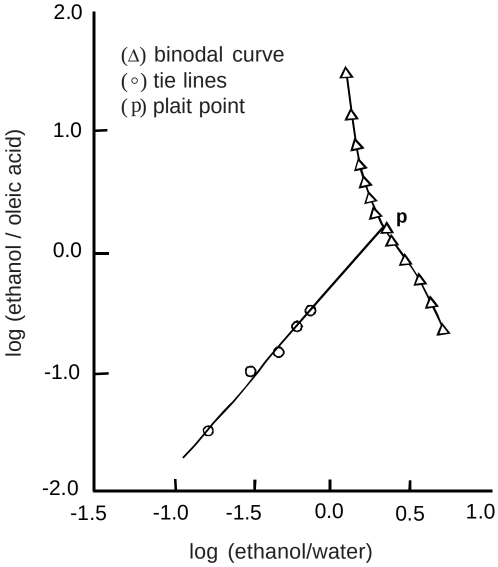


Fig 5 Hand type ternary diagram for plait point determination of the ethanol-water-oleic acid system.

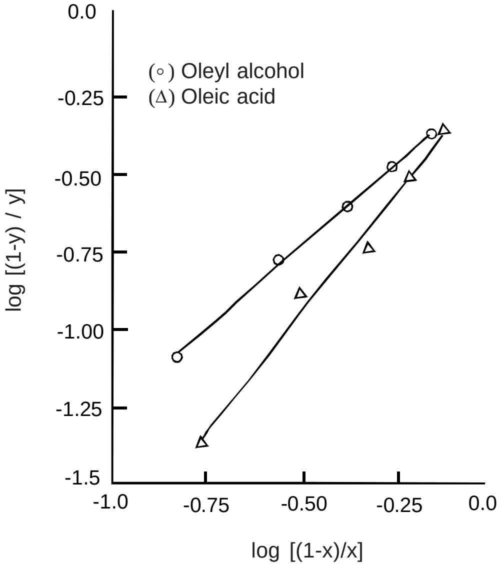


Fig 6 Othmer-Tobias plot of tie lines data for ethanol-water-solvent systems.

K_D values ranging from 0.5 - 0.6 and for oleic acid, it ranges from 0.2 - 0.6 for various ethanol concentration in feed. The separation factors for ethanol-water-oleyl alcohol and ethanol-water-oleic acid systems are considerably greater than 6. Oleyl alcohol and oleic acid give the separation factors (α) ranging from 14.3 - 57.8 and 6.3 - 26.0, respectively, for various ethanol concentration in feed. This indicates that ethanol has preferential solubility in solvents as desired in the extraction process.

Distribution of ethanol between solvent and water may be correlated graphically according to Hand's plot (Perry *et al* 1984). This reduces the number of experimental data required; moreover, it allows a graphical determination of the plait points. Extrapolation of the tie line curves crosses the solubility curves at the plait points, as shown in Fig 4 and 5. The plait point compositions for ethanol-water-oleyl alcohol and ethanol-water - oleic acid systems are obtained graphically by means of Hand's plot which are mentioned in Table 1 and 2.

The tie lines were satisfactorily correlated by the Othmer-Tobias method on a mass fraction basis, and their coordinates for ethanol-water-oleyl alcohol and ethanol-water-oleic acid systems are presented in Fig 6. This figure shows log [(1-y)/y] plotted against log [(1-x)/x], where y is the weight fraction solvent in the organic phase and x is the weight fraction water in the aqueous phase. From this figure it is seen that the relation indeed results in the straight lines. It is expected that both Othmer-Tobias plot and Hand's correlation would yield tie lines as straight lines (Hand 1930).

Selection of solvents for extraction of ethanol from dilute aqueous solution should be guided by considerations of selectivity with respect to water (separation factor), as well as equilibrium distribution coefficient for ethanol. It can be observed from Table 3, that oleyl alcohol is the better of the two solvents and may be regarded as a separating agent for dilute aqueous ethanol solutions.

Conclusion

Liquid-liquid phase equilibrium data have been measured for ethanol-water-oleyl alcohol and ethanol-water-oleic acid ternary systems. The binodal curves, tie lines, distribution coefficients and separation factors have been determined. Hand's method has been used to correlate tie lines and to calculate coordinates of plait points. Tie line data were satisfactorily correlated by the Othmer-Tobias method on a mass fraction basis, and their plot would yield tie lines as straight lines. The binodal region of oleyl alcohol system has appeared to be slightly broader than that of oleic acid system. The distribution coefficients of ethanol for oleyl alcohol and oleic acid systems are greater than 0.5 and 0.2, respectively and the separation factors of oleyl alcohol and oleic acid systems are greater than 14 and 6, respectively. It is concluded that oleyl alcohol may be considered a separating agent for dilute aqueous ethanol solutions.

Acknowledgement

Authors are grateful to the Ministry of Science and Technology, Bangladesh for granting an NST fellowship to one of the authors (Mr M N Nabi)

References

Ananthanarayanan P, Rao P B 1968 Ternary liquid equilibria of the water-phosphoric acid-isoamyl alcohol, cyclohexanol, or methyl isobutyl ketone systems at 35˚C. *J Chem Eng Data* **13** 194-196.

Arda N, Sayar A A 1992 Liquid-liquid equilibria of water-tetrahydrofuran-1-methylcyclohexanol and of water tetrahydrofuran-2-methylbutylethanoate at the temperature (293.16 ± 0.30) K and pressure (101.325 ± 0.070) k Pa. *J Chem Thermodynamics* **24** 145-149.

Botto G J, Agaras H H, Marschoff C M 1989 Liquid-liquid equilibrium data for the system water-benzonitrile-methanol. *J Chem Eng Data* **34** 382-384.

Briones J A, Mullins J C, Thies M C 1994 Liquid-liquid equilibria for the oleic acid-β-sististerol-water system at elevated temperatures and pressures. *Ind Eng Chem Res* **33** 151-156.

Feki M, Fourati M, Chaabouni M M, Ayedi H F 1994 Purification of wet process phosphoric acid by solvent extraction liquid-liquid equilibrium at 25 and 40˚C of the system water-phosphoric acid-methylisobutylketone. *Can J Chem Eng* **72** 939-944.

Gomez Marigliano A C, Gramajo De Doz M B, Solimo H N 1998 Influence of temperature on the liquid-liquid equilbria containing two pairs of partially miscible liquids water-furfural-1-butanol ternary system. *Fluid Phase Equilibria* **153** 279-292.

Hand D B 1930 Dineric distribution. *J Phys Chem* **34** 1961-2000.

Hegazi M F, Salem A B 1983 Ternary data for the acetic acid-water-mesityl oxide system. *J Chem Tech Biotechnol* **33A** 145-150.

Katayama H, Hayakawa T, Kobayashi T 1998 Liquid-liquid equilibria of three ternary systems: 2-propanone-glycerol-methanol, 2-butanone-glycerol-ethanol, and 2-butanone-glycerol -2-propanol in the range of 283.15 to 303.15 K. *Fluid Phase Equilibria* **144** 157-167.

Letcher T M, Ravindran S, Radloff S E 1991 Liquid-liquid equilibria for mixtures of an alkanol-methyl tert-butyl ether-water at 25˚C. *Fluid Phase Equilibria* **69** 251-260.

Loudon G M 1995 *Organic Chemistry*. The Benjamin Cummings Publishing Company, Inc, California, USA, 3rd ed, pp 346-358

Maeda K, Yamada S, Hirota S 1997 Binodal Curve of two liquid phases and solid-liquid equilibrium for water-fatty acid-ethanol systems and water-fatty acid-acetone systems. *Fluid Phase Equilibria* **130** 281-194.

Munson C L, King C J 1984 Factors influencing solvent selection for extraction of ethanol from aqueous solutions. *Ind Eng Chem Process Des Dev* **23** 109-115.

Othmer D F, Tobias P E 1942 Tie line correlation. *Ind Eng Chem* **34** 693-696.

Perry R H, Green D W, Maloney J O 1984 *Perry's Chemical Engineers' Hand Book*. Mc Graw-Hill International Editions, New York, USA, 6th ed, p 15-25.

Rahman M A, Rahman M S, Nabi M N 2001 Extraction of ethanol from aqueous solution by solvent extraction-liquid-liquid equilibrium of ethanol-water-1-butanol, ethanol-water-1-pentanol and ethanol-water-1-hexanol systems. *Indian J Chem Technol* **8** 385-389.

Weast R C, Astle M J 1982-83 *CRC Handbook of Chemistry and Physics*. CRC Press, Inc, Boca Raton, Florida, USA, 63rd ed, pp E50-53.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 414 - 417*

ELECTROCAPILLARY AND FLOTATION STUDIES USING POTASSIUM ETHYLXANTHATE, DITHIOPHOSPHATE COLLECTORS AND THEIR MIXTURE.

M Riaz,* Faridullah Khan, Mumtaz, Nazir Jan and Naeem Pirzada

PCSIR Laboratories, PO Peshawar University, Jamrud Road Peshawar, Pakistan

(Received October 30, 2002; accepted January 27, 2003)

The sufrace tension measurements were carried out on dropping mercury electrode (dme) in 0.1 M sodium tetraborate buffer solution, with potassium ethylxanthate (KEtx) and dithiophosphate (Dtp) added separately or in combination under comparable conditions. The electrocapillary curves determined as function of potential indicating reduction in surface tension by the addition of KEtx and Dtp. Synergistic behaviour was also studied by comparing the decrease in surface tension of individual collectors with that of their mixtures at various mole ratios and potentials. Flotation studies were also conducted on heazlewoodite (Ni_3S_2) with these collectors separately and in combination to study the synergistic effect.

Keywords: Electrocapillary, Flotation, Collectors

Introduction

Synergism may be defined as the enhanced effect obtained from the use of a combinations of reagents relative to their individual action. In flotation, synergistic effect between collectors and frothers have long been recognized in plant practices (Taggart 1945), though little attention have been paid to these in laboratory studies. Exception to this area, for example, the investigation reported by Glembotskii (1958) on the use of mixtures of collectors of same type but of different hydrocarbon chain length or degree of branching. The work of Mingion (1984) on the use of dithiophosphates in conjunction with xanthates and sodium mercaptobenzothiozole in the flotation of platinum group metals, and the work of Pomianowski and Powlikowski - Czubak (1967), who have presented the results the tensammetric measurements on mercury and of flotation using KEtx with dodecyltrimethyl ammonium bromide. The study of synergism between xanthate and carbamate, sodium sulphide and carbamate was also carried out (Critchely and Riaz 1991; Riaz and Critchley 1993; Riaz *et al* 1997; Riaz *et al* 2001) on dropping mercury electrodes and other metal electrodes. The study of such effects between KEtx and dithiophosphate (Dtp) in reaction with mercury electrode and flotation studies of heazlewoodite (Ni_3S_2) synergised and supplied by Johnson Matthey Research, Ltd. is described in the present contribution. Correlation was obtained between flotation recoveries obtained in a modified Hallimond tube and simple measurements of surface tension carried out on a dropping mercury electrode at controlled potentials (in electrocapillary phenomenon).

Experimental

Reagents. All the chemicals used in the investigation were of analytical grade. The KEtx and Dtp were freshly recrystallized for each experiment, single distilled water being used through out. Unless otherwise stated, all experiments were carried out in an electrolyte of 0.1M sodium tetraborate which gives a constant pH of 9.2. The pH was adjusted as necessary by addition of NaOH or H_2SO_4.

Surface tension was measured by dropping mercury electrode by means of drop weight method. The apparatus was calibrated against standard values for the surface tension of mercury in contact with 0.1M KC1 solution. Potentials were measured with a saturated calomel reference electrode (SCE), and all potentials are given relative to this scale. The dropping mercury electrode consisted of an extra long capillary and large mercury head to give as constant mercury flow rate as possible. The lower tip of capillary was immersed in test solution contained in the cell. The volume of the cell was 100 cm^3 which housed the working electrode connected by a side tube containing a sintered glass frit and an agar-salt bridge to a side tube which formed the saturated calomel reference electrode. The potential across the cell was controlled by general purpose polarograph E.I.I.Cambridge Model 0410 and digital multimeter, Thander TM 355.All potentials were measured with respect to a saturated calomel electrode, whose potential may be taken as 241.2 mV with respect to the saturated hydrogen scale of potential. The solutions were deoxygenated using nitrogen gas that had been scrubbed in vanadous chloride. A continuous nitrogen flow was maintained though the experiments.

---

*Author for correspondence

For each experiment, the dme was polarized to a fixed potential relative to the SCE and time taken for at least 10 drops to form which was measured with a stop watch. Measurements were made starting from the negative potentials (-1.6V) at 100 mV intervals. All potentials were repeated at least three times to check experimental accuracy and reproducibility. The experimental determination of drop time in 0.1M borate buffer gives reproducibility slightly better than ± 1 second over a time interval of about 120 seconds for the formation of 10 drops.

Flotation tests were performed in a Hallimond tube (height, 150 nm and internal diameter, 35 nm) with a magnetic stirrer, maintaining a constant speed for all the trials. Nitrogen gas was used at constant flow rate for flotation. A detachable mineral receiver was held in place by a general glass joint so that by changing receivers as required, the kinetic of flotation could be followed. The 5 grams samples of heazlewoodite used for the flotation studies were freshly ground (- 200, + 100 μm) and kept in vacuum out of air contact.

Results and Discussion

The electrocapillary curves for mercury determined in 0.1 M borate solution and in the presence of various additions of KEtx and Dtp are shown in Fig 1 and 2. The mercury surface is initially observed as positively charged. On reducing this charge by means of applied potential, the surface tension increases, goes through maximum and then decreases. The maximum occurs at a potential at which the charge density changes from positive to negative values passing through zero. The potential at electrocapillary maximum (E.C.M.) is also known as the potential of zero charge (P.Z.C.) given by the symbol Eq = 0. On other side of P.Z.C, where the surface is either positively charged or the negatively charged, counter ions are adsorbed on the surface. The variation of surface tension with potential in the absence of electro active species, is presumably a result of orientation effects among water dipoles due to the surface charge on the mercury as contact adsroption of sodium or borate is unlikely. This is supported by the observation that the point of inflexion of curve coincides with P.Z.C. Electrocapillary curves may be differentiated with respect to the potential to give the surface charge (dr/dE= - qs) where, qs is surface charge in electrolyte and redifferentiated to give the differential surface capacitance ($dr^2/dE^2 = C$).

With the addition of KEtx and Dtp, the curve shows variation of surface tension to a varying degrees depending on the nature and concentration of collectors. The E.C.M. are shifted to more positive potentials by about 60 mV for a ten fold degree in concentration. This is in agreement of surface capacitance reported by Hunter (1985) for different xanthate concentrations. Equating the decrease in surface tension to the extent of adsorption, it is noteworthy that measurable adsorption takes place at potentials several hundred millivolts negative to E.C.M. Where as, on negative charged surface, anion adsorption would not be normally be expected to be significant. It may have been caused either by the weak affinity of sulphur in the polar group for mercury or by the chemisorption in which mercury atoms remain a part of metallic phase even after interaction with these collectors. The decrease in surface tension becomes greater with increasing concentration and applied potential. The magnitude of depression being proportional to the surface concentration of adsorbed xanthate or thiophosphate on the surface caused erratic be-

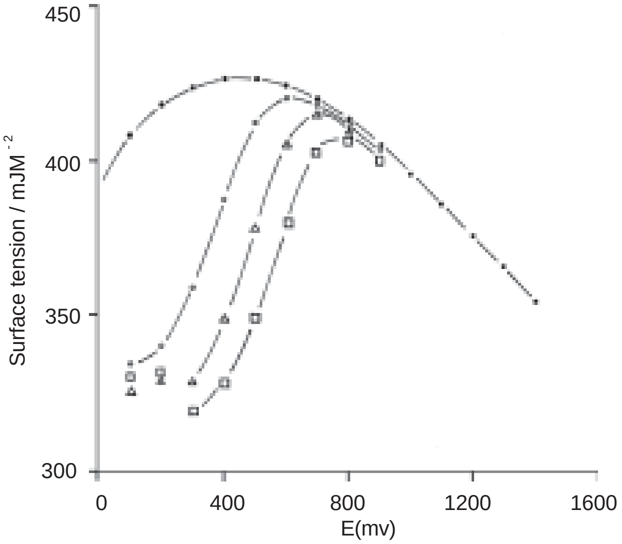


Fig 1. Surface tension of mercury in 0.1M borate solution in presence and absence of KEtx 10^{-4}M (○), KEtx 10^{-3}M (△), KEtx 10^{-2}M (□)

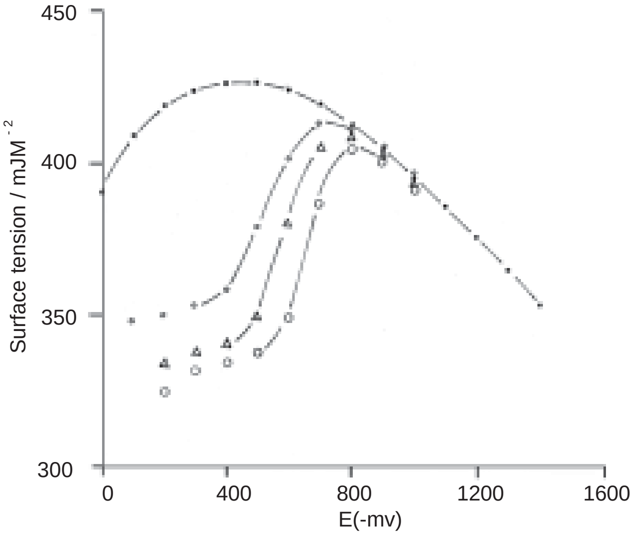


Fig 2. Surface tension of mercury in 0.1M borate solution in presence and absence of Dtp 10^{-4}M (○), Dtp 10^{-3}M (△), D 10^{-2}M (○).

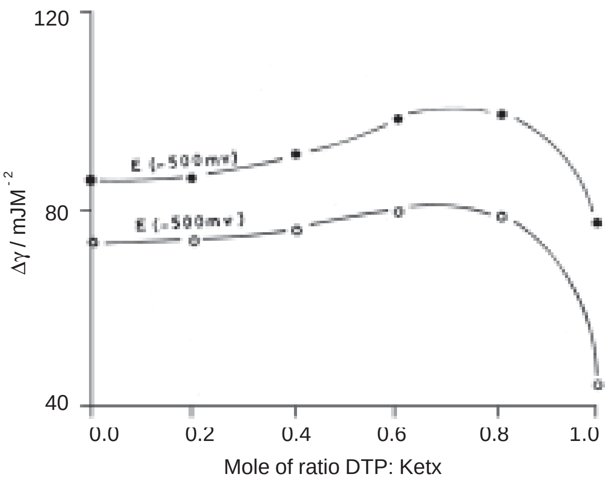


Fig 3. Change in surface tension of mercury on addition of collector as function of the ratio of KEtx Dtc (10^{-2}M).

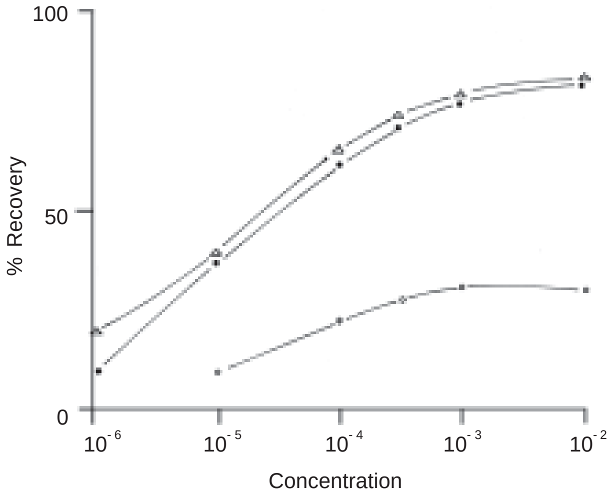


Fig 4. Cumulative % age recoveries as a function of concentration for flotation of Ni_3S_2 with use Dtc (o), KEtx (•) and KEtx + Dtp equimolar mixture (Δ).

havior in the curves. However, at potentials more negative than - 900 mV (S.C.E.), all the curves tend to coincide with that of electrolyte.

A collectors mixtures show synergism when they contain a greater lowering of surface tension at a given total concentration than that of separate components of mixture at the same concentration. In Fig 3, the change in surface tension (measured at fixed potential) brought about by the addition of a mixture of PEtx and Dtp to a total concentration of 10^{-2}M is plotted as a function of the ratio of the two collectors. It can be seen that, the decrease in surface tension is significantly greater than that would be expected from a linear interpretation from the results for the separate reagents. The maximum synergistic effect occurring at an approximately 7:3 concentration of Dtp and KEtx collectors, respectively. The observed synergistic effect could be, as a result of molecular interaction between the components of mixture or the mercury surface charge modification. From the present work, it can be suggested that the component KEtx adsorable at lower potential, modifies the mercury surface charge for other component. Dtp normally adsorable at higher potentials, resulting in a greater reduction of surface tension.

Flotation recoveries obtained with KEtx and Dtp separately at a concentration of $5x10^{-2}$ M and for mixture (1 : 1) at the same

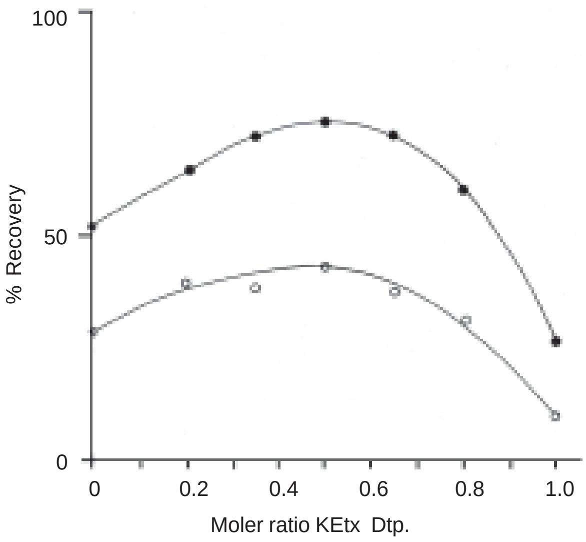


Fig 5. Recovery as a function of molar ratio of KEtx to Dtp at constant total concentration of collector and for flotation time lower curve (10 min), upper curve (20 min).

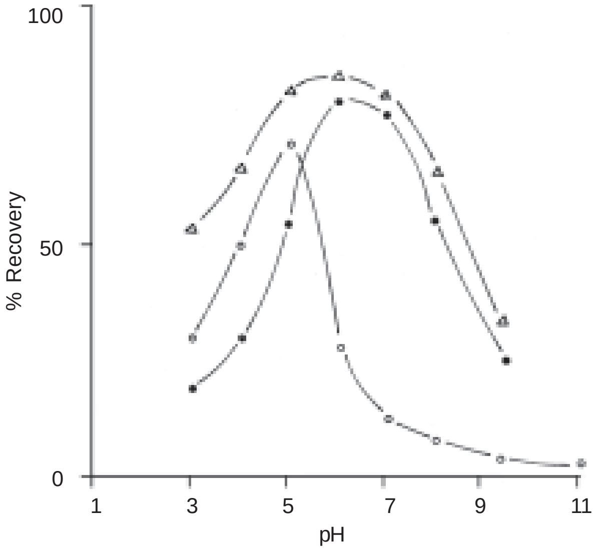


Fig 6. Recovery as a function of pH for flotation of Ni_3S_2 with KEtx (•), Dtp 10^{-4}M (o) and 1: 1 molar ratio mixture at constant total concentration 10^{-4} (Δ).

total concentration for the flotation time of 20 minutes as a function of concentration are given in Fig 4, in which the synergistic effect is evident. Recoveries after 10 and 20 minutes for the mixed collectors are shown as a function of the molar ratio KEtx:Dtp in Fig 5, in which the synergistic effect is clearly evident, this is at a maximum at ration of 1: 1. In Fig 6 flotation recoveries are plotted as a function of pH for KEtx and Dtp separately and for a mixture at a molar ratio 1 : 1 and the same total collector concentration. It is apparent that the synergistic effect extends over the whole pH range with in which significant flotation is observed.

It is evident from these limited experiments that synergism between collectors is important factor in the selection of reagents in flotation. It has been demonstrated that its occurrence can be readily explored by quite simple electrocapillary measurements, which can be related to the recoveries obtained in flotation. By applying these methods to other combinations of collectors and other mineral species, it should be possible to obtain a fuller understanding of factors that control synergism, both in general terms and in particular systems. The methods should also be capable of developing into a useful industrial tool for the improvement of plant performance thus it is hoped to develop this asepect of the work further.

References

Critchley J K, Riaz M 1991 Study of synergism between xanthate and dithiocarbamate collectors in flotation of heazlewoodite. *Trans Instn Min Metall (Sect. C: Mineral Process. Extr. Metall),* 100, C_{55} - C_{57}

Glembestskii A A 1958 The combined action of collectors during flotation. *Tsvetnye Metally* **31**, (4) 6 - 14, (Russian Text).

Hunter C J 1985 Cyclic voltametery studies of sulphide minerals Ph.D Thesis, Brunel, The University of West London, UK.

Mingione P A 1984 . Reagents in the Minerals Industry. In:*Use of Dialkyl and Diaryl Dithiophosphate Promoters as Mineral Flotation Agents.* eds. Jones M J and Oblatt Revised (London, UK, IMM, 19 - 24).

Pomianowski A, Pawlikowski - Czubak 1967 Electrical surface characteristics of the mercury/solution/air system containing xanthates and dodecyltrimethyl ammonium bromide. *Przemyst Chem* **46** pt 8, 481 - 5 (Polish text).

Riaz M, Critchley J K 1993 Surface tension measurement on dropping mercury electrode using thiol-collectors and their mixtures. *Pak J Sci Ind Res* **36** (4) 123 - 125.

Riaz M, Mumtaz, Kamin K 1997 The catalytic effect of sulphur on sodium dithiocarbamate oxidation in reaction with platinum and heazlewoodite. *Pak J Sci Ind Res* **40** (1 - 4) 23 - 26.

Riaz M, Rahman A, Mumtaz, Kamin K, Asadullah J 2001 Synergistic effect between thiol collectors in reaction with sulphide minerals. *Pak Sci Ind Res* **44** (5) 257 - 262.

Taggart AG 1945 *Handbook of Mineral Dressing.* John Willey, New York, USA.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 418 - 423*

THE DISTRIBUTION OF Mn, Zn, Cu, Cr, Ni, AND Pb AROUND TWO MAJOR REFUSE DUMPSITES IN BENIN CITY, NIGERIA

E E Ukpebor[a]**, P O Oviasogie** [b]**, C A Unuigbe** [a]*

[a] *Chemistry Department, University of Benin, Benin City, Nigeria*

[b] *Chemistry Department, Nigeria Institute for Oil Palm Research, PMB 1030, Benin City, Nigeria*

(Received September 11, 2001; accepted January 29, 2003)

The concentration of Zn, Pb, Mn, Cu, Cr and Ni around two major refuse dumpsites in Benin City have been determined. This was done in order to ascertain the suitability of these area of land for residential and agricultural purposes when eventually reclaimed. In all, 18 soil samples were collected at distances of 0 m, 50 m and 100 m (9 top soil; 0 to 15 cm and 9 bottom soil; 15 to 30 cm) from each dumpsite. Sample solutions were prepared and analysed using atomic absorption sepectrophotometry. Results obtained indicate that top-soil samples from Ugbowo dumpsite contain as much as 1.10 - 8.88 mg/kg Mn, 0.68 - 2.30 mg/kg Zn, 5.90 - 8.70 mg/kg Cu, 0.08 - 0.16 mg/kg Cr, 0.50 - 77 mg/kg Ni and 0.10 - 0.45 mg/kg Pb. Bottom soil samples from the same dumpsite gave ranges of 4.44 - 15.26 mg/kg Mn, 0.84 - 6.59 mg/kg Zn, 5.30 - 7.70 mg/kg Cu, 0.11 - 0.20 mg/kg Cr, 0.66 - 1.57 mg/kg Ni and 0.20 - 0.60 mg/kg Pb. For Evbuotubu dumpsite, concentration ranges obtained for the top soil samples are 5.72 - 18.33 mg/kg of Mn, 2.10 - 5.23 mg/kg of Zn, 1.96 - 12.22 mg/kg of Cu, 0.22 - 0.56 mg/kg of Cr, 0.27 - 0.83 mg/kg of Ni and 0.72 - 1.20 mg/kg of Pb. Bottom soil samples gave concentration ranges of 3.24 - 17.96 mg/kg of Mn, 1.46 - 6.20 mg/kg of Zn, 4.33 - 10.93 mg/kg of Ni and 0.69 - 1.51 mg/kg Pb. The heavy metal levels were found to decrease in both top and bottom soils with distance from the dumpsites.

Key words: Heavy metals, Top soil samples, Absorption spectrophotometry.

Introduction

Benin city which lies between latitudes 6°, 00'N and longitudes 5°, 40'E is located in the Southern part of Nigeria. The ancient city is urban and has witnessed an overwhelming influx of people from the rural areas in the last few decades. This has resulted in a tremendous increase in population in the city. Population explosion is always inevitably accompanied with environmental pollution. In order to meet man's daily myriad demands, large quantities of solid wastes are generated from industrial, domestic and commercial activities. If not properly disposed and managed, the resulting environmental impact from these wastes can be disastrous.

As a result of prohibitive cost and manpower requirement to operate standard solid waste management machines such as incinerators, waste disposal and management in Benin City is by the less attractive method of open dumping in designated locations. Population explosion in the city and other factors have necessiated the re-developing of some of these dumpsites covering a expanse land for residential and agricultural purposes. It is, therefore, essential that the levels of heavy metals in these dumpsites are assayed, because uncontrollable inputs of heavy metals are undersirable. Once accumulated in the soil, these elements are generally very difficult to remove and potentially harmful effects may arise in the future.

Soil metal contamination has occurred since prehistoric times, but the extent and pace of contamination has increased during the last century as a result of rapid industrialization and population explosion. Toxic metals are of considedrable environmental concern due to their toxicity and accumulative behaviour (Purves 1985). Trace quantities of some of the heavy metals are essential for animal and plant growth. However, they are easily assimilable and tend to accumulate in materials in the environment (Nurberg 1984). Metal contamination of soils became a world-wide concern when it was observed that rice paddy fields irrigated with wastewaters from a Zinc mine caused excessive cadmium (Cd) intake and adverse health effects in farmers who had consumed rice grown in this contaminated soils (Kobayashi 1978). This first observation of human disease caused by a heavy metal in the environment has stimulated research on the potential adverse effect of Cd and other metals in soils and in agricultural and dietary systems. During the 1980s, the risks of young children suffering from neuropsychological effects because of excessive lead (Pb) ingestion appeared to be more serious than had been previously recognized (Needlemann *et al* 1979; Needlemann *et al* 1990). Increased bioavailability of heavy metals may inhibit root growth and uptake of macronutrients by trees and

---

*Author for correspondence

these effects have been shown to be synergistic (Burton *et al* 1983; Breckle and Kahle 1992). Most recently, it has been reported (Dudka *et al* 1996) that addition of Pb - Zn smelter flue dust strongly contaminated the test soil with Cd, Pb and Zn, although there were relatively low metal concentration in crop plants, the crop yield reduction indicated the presence of phytotoxic conditions in the studied soil.

As a result of the potentially harmful effects of long-term accumulation of heavy metals on plant growth, the evaluation of ecological significance of heavy metal pollution requires most importantly an assessment of the relative concentration level of the metals. The present study was therefore, focused on establishing the levels of Mn, Zn, Cu, Pb, Cr and Ni in the soil around two major refuse dumpsites in Benin City.

Materials and Methods

With the aid of soil auger and a trowel, 18 composite soil samples were collected at the distance of 0 m, 50 m and 100 m (9 top soil; 0 to 15 cm and 9 bottom soil; 15 to 30 cm) from each dumpsite (Fig 1). The soil samples were stored in polyethylene bags and labelled property.

The samples collected were air dried, ground in an agate mortar and then sieved through a 1.73 mm nylon sieve. Soil pH was determined using H_2O according to Folson *et al* (1981). The soil/solution ratio was 1:2. Soil organic carbon was determined by Walkey Black rapid dichromate oxidation technique (Nelson and Sommers 1982) with the use of correction factor 1.3 to account for incomplete oxidation of organic compound and a multiplying factor 1.724 to convert organic carbon to organic matter (%). Particle size analysis was achieved according to the method of Bouyoucos (1962).

Metal determination. A 1g sub-sample of the processed soil was weighed into a 125 cm^3 hard - glass digestion tube, a few drops of high-purity HNO_3 were added slowly. After the effervescence, 5 cm^3 of high-purity HNO_3 and 15 cm^3 of $HClO_4$ were added slowly and kept overnight. The samples were then heated in a digester at 120°C for 3 hours. The contents were allowed to cool for 15 minutes after the appearance of white fumes, filtered into a 100 cm^3 volumetric flask and diluted to volume with distilled water (Allen *et al* 1974). Concentrations of Mn, Zn, Cu, Cr, Ni and Pb were determined using a Varian spectra AAIO Atomic Absorption Spectrophotometer.

Results and Discussion

The levels of selected physiochemical properties of the soils from the two refuse dumpsites are shown in Table 1, while the measured concentration ranges, the average levels and standard deviations for Mn, Zn, Cu, Cr, Ni and Pb in both bottom and top soil samples from Evbuotubu and Ugbowo refuse dumpsites are summarized in Table 2 and 3. Soil organic matter was observed to be generally higher at Ugbowo dumpsite than at Evbuotubu dumpsite. This may be attributed to varied rates of microbial decomposition or degradation associated with different types, quality and quantity of waste in the two locations. A number of organic wastes, such as tree bark, leaf mold, city and urban refuse, sewage sludge and sawdust simultaneously undergo humification in both controlled systems (composing) and open refuse dumpsites (Inbar *et al* 1990).

Mn gave the highest level with a range of 3.24 - 17.96 mg/kg and a mean of 12.22 mg/kg at the base (0 m) of the dumpsite at Evbuotubu, while the total concentration of Mn reduced to a mean of 6.07 mg/kg at 100 m away from the site. The reduction in the amount of Mn with distance from the dumpsite also exhibited an increase in pH (Table 1). The high concentration of Mn at the base 0 m of the site corresponds to a lower pH of 4.8. High organic matter in the soil causes a flush of microbial activity, which adds complexing agents to the soil and affects the redox condition of the soil. Controlled oxidation - reduction experiments have shown that more Mn is present in soil at low pH and Eh (reducing conditions) than at high pH (Shuman 1988). This same trend was observed at Ugbowo dumpsite where the amount of Mn decreased with distance from the dumpsite as the pH increased. On the other hand, there may have been little lateral migration of the waste containing sources of Mn.

Cu had the next highest concentration with a range of 1.42 - 6.20 mg/kg and a mean of 8.16 mg/kg just at he periphery (0 m) of the dumpsite at Evbuotubu. At Ugbowo, total concentration of Cu ranged between 5.90 and 8.0 mg/kg (Table 3) also at the base of the dumpsite. The concentration of Cu decreased with distance away from the dumpsites. The relatively high organic matter content of the soil at both locations associated with increased Cu concentrations is consistent with previous reports (Ducaroir *et al* 1990; Baker 1990; Ramos *et al* 1994), that even in metal speciation studies, the greater amount of Cu occurs in the organic fraction. Since the refuse dumpsites contain high organic matter, it could be opined that the distribution of the metals studied are affected basically by the organic matter content and the soil pH.

Zn had a mean concentration of 3.28 mg/kg at Evbuotubu and 2.79 mg/kg at Ugbowo dumpsite. Zn has been shown to occur mostly in the residual fraction (87-90 %) even in acid soils with high loadings of organic material or sludge (Xiang *et al* 1995). Similarly, Chlopecka *et al* (1996) reported a non-correlation between the total concentration of Zn and organic fraction associated with increasing contamination of soils in areas where metallurgical industries are located in Poland.

Table 1

Selected physicochemical properties of the soils from the two refuse dumpsites

Distance (m) from dumpsite	Depth (cm)	% C	% Organic matter	pH	% Sand	% Silt	% Clay
Ugbowo							
0	0 - 15	2.91	5.01	4.70	79.6	7.00	13.40
50	0 - 15	2.64	4.55	4.50	80.4	7.90	11.70
100	0 - 15	1.77	3.05	5.20	84.9	6.10	9.00
0	15 - 30	1.18	2.03	5.00	80.1	9.20	10.70
50	15 - 30	1.13	1.94	5.60	83.5	4.20	12.30
100	15 - 30	1.05	1.81	5.70	82.7	7.50	9.90
Evbuotubu							
0	0 - 15	1.56	2.68	4.80	84.90	3.90	11.20
50	0 - 15	1.39	2.39	5.30	84.30	7.20	8.50
100	0 - 15	1.24	2.13	5.60	85.70	3.90	10.40
0	15 - 30	0.97	1.67	5.20	82.10	5.10	12.80
50	15 - 30	0.95	1.63	5.40	80.50	8.80	10.70
100	15 - 30	0.88	1.51	5.40	83.20	5.90	10.90

Table 2

Concentration of Mn, Zn Cu, Cr, Ni and Pb in top and bottom soil samples around the Evabotubu refuse dumb site

Distance from		Concentration mg/kg					
dumpsite	Top soil	Mn	Zn	Cu	Cr	Ni	Pb
0 m	Average conc.	12.02	3.22	7.22	0.44	0.64	0.98
	S.D	6.32	1.74	5.13	0.19	0.32	0.24
	Range	5.72 - 18.33	2.10 - 5.23	1.96 - 12.22	0.22 - 0.56	0.27 - 0.83	0.72 - 1.20
	Bottom soil						
	Average conc.	12.22	3.28	8.16	0.40	0.78	1.22
	S.D	7.88	2.56	3.43	0.19	0.49	0.46
	Range	3.24 - 17.96	1.42 - 6.20	4.33 - 10.93	0.18 - 0.52	0.23 - 1.17	0.69 - 1.51
50 m	Top soil						
	Average conc.	8.23	1.62	5.29	0.22	0.36	0.64
	S.D	5.07	0.61	1.03	0.03	0.23	0.18
	Range	2.46 - 12.00	0.93 - 2.07	4.24 - 6.30	0.18 - 0.24	0.13 - 0.58	0.47 - 0.83
	Bottom soil						
	Average conc.	8.12	2.04	6.17	0.28	0.32	0.72
	S.D	3.42	1.22	3.56	0.03	0.16	0.41
	Range	4.26 - 10.98	1.09 - 3.41	3.99 - 10.28	0.26 - 0.32	0.14 - 0.43	0.25 - 1.00
100 m	Top soil						
	Average conc.	5.33	0.76	2.78	0.99	0.21	0.31
	S.D	2.55	0.59	1.09	0.01	0.03	0.17
	Range	3.42 - 8.23	0.14 - 1.31	1.98 - 4.02	0.08 - 4.02	0.19 - 0.24	0.13 - 0.47
	Bottom soil						
	Average conc.	6.07	0.91	2.42	0.18	0.28	0.33
	S.D	1.07	0.09	1.40	0.05	0.02	0.16
	Range	5.18 - 7.25	0.18 - 0.99	1.26 - 3.97	0.13 - 0.22	0.26 - 0.30	0.23 - 0.51

Table 3

Concentration of Mn, Zn Cu, Cr, Ni and Pb in top and bottom soil samples around the Ugbowo refuse dumb site

Distance from Dumpsite		Concentration mg/kg					
	Top soil	Mn	Zn	Cu	Cr	Ni	Pb
0 m	Average conc.	6.07	1.27	6.83	0.13	0.61	0.31
	S.D	4.32	0.89	1.62	0.04	0.13	0.19
	Range	1.10 - 8.88	0.68 - 2.30	5.90 - 8.70	0.80 - 0.16	0.50 - 0.75	0.10 - 0.45
	Bottom soil						
	Average conc.	9.45	2.79	6.17	0.16	0.97	0.44
	S.D	5.45	3.29	1.33	0.05	0.52	0.21
	Range	4.44 - 15.26	0.84 - 6.59	5.30 - 7.70	0.11 - 0.20	0.66 - 1.57	0.20 - 0.60
50 m	Top soil						
	Average conc.	4.10	0.36	3.22	0.17	0.47	0.22
	S.D	1.01	0.56	1.14	0.06	0.11	0.16
	Range	3.08 - 5.09	0.02 - 1.01	2.06 - 4.34	0.01 - 0.12	0.38 - 0.59	0.19 - 0.39
	Bottom soil						
	Average conc.	3.96	0.39	4.13	0.06	0.53	0.25
	S.D	0.87	0.58	0.65	0.04	0.11	0.14
	Range	3.03 - 4.76	0.02 - 1.05	3.50 - 4.80	0.02 - 0.07	0.42 - 0.64	0.13 - 0.41
100 m	Top soil						
	Average conc.	2.80	0.14	2.42	0.04	0.31	0.11
	S.D	1.06	0.12	1.32	0.02	0.15	0.07
	Range	1.96 - 4.01	0.06 - 0.20	1.35 - 3.89	0.02 - 0.07	0.19 - 0.48	0.06 - 0.19
	Bottom soil						
	Average conc.	3.02	3.03	2.74	0.04	0.38	0.14
	S.D	1.12	0.14	1.23	1.23	0.16	0.10
	Range	2.07 - 4.26	0.08 - 0.33	1.92 - 4.16	0.03 - 0.06	0.21 - 0.52	0.07 - 0.26

The pattern of decrease in metal concentration of Ni, Pb and Cr away from the two dumpsites were equally obtained (Table 2 and 3). The similarities in the distribution pattern of these heavy metals at the two refuse dumpsites is as a result of similarities in the composition of the solid waste dumped at both locations, since the wastes are from different quarters of the same ancient city with the populace have identical dietary pattern and living conditions. Results available equally indicate that metal concentrations were slightly higher at Evbuotubu dumpsite which is attributed to high population density at Evbuotubu. This means the utilization of more materials and the generation of more refuse.

Correlation analysis was carried out to determine the extent of relationship between the elements investigated (Table 4 and 5). The correlation matrix shows that the highest correlation was obtained between Mn and Ni (r = 0.92) at Evbuotubu dumpsites. The high level of organic matter present in the soils suggests amongst other things the presence of humic substances (humic and fulvic acids). Generally, phenolic compounds present in these substances enhance sorption of metallic cations such as Ni, on soil materials containing high concentration of Mn (Gagnon *et al* 1992). Increased competition for complexing or adsorption sites are perhaps responsible for high correlation between Cu and Ni (r = 0.86) obtained at the Ugbowo dumpsite. Correlation decreases and increases between the various metals studied are presented in Table 5. The entire correlation increases and/or decreases between the metals can be better understood by postulating a scheme of what happens in a typical waste deposit. Since waste deposits contain a complex mixture of different compounds, their morphology is also very variable and over time the wastes change considerably. The processes are in many case similar to those found in soil formation where organic material degrades by biologically mediated anaerobic and aerobic processes (Bozkurt *et al* 1999). There is a strong competition for the metals by the organic acids and between the metals for other complexing agents. Also colloids formed by the release of the little soluble part of the solid humus phase can carry considerable amounts of these metals which have been sorbed. It is thus not certain that even reducing phase there will be negligible release of the metals of concern (Zn, Mn, Cu, Cr, Pb and Ni) (Bozkurt *et al* 1997).

Comparison of data obtained in this study with previous results concerning heavy metal pollution in road side sedi-

Table 4

Correlation between the elements Mn, Zn Cu, Cr, Ni and Pb in both layers (Evbuotubu dumpsite)

	Mn	Zn	Cu	Cr	Ni	Pb
Mn	1.00	0.56	0.47	0.65	0.92	0.77
Zn		1.00	0.51	0.61	0.55	0.72
Cu			1.00	0.59	0.50	0.67
Cr				1.00	0.66	0.82
Ni					1.00	0.74
Pb						1.00

Table 5

Correlation between the elements Mn, Zn Cu, Cr, Ni and Pb in both layers (Ugbowo dumpsite)

	Mn	Zn	Cu	Cr	Ni	Pb
Mn	1.00	0.73	0.75	0.63	0.76	0.83
Zn		1.00	0.78	0.85	0.80	0.82
Cu			1.00	0.67	0.86	0.80
Cr				1.00	0.60	0.58
Ni					1.00	0.84
Pb						1.00

ments and soil in the same city (Ihenyen 1998; Ndiokwere 1984) indicate very much lower concentrations in this study. While the highest concentration of 1.22 mg/kg Pb was obtained in the present study, previous studies gave 753.14 ppm Pb (Ihenyen 1998) and 11.70 ppm (Ndiokwere 1984). One main reason that may explain these differences in the levels of heavy metals obtained previously and now is that most of these metals especially Pb and Zn are directly associated with emissions from vehicles exhaust which run solely on leaded gasoline, activities of road side mechanics along motorways and the presence of these metals as additives which form components of some lubricating oils. The dumpsites investigated in this study are located in areas remote from high human activities covering a distance about 4km from a major road. In addition, a substantial part of waste dump at the sites are food waste and other household waste. It is important to emphasize that more remote agricultural areas and settlements may also be receiving contaminating metals, not only from industries, but also from sewage sludge, fertilizers and gasoline used in powering local milling machines. It has been estimated that 2 - 4% of arable soils in Poland are contaminated at least to some extent by Cd, Pb, and Zn due to these mentioned activities (Kabata - Pendias *et al* 1992). The values obtained in this study are, however, similar to those reported for soils at Ekpan (Omgbu and Kokogho 1993), but lower in concentration.

Table 6

Environmental quality criteria in the UK. Soil quality criteria recommendations to the National government (Visser 1993)

Element	Soil (mg / kg) Threshold Domestic gradens, play areas	Soil (mg / kg) Threshold Landscapes buildings
Cd	3	15
Cr	600	1000
Cu	-	130
Pb	500	2000
Ni	-	-

Table 7

Environmental quality criteria in Canada. Interim environmental quality criteria for contaminated sites. Recommendations to sub-national authorities (CCME 1991)

Element	Soil (mg / kg) Agriculture	Soil (mg / kg) Residential	Soil (mg / kg) Commercial/ Industrial
Cd	3	5	20
Cr	750	250	800
Cu	150	100	500
Pb	375	500	1000
Ni	150	100	500

Conclusion

Soil contaminated with heavy metals are not only a problem with respect to plant nutrition and the food chain, they may constitute a direct health hazard as well. However, levels of heavy metals obtained in this study when compared with standards giving critical concentrtion of various pollutants in soils (Table 6 and 7), suggest no serious environmental problems at the moment. The dumpsites can, therefore, be effectively utilized for residential and agricultural purposes when eventually reclaimed. It is equally strongly recommended that dumping of refuse in these locations be discontinued and the sites be allowed to go follow for a period of time. Furthermore, it is suggested that further studies be carried out in the dumpsites to ascertain the forms or species in which the heavy metals occur. This will equally guarantee the safe use or otherwise of the decomposing wastes as soil amendment materials especially in organic farming.

Acknowledgement

We are indebted to Mr. Cyril Ishiekwene who carried out the statistical analysis.

References

Allen S E, Grinshaw H W, Parkinson J A, Quarmby C 1974 *Chemical Methods of Analyzing Ecological Materials.* London, UK, Oxford Blackwell Scientific Publication. p 565.

Baker D E 1990 Copper. *In*: *Heavy Metals in Soil.* eds Alloway B J, John Wiley and Sons, New York, USA, p 151 - 174.

Bozkurt S, Aulin C, Moreno L, Neretnieks I 1997 Long - Term release of toxic metals from waste deposits. *In*: Proceedings of the Sardina 97 6th Inter. Landfill Symposium. Christensen T H, Cossa R, Stegmann R (eds). **1** 257 - 266.

Bozkurt S, Moreno L, Neretnieks I 1999 Long - term fate of organics in waste deposits and its effect on metal release. *The Science of the Total Environment* **228** 135 - 152.

Breckle S W, Kahle H 1992 Effects of toxic metals (Cd, Pb) on growth and mineral nutrition of beech *(Fagus sylvatica* L). *Vegetation* **101** 43 - 53.

Burton K W, Morgan E, Roig A 1983 The influence of heavy metals upon the growth of sitka-spruce in South Wales forests. *Plant Soil* **73** 327 - 336.

Bouyoucos G J 1962 Improved hydrometer method for making particle size analysis of soils. *Agron J* **54** 464 - 465.

CCME 1991 *Interim Canadian Environmental Quality Criteria for Contaminated Sites*. Report CCME EPC - CS3.

Chlopecka A, Baron J R, Wilson M J, Kay J 1996 Forms of cadmium, lead and zinc in contaminated soils from Southwest Poland. *J Environ Qual* **25** 69 - 79.

Ducaroir J, Cambier P, Leydecker J, Prost R 1990 Application of soil fractionation methods to the study of the distribution of pollutant metals. *Z Pflanzeneraehr Bodenkd* **153** 349 - 385.

Dudka S, Piotrowska M, Terelak H 1996 Transfer of cadmium, lead and zinc from industrially contaminated soil to crop plants a field study. *Environmental Pollution* **94** (2) 181 - 188.

Folson B L, Lee C R, Bates D J 1981 Influence of disposal environment on availability and plant uptake of heavy metals indredged material. *Tech Rep El* **81** 21 US Army, Washington DC, USA.

Gagnon C, Arnac M, Brindle J 1992 Sorption interactions between trace metals (Cd and Ni) and phenolic substances on suspended clay minerals. *Wat Res* **26** (8) 1067 - 1072.

Ihenyen A 1998 Assessment of heavy metal pollution in roadside sediments in Benin City, Nigeria. *Geologia* Tom 24 Zeszyt 3 187 - 196.

Inbar Y, Chen Y, Hadar Y 1990 Humic substances formed during the composting of organic matter. *Soil Sci Soc Am J* **54** 1316 - 1323.

Kabata - Pendias A, Dudka S, Chlopecka A, Gawinowska T 1992 Background levels and environmental influences on trace metals in soils of the temperate humid zone of Europe. In:*Biogeochemistry of Trace Metals.* Adriano D C, Lewis Publ, Boca Raton, Florida, USA, p 61 - 84.

Kobayashi J 1978 Pollution by cadmium and itai-itai diseases in Japan. In: *Toxicity of Heavy Metals in Environment*, ed. Oechme F W, Marcel Dekker, New York, USA, pp 199 - 260.

Ndiokwere C L 1984 A study of heavy metal pollution from motor vehicle emission and its effects on roadside soil, vegetables and crops in Nigeria. *Environ Sci and Techn (Series B)* **7** 35 - 42.

Needlemann H L, Gunnoe C E, Leviton A, Reed R, Peresie H, Maler C, Barrel P 1979 Deficit in psychological and classroom performance of children with elevated lead levels. *New England J Med* **300** 689 - 695.

Needlemann H L, Schell A, Bellinger D, Leviton A, Allerd E N 1990 The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *New England J Med* **322** (2) 83 - 88.

Nelson D W, Sommers L E 1982 Total carbon, organic carbon and carbon organic matter. In: *Methods of Soil Analysis*, eds page A Z *et al*, Part 2 2nd ed ASA. SSSA, Madison Wisc.

Nurnberg H W 1984 The voltametric approach in trace metal chemistry of natural waters and atmospheric precipitation. *Analyt Chim Acta* **164** 1 - 21.

Omgbu J A, Kokogho M A 1993 Determination of Zn, Pb Cu and Hg in soils of Ekpan, Nigeria. *Environment International* **19** 611 - 613.

Purves D 1985 Trace element contamination of the environment. Amsterdam, Elsevier.

Ramos L, Hernandez L M, Gonzalez M J 1994 Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Donana National Park. *J Environ Qual* **23** 50 - 57.

Shuman L M 1988 Effect of organic matter on the distribution of manganese, copper, iron and zinc in soil fractions. *Soil Science* **146** (3) 192 - 198.

Visser W J F 1993 Contaminated land policies in some industrialized countries. *TCB report* RO2.

Walkley J T, Black A 1934 An examination of the degtejareff method of determining soil matter and a proposed modification of the chromic acid titration method. *Soil Sci* **37** 29 - 38.

Xiang H F, Tang H A, Ying Q H 1995 Transformation and distribution of forms of zinc in acid, neutral and calcareous soils of China. *Geoderma* **66** 121 - 135.

Pak. J. Sci. Ind. Res 2003 ***46****(6) 424 - 431*

SIMULATION OF CHLORIDE TRANSPORT BASED DESCRIPTIVE SOIL STRUCTURE

M Mahmood-ul-Hassan*, M S Akhtar, S M Gill and G Nabi

Land Resources Research Program, National Agricultural Research Centre, Islamabad-45500, Pakistan

(Received October 5, 2001; accepted March 24, 2003)

There is a need of environmental implications of rapid appearance of surface by applying chemical at depths below the vadose zone (tile line or shallow groundwater) for developing better insight into solute flow mechanism through the arable lands. Transport of chloride, a respresentative non-adsorbing solute, through a moderately structured silty clay loam soil (Gujranwala series, Typic Ustochrepts) and an un-structured sandy loam soil (Nabipur series, Typic Camborthid) was characterized and two existing models viz. convection dispersion equation (CDE) and preferential flow models were tested. The flux average of solute concentration in the outflow as a function of cumulative drainage was fitted to the models. The CDE fitted, relatively, better in the non-structured soil than in the moderately structured soil. Dispersivity value determined by CDE was very high for the structured soil which is physically not possible. The preferential flow model fitted well in the Gujranwala soil, but not in the Nabipur soil. The breakthrough characteristics i.e. drainage to peak concentration (Dp), symmetry coefficient (SC), skewness, and kurtosis were compared. Chloride breakthrough was earlier than expected based on piston flow. It indicated preferential flow in both the soils, yet, immediate appearance of the tracer in the Gujranwala soil demonstrated even larger magnitude of the preferential flow. Breakthrough curves' parameters indicated a large amount of the solute movement through the preferred pathways bypassing the soil matrix in the Gujranwala soil. The study suggests that some soil structure parameters (size/shape and degree of aggregation) should be incorporated in the solute transport models.

***Key words*: Soil structure, Solute transport, Simulation, Dispersitivity, Preferential flow.**

Introduction

Loss of agricultural chemicals from agro-ecosystems and the subsequent groundwater contamination demand better understanding of water and solute movement in the root and vadose-zone. Simulation models are widely used for predicting water and solute movement through unsaturated soil (Steenhuis *et al* 1994; Hatfiel *et al* 1997). Discrepancies between model results and the actual field measurements often occur (Jury and Fluhter 1992; Steenhuis *et al* 1994). Many recent studies have depicted rapid increase in concentrations of surface when applied agro-chemicals in tile lines or shallow groundwater shortly after application (Mohanty *et al* 1998). In other studies, travel times of adsorbed and non-adsorbed chemicals have been found to be the same (Flury *et al* 1994; Camobreco *et al* 1996).

The classical convection-dispersion equation used for water and solute movement through the porous medium is valid as long as the porous medium is homogeneous and solute moves with a horizontally uniform wetting front (Khan and Jury 1990; Hatfield *et al* 1997). However, validity of this equation for field application has been challenged in the recent past due to soil textural and structural heterogeneity (Bouma 1991). Some pedological features viz. macropores, continuous inter-aggregated voids, earthworm burrows, decayed root channels and other geometric anomalies, have entirely different hydraulic properties than soil matrix and act as preferential flow pathways (Gupta *et al* 1999). The preferential pathways are small fractions of total porosity through which solutes travel rapidly, by passing the soil matrix (Radulovich *et al* 1992), causing a rapid and accelerated breakthrough (Buchter *et al* 1995; Gaber *et al* 1995).

Accurate estimation of water and solute velocities in soil profile is essential for the prediction of sub-soil and groundwater contamination. Solute transport can accurately be predicted once breakthrough curves over a range of flow rates have been established, which is cumbersome and impractical under field conditions. The soil structure description available in the soil survery reports can be correlated with the magnitude of preferentially-transported solutes and hence, possibly forms the basis to simulate models for agricultural chemicals loss. Objectives of the leaching study were to develop relationship between soil structure and magnitude of preferential flow and test applicability of the existing models for one-dimensional transport of non-adsorbing solute using $C1^-$ as tracer.

Models. Convection dispersion equation. The well-known convection-dispersion model assumes that dispersion process is formally equivalent to the diffusion. Even though the dispersion is a convective transport process and solute

---

*Author for correspondence; E.mail: mmh@isb.comsats.net.pk samples all pore spaces with an average velocity with dispersion around the front. The convection-dispersion equation for one-dimensional transport of adsorbing and non-adsorbing solutes in one or two domains has been solved for several boundary conditions (Parker and van Genuchten 1984; Marshall *et al* 1996). A constant adsorption partition coefficient is employed to solve the differential equation for adsorbing solutes and movement of solutes is scaled with a retardation coefficient, R. Thus, the average velocity is R times slower and time of arrival is R times longer compared to a non-adsorbing solute. In the one-domain model, the whole profile is assumed to take part in the transport of the solutes. In the two-domain model, the liquid phase is partitioned into mobile and immobile domains and the solute exchange between the two liquid regions is modeled as a first-order process (Parker and van Genuchten 1984).

Preferential flow model. The preferential flow model assumes that the flow through the macropores is fast and no interaction takes place with the soil matrix. This model is simple and requires minimum parameters to be fitted (Steenhuis *et al* 1994). It is assumed that the flow in the distribution layer can be described with the linear reservoir theory (Gelhar and Wilson 1974) and that no interaction with the soil matrix takes place below the distribution layer. The cumulative loss of solutes, L, in the preferentially moving water from a soil with a distribution layer of thickness D, can be written as (Steenhuis *et al* 2001).

$$L = Mo\,[1\text{-}exp\,(-\,\frac{Y}{W})]\ \ldots\ldots\ldots\ldots\ldots\ \mathbf{(1)}$$

Where,

W = Apparent water content and equals $D(\rho\ k_d + \theta_s)$,
Y = The cumulative amount of percolation since the application of solute,
Mo = Initial amount of solute applied.

This equation is similar in form to that used by the U.S. Environment Protection Agency (1992) in predicting the loss of metals from the incorporation zone of sludge. The preferential flow model has been used to predict the loss of $C1^{-}$, pesticides, blue dye and metals when the matrix flow in the vadose zone could be neglected (Steenhuis *et al* 1994; Steenhuis *et al* 2001).

Materials and Methods

Site description. The soils were located at longitude 72.1°E and latitude 34.4°N in Potohar plateau (Pakistan) in sub-humid continental climate developed in Subrecent floodplain of Korang River (Khanzada 1976). Two soils-Nabipur, a sandy loam Typic Camborthid and Gujranwala (silty clay variant), silty clay Typic Ustochrepts were selected for the study. The Nabipur soil is deep, well drained, moderately calcareous and loam developed on level to nearly level position of the floodplain. It has very friable, massive and sandy loam top-soil underlain by friable loam B horizon with weak, coarse and sub-angular blocky structure. The Gujranwala (silty clay variant) is very deep, well drained and non-calcareous and the soil is developed in nearly leveled parts of convex slopes. The soil has moderate and medium sub-angular blocky silty clay loam surface and moderate, coarse and medium, sub-angular blocky silty clay 'B' horizon. The Nabipur soil has been under rain-fed wheat-maize cropping with annual moldboard tillage operation while the Gujranwala soil remained untilled for the last 4 years.

Excavation and Preparation of soil columns. Six intact soil columns, three for each soil, were extracted by hand-excavating and carving leaving soil pedestals in the centre of the soil pit. The pedestals were carefully trimmed to closely fit in the 260 mm diameter and 390 mm long PVC pipes. The space (≈ 10 mm) between the PVC pipe and the pedestals was filled with melted paraffin wax. The columns were transported to the laboratory. Undisturbed soil cores were also taken from 30 to 80 mm, 130 to 180 mm and 230 to 280 mm depths to determine the soil bulk density. Total porosity was calculated, assuming particle density 2.65Mg/m^3. Bottom and top of the columns were trimmed and smoothed in the laboratory. Further, 5 to 7 mm of bottom soil was removed and 0.05 to 0.02 mm fine sand was filled and covered by the nylon gauze sheet to ensure good hydraulic contact between the column and collection chamber. Finally, a perforated aluminum sheet was fixed at the bottom to firmly support the sand and the nylon gauze sheet. The sand had 3.4 mm/s saturated hydraulic conductivity and 1.52 Mg/m^3 bulk density. The nylon gauze sheet and aluminum sheet had 81 mesh openings. The column rested on a collection chamber, sealed with silicon rubber sealant. Polythene drain tube was fixed to both the holes. The collection chamber had attached two drinage tubes, one was used to drain leachate to sampling bottle and the other served as a peizometer. Each column had two microtensiometers fixed at 70 and 220 mm below the soil surface to ensure constant saturation.

Each column was slowly saturated from the bottom through the drain tube attached to the chamber. Saturation was achieved in 4 days by raising the water reservoir 100 mm in a day until water appeared at the surface. Water was kept ponding for further 48 h to ensure complete saturation. During saturation one drain tube attached to the chamber in order to bleed air. To maintain the constant ponding on the surface of the column, a water supply reservoir (Mariotte siphon) with

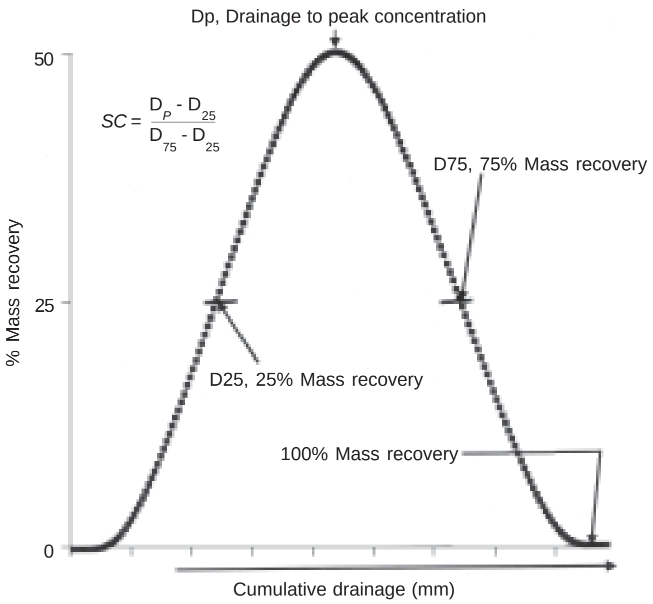


Fig 1. A hypothetical symmetrical distribution indicating symmetry coefficient as 1.

adjustable elevation was conected directly to the surface of the column. Saturated hydraulic conductivity (Ks) was measured with a constant head method by maintaining water level 30 mm above the column surface. Mean flow velocity (V) was calculated from Ks, assuming that water flux passed through all the water-filled pores.

Leaching experiment. Saturated columns were flushed with two-pore volume of 15 mM $LiNO_3$ solution at 30 mm head to displace interstitial anions with NO_3. Application of $LiNO_3$ solution ended at steady state condition with inflow equal to the outflow. Then the columns were leached with 15 mM Cl^- using LiC1 solution. When effluent $C1^-$concentration reached approximately 15 mM, the application of LiC1 solution stopped and the $LiNO_3$ solution started again to displace $C1^-$. Finally, $LiNO_3$ leaching stopped when effluent $C1^-$ concentration dropped below 0.02 mM. The effluent $C1^-$ concentration and effluent volumes were recorded. Chloride concentration was determined using the Fisher Accumet 950 pH/Ion meter using $C1^-$ specific electrode.

Parameter estimation. The breakthourgh curves (BTCs) depicted relative concentration (C/Co) versus percolate depth (drainage volume per unit surface area). Solute flow parameters were calculated from the breakthrough data by using convection-dispersion and the preferential flow models. Other indicators of preferential flow included symmetry coefficient (SC), percolate depth to peak concentration (Dp), and skewness and kurtosis of the curves (discussed later).

The CDE was executed using CXTFIT (Toride *et al* 1995). By assuming one domain vertical transport of Cl^- without adsorption solute velocity (V) and dispersion (D) were obtained. The simple preferential flow model (equation 2) (Steenhuis *et al* 1994; Steenhuis *et al* 2001) yielded apparent water content (W), in which depth of water was required to leach 50% of mass applied.

$$1n\,(1-\frac{L}{M_o}) = \frac{1}{W}\,Y \ldots\ldots\ldots\ldots\ldots\ \mathbf{(2)}$$

In $(1\text{-}L/M_o)$ was plotted against drainage (Y), where L was successive cumulative solute mass loss corresponding to respective cumulative drainage depth. Using a linear regression with Y as the dependent variable and 1n(1-L/Mo) as the independent variable without intercept, W was the inverse of the slope. In both the models r^2 depicted goodness of fit.

Symmetry coefficient (SC) of curve proposed by Hatfield *et al* (1997) was modified by replacing time with cumulative drainage (Fig 1). It was a ratio of the two differences: (a) the difference between drainage to peak concentration and 25% mass loss and (b) the difference between drainage to 75% mass loss and to peak concentration. Skewness and kurtosis of the curves were calculated by using PROC NPARIWAY (SAS Inc 1996).

Table 1
Physical properties of soil columns

Soil	Column	Bulk density	Total porosity	Ks[†]	Velocity	Macropores	
		(Mg/m^3)	(m^3/m^3)	(mm/day)		Surface	Bottom
Nabipur	1	1.57	0.41	17.50	42.90	1	2
	2	1.54	0.42	29.90	71.20	2	1
	3	1.58	0.40	16.40	41.00	0	4
Gujranwala	1	1.48	0.44	28.10	63.90	0	6
	2	1.45	0.45	31.90	70.90	1	7
	3	1.51	0.43	27.20	63.30	3	5

[†] Saturated hydraulic conductivity.

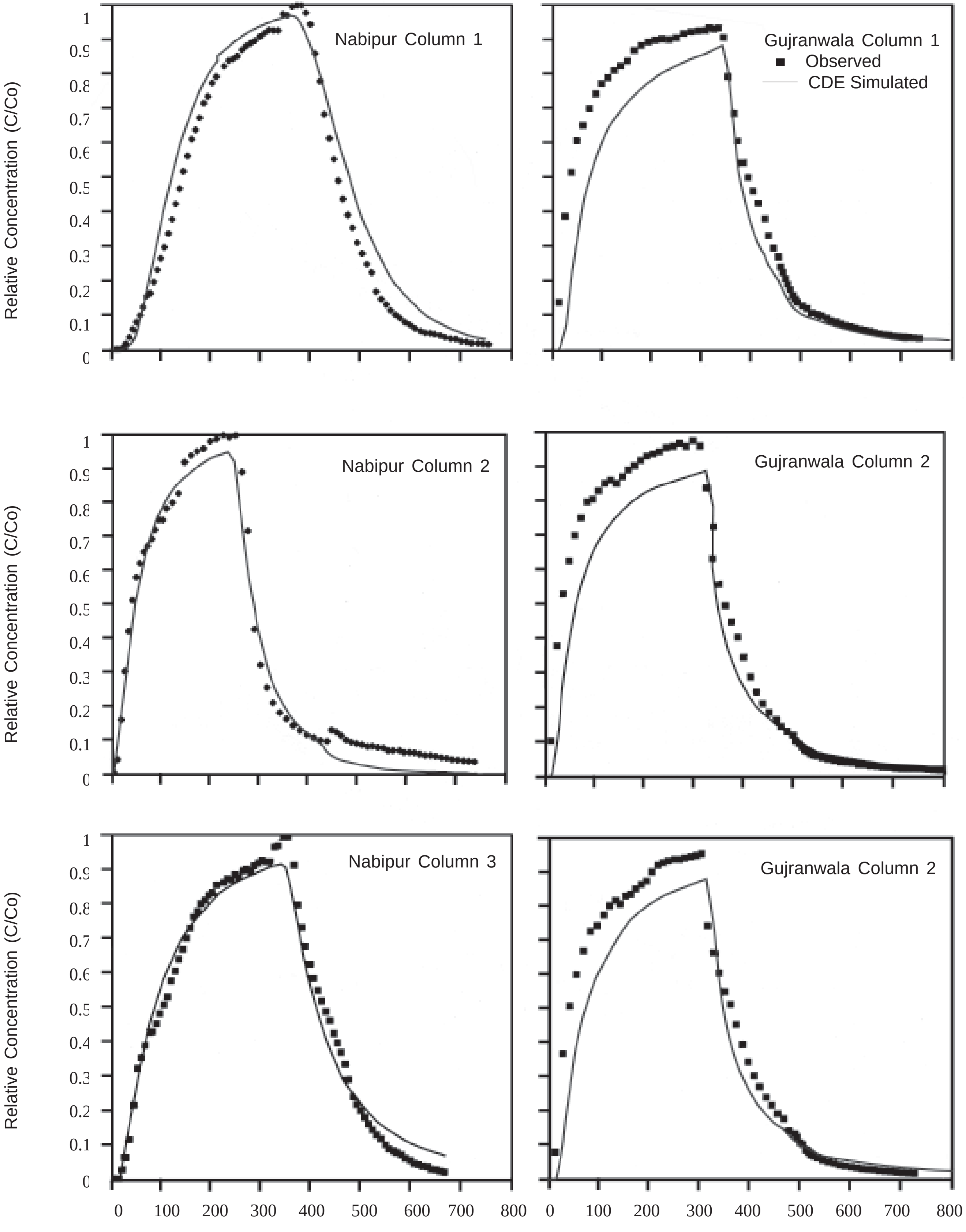


Fig 2. Chloride breakthrough in the Nabipur and Gujranwala soil columns.

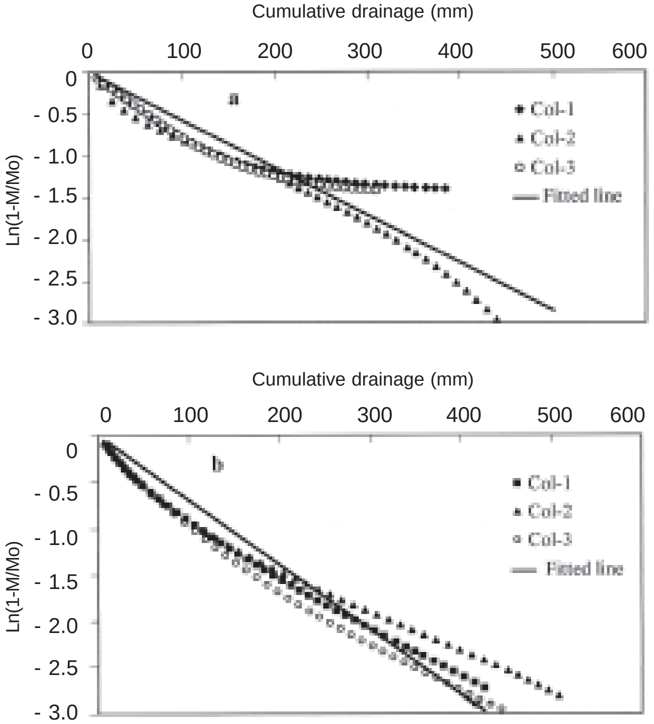


Fig 3. Preferential Flow Model (In (1M/Mo) vs cumulative outflow) fitted in (a) Nabipur and (b) Gujranwala soil columns.

Results and Discussion

Soil physical characteristics. Columns extracted from Nabipur soil had greater bulk density than those extracted from Gujranwala soil (Table 1). The Nabipur soil, was sandy loam and weakly structured, with an average bulk density of 1.51, 1.61, and 1.56 Mg/m^3 in the Ap, Bwt, and Bt horizons, respectively. The corresponding horizons in the moderately structured silty clay soil (Gujranwala) had a bulk density of 1.48, 1.49, and 1.51 Mg/m^3. A relatively greater bulk density of Bw horizon of the Nabipur soil than that of the Gujranwala soil was noticeable and can be ascribed to mechanical compaction of the sandy loam material. However, column bulk density, on the whole, was very close to the calculated average of the profile. The column 2 of Nabipur soil had lower bulk density than the column 1 and 3. It is interesting to note that the Gujranwala soil contained a greater number of visible macropores than the Nabipur soil. Consequently, the greater porosity and probably pore continuity in the Gujranwala soil columns resulted in larger hydraulic conductivity than the Nabipur columns.

Chloride breakthrough. In both the soils, C1$^-$ breakthrough occurred earlier than one pore volume (Fig 2). In all the Gujranwala soil columns, C1$^-$ breakthrough was almost immediate. Initially, slope of the breakthrough curve was steep and relative concentration (C/Co) reached 0.5 only after 40 mm of cumulative drainage. Afterwards, the slope of the curve declined relative to the initial slope and C/Co reached to 0.75 with another 40 mm cumulative drainage. The peak C/Co (0.95) in the Gujranwala soil columns was obtained with 300 mm cumulative drainage. During the flushing phase, when chloride application had stopped and C1$^-$ free water had started leaching, there was an immediate and sharp decline in percolate Cl$^-$. In contrast, the C1$^-$ breakthrough in the Nabipur soil columns was delayed by approximately 25 mm, and the concentration ratio of 0.5 was attained after 125 mm percolate depth and 0.75 with 200 mm. The peak C/Co of 0.95 was obtained after 350 mm cumulative drainage. Moreover, the concentration ratio decreased gradually in this soil after termination of C1$^-$ application and there was less tailing in the Nabipur soil as compared to that of the Gujranwala soil.

Table 2

Characteristics of breakthrough curves

Soil	Column	Dp[†] (mm)	Tp[‡] (h)	SC[§]	Skewness	Kurtosis
Nabipur	1	370	24.00	5.70	0.29	1.56
	2	250	10.50	10.80	0.77	1.09
	3	340	22.00	7.00	0.01	1.51
Gujranwala	1	300	13.00	16.90	0.56	1.41
	2	250	12.00	10.10	0.67	1.31
	3	290	13.00	15.10	0.59	1.38

[†] Drainage to peak concentration; [‡] Time to peak concentration; [§] Symmetry coefficient.

Curve shape parameters. The drainage to peak concentration (Dp), symmetry coefficient (SC) and skewness of C1$^-$ BTCs provided good comparison between the Nabipur and the Gujranwala soils (Table 2). A peak C1$^-$ concentration was achieved with lesser drainage in the Gujranwala soil columns compared to the Nabipur. Except for the column 2, drainage to peak concentration in Nabipur soil columns was 50 to 75 mm

Table 3

Summary of CDE[†] and preferential flow model results

Soil	Columns	Convection-Dispersion equation				Preferential flow model	
		D (cm^2/h)	V (cm^2/h)	λ (cm)	r^2	W (cm)	r^2
Nabipur	1	14.50	3.21	4.52	0.96	20.96	0.64
	2	172.20	9.89	17.41	0.98	15.11	0.94
	3	64.80	4.05	16.00	0.98	17.48	0.83
Gujranwala	1	255.40	6.94	36.80	0.97	14.37	0.96
	2	230.10	8.53	26.98	0.98	16.58	0.93
	3	156.20	7.33	21.31	0.96	13.81	0.95

[†] Convection-Dispersion equation.

which was greater than that of Gujranwala columns. Breakthrough curves from the Nabipur soil columns were relatively symmetrical and were less skewed as compared to the Gujranwala soil columns. The symmetry coefficient value in the Nabipur soil column curves was half that of the Gujranwala columns. Mean kurtosis values for both the soils were similar but the Nabipur soil took twice the time (1400 min) to achieve the crest as compared to the Gujranwala (740 min). The Nabipur soil column 2 behaved differently than the other two columns from the same soil.

The solute breakthrough occurred immediately in the structured (Gujranwala) soil and after 25 mm of drainage in the un-structured (Nabipur) soil (Fig 2). Further, in un-structured soil, the percolate amount was less than 0.3 pore volume whereas, under uniform flow exactly one pore volume of incoming solute would have been required to replace the pre-existing solute and breakthrough at outflow end by assuming zero dispersion (van Genuchten 1981). In a homogeneous cylindrical soil column, solute mixed completely in radial direction before it reached to the outflow end in the vertical direction. Therefore, the early breakthrough of the solute indicated the occurrence of preferential flow through all the columns of both the soils although the magnitude was greater in the structured than in the un-structured soil.

Preferential flow was caused by wetting front instability (DeRooij and DeVaries 1996), funnel flow in layered soils (Kung 1990) and flow through macropore by-passing the soil matrix (Sollins and Radulovich 1988; Gupta *et al* 1999). Macro-pores flow, through non-capillary inter-pedal void spaces, was associated with pedological cracks, decayed root channels and other structural anomalies essentially present in intact soil columns (Sollins and Radulovich 1988). The immediate breakthrough in case of the Gujranwala soil could be due to preferential flow through inter-ped void spaces or macropores. These results corroborated with the structural conditions of the soils as macropores resulted in greater inter-aggregate infiltrability than intra-aggregate infiltrability (Gupta *et al* 1999).

Model fitting. Convection dispersion and preferential flow models have been compared. The Convection-Dispersion equation used one-dimensional mode by assuming zero retardation (R) as $C1^-$ is non-adsorbing. The model parameters mean i.e. pore velocity (V), apparent dispersion coefficient (D) and r^2 (indicates the fitness of the model) were determined by using CXTFIT computer program (Toride *et al* 1995). Dispersivity (λ), solute dispersion to mass transfer per unit time or drainage outflow in a unit cross-sectional porous area, is D/V (Jury *et al* 1991). Except for one column, mean pore velocity of the un-structured soil was approximately two times un-structured soil (Table 3), indicating larger flow through non-capillary porosity. Dispersion in the structured soil columns was larger than the un-structured soil but was highly variable. Surprisingly, in all the three Gujranwala soil columns best-fit solution ($r^2 > 0.96$) was achieved at $D > 150$ cm^2/h. This large D value implied no mass transfer of water had occurred and the movement of $C1^-$ was solely due to diffusion. This resulted in extremely high dispersivity values (21 - 37 cm) that were physically impossible. Dispersivity ranged from 4.5 to 17.4 cm for the non-structured soil, which were within acceptable limits (Jury *et al* 1991). Therefore, although the CDE model simulated the general shapes of the BTC, except the initial breakthrough and the peak, it predicted an erroneous dispersivity in the structured soil.

In contrast, the preferential flow model was better fit in the structured soil than in the un-structured soil as indicated by a fairly straight line in the later case (Fig 3). If the preferential flow model is valid then the data should plot reasonably well as a straight line. The regression results showed that the data fit the preferential flow model very well (Table 3). The r^2 for the three columns from the Gujranwala soil was 0.93 or higher and with an exception, it was 0.83 or less for the Nabipur soil. One column in Nabipur soil did fit to a straight line, which reflects either an artifact or natural variability.

In the Gujranwala soil, the conductivity of the matrix was relatively low than in the Nabipur soil. Thus, no exchange of solutes took place between macropores and matrix for the Gujranwala soil which was assumed in the preferential flow model (Steenhuis *et al* 1994). This is not true for the Nabipur soil, showing a deviation from the straight line probably because of increasing concentration (Steenhuis *et al* 2001). The theory assumes that the mixing is instantaneous and that there is no delay in travelling time from the distribution zone to the bottom of the column. In this study, we plotted part of the data set (natural log of mass of Cl^- remaining vs. the cumulative outflow) starting immediately after the effluent Cl^- had reached maxima as mixing was not instantaneous in this case. Therefore, the initial deviation from straight line is not depicted in the graph.

Curve shape parameters. The curve parameters i.e. drainage to peak concentration (Dp), symmetry coefficient (SC) and skewness provided comparison between the Nabipur and Gujranwala soil columns (Table 2). Compared to the Nabipur soil, the peak Cl^- concentration in the Gujranwala soil columns was attained with less drainage due to inter-void spaces conducting greater solute compared to matrix. This phenomenon is related to differences in soil structure. However, time to peak concentration had greater magnitude of difference between the two soils compared with drainage to peak concentration because higher flow rate in the structured soil also allowed more water to drain in given time. As such the Nabipur soil columns required twice the time to attain the peak Cl^- concentration than the Gujranwala soil while the difference in drainage was not so high.

The peak concentration would coincide with loss of 50% mass in a symmetrical bell-shaped curve. A symmetry coefficient close to one indicated the symmetricl distribution and value >1 indicate preferential flow. The Gujranwala soil had about two times larger SC than the Nabipur soil. The faster translocation of mass in the Gujranwala soil compared with the Nabipur soil was obvious as the peak concentration in the Gujranwala soil columns coincided with about 75% of the total mass loss, which was about 60% in the Nabipur soil. Kurtosis values of BTCs, another quantitative indicator of preferential flow (Hatfield *et al* 1997), were slightly higher in the Nabipur soil than in the Gujranwala soil. The reported results are contrarily to Hatfield *et al* (1997) to the extent that kurtosis is a better numeric indicator of preferential flow than skewness and SC, whereas we found DP, skewness, and SC better numeric indicators than kurtosis.

Conclusion

Comparison of calculated and observed first arrival times and BTCs indicated that perferential flow occurred in all the columns from both the soils. However, the magnitude of preferential flow was higher in the Gujranwala soil than in the Nabipur soil. Drainage to peak concentration, symmetry coefficient, and skewness of the BTC were quantitative parameters for perferential flow and their statistical comprison has potential for field application. Further, the CDE described well the solute transport through the un-structured soil but failed in case of the structured soil while the reverse was true for the preferential flow model. The study indicates a need for incorporation of soil structure parameters (size/shape and degree of aggregation) in the solute transport models in order to improve simulation.

References

Bouma J 1991 *Influence of soil macroporosity on environmental quality. Advances in Agronomy,* Academic press, San Diego, CA, USA, Vol 46 pp 1 - 37.

Buchter B, Hinz C, Flury M, Fluhler H 1995 Heterogenous flow and solute transport in an unsturated stony soil monolith. *Soil Sci Soc Am J* **59** 14 - 21.

Camobreco V J, Richards B K, Steenhuis T S, Peverly J H, McBride M B 1996 Movement of heavy metals through undisturbed and homogenized soil columns. *Soil Sci* **161** 740 - 750.

DeRooij G H, DeVaries P 1996 Solute leaching in a sandy soil with a water repellent surface layer: A simulation. *Geoderma* **70** 253 - 263.

Flury M, Fluhler H, Jury W A, Leuenberger J 1994 Susceptibility of soils to preferential flow of water: A field study. *Water Resour Res* **30** 1945 - 1954.

Gaber H M, Inskeep P W, Comfort S D, Mraith J M 1995 Non-equilibrium transport of atrazine through large intact soil cores. *Soil Sci Soc Am J* **59** 60 - 67.

Gelhar L W, Wilson J L 1977 Groundwater quality modelling. *Groundwater* **12** 399 - 408.

Gupta A, Destouni G, Jensen M B 1999 Modelling tritium and phosphorous transport by preferential flow in structured soil. *J Contam Hydrol* **35** 389 - 407.

Hatfield K K, Warner G S, Guillard K 1997 Bromide and FD and C Blue No 1 dye movement through intact and packed soil columns. *Transactions ASAE* **40** 309 - 315.

Jury W A, Gardner W R, Gardner W H 1991 *Soil Physics.* John Wiley and Sons Inc, New York, USA.

Jury W A, Fluhter H 1992 Transport of chemical through soil mechanisms, models and field application. *Adv Agron* **47** 141 - 201.

Khan A H, Jury W A 1990 A laboratory study of the dispersion scale effect in column out-flow experiments. *J Contam Hydrol* **5** 119 - 131.

Khanzada S K 1976 *Soils and Capability.* National Agricultural Research Centre, Islamabad. Soil Survey of Pakistan, Ministry of Food and Agriculture, Pakistan.

Kung K J S 1990 Preferential flow in a sandy vadose soil: 1 Field observation. *Geoderma* **46** 51 - 59.

Mohanty B P, Bowman R S, Hendrickx J M H, van Genuchten M Th 1998 Preferential transport of nitrate to a tile drain in an intermittent-flood-irrigated field: Model development and experimental evaluation. *Water Resour Res* **34** 1061 - 1076.

Parker J C, van Genuchten M Th 1984 Determining transport parameters from laboratory and field trace experiments. *VA Agric Exp Stn Bull* 84 - 93.

Radulovich R, Sollin P, Baveye P, Solorzano E 1992 Bypass water flow through unsaturated microaggregated tropical soils. *Soil Sci Soc Am J* **56** 721 - 726.

SAS Institute, INC 1990 Version. *SAS/STAT User's Guide,* SAS Institute, Inc. Cary, NC, USA, 64th ed.

Sollins P, Radulovich R 1988 Effect of soil physical structure on solute transport in a weathered tropical soil. *Soil Sci Soc Am J* **52** 1168 - 1173.

Steenhuis T S, Boll J, Shalit G, Selker J S, Merwin I A 1994 A simple equation for predicting preferential flow solute concentrations. *J Environ Qual* **23** 1058 - 1064.

Steenhuis T S, Kim Y J, Parlange J Y, Akhtar S M, Richards B K, Kung K J S, Gish T J, Dekker L W, Ritsema C J, Aburime S A 2001 An equation for describing solute transport in field soils with preferential flow paths. In: Preferential Flow: Water Movement and Chemical Transport in the Environment. *Proc ASAE 2nd Int Symp Preferential flow.* Honolulu, HI, USA, Jan 3-5, 2001 pp 137 - 140.

Toride N, Leij F J, van Genuchten M Th 1995 *The CXTFIT Code for Estimating Transport Parameters from Laboratory or Field Tracer Version 2.0.* Research Report No. 137, US Salinity Laboratory, U S Department of Agriculture, California, USA.

USEPA 1992 *Another Look National Survey of Pesticide in Drinking Water Wells.* Phase II Report NTIS Doc PB92-120883 U S Environmental Protection. Agency, Washington, DC, USA.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 432 - 435*

Studies of the Polynuclear Complexes of Labile Ligands of Vitamin B$_1$ and Zn (II), Cd (II) and Hg (II) with Fe (III)

James O Ojo

Department of Chemistry, Federal University of Technology, PMB 704 Akure, Nigeria

(Received January 3, 2002; accepted October 4, 2003)

The ligands (complex salts) of vitamin B$_1$ (H Vit.) and the chlorides of Zn, Cd and Hg with the general formula, $[HVit]^{+2}$ $[MCl_4]^{-2}$ were prepared and their interactions with iron (III) investigated. It was found that the complex salts of Zn and Cd produced the dinuclear complexes and that of mercury produced a complex without the thiamine moiety. The possible reason for the absence of a Hg complex similar to those of Zn and Cd may be that large size of mercury ion. The complexes were characterized by elementary analyses, infrared and visible spectra, magnetic moment and conductivity measurements.

Key words: Vitamin B$_1$, Ligands, Elementary analyses, Conductivity measurements, Dinuclear complexes.

Introduction

Vitamin B$_1$ also called thiamine chloride hydrochloride (Fig 1) is very important in humans. Its potential as a ligand is being exploited in coordination chemistry chiefly because of its wide variety of coordination sites (Talbert *et al* 1970). The great success achieved in the exploitation of the coordination chemistry of vitamin B$_1$ is owed to the recovery of acetate catalyzed removal of the pyrimidinyl hydrogen ion of NH_2 group of vitamin B$_1$ moiety (Adeyemo and Shamin 1983a). Many of the reported complexes of thiamine are bounded by metals through the N(1′) of the pyrimidine ring (Adeyemo *et al* 1983b).

Recently, a number of complexes have been reported (Casas *et al* 1995) indicating the metal bonding through the oxygen of the hydroxyethyl group. Also of great interest are the reported polymetallic complexes (Adeyemo 1986; Ojo 2001).

Polymetallic complexes of vitamin B$_1$ involving Fe (III) have not been investigated as yet. This communication now reports dinuclear complexes of vitamin B$_1$ involving Zn (II), Cd (II) with Fe (III) and discusses the inability of Hg (II) to produce similar dinuclear complex.

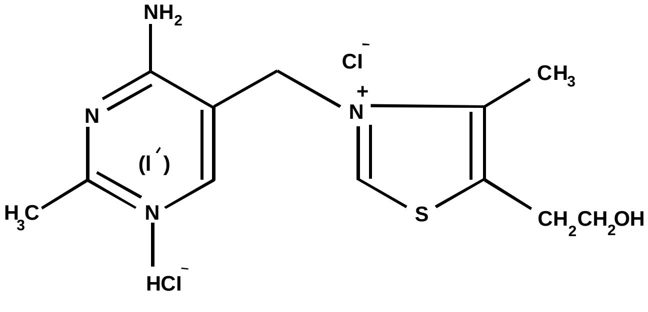


Fig 1. The structure of thiamine chloride hydrochloride (Vitamin B$_1$).

Materials and Methods

Preparation of the labile ligands (complex salts). Ligand were prepared by adding a solution of thiamine chloride hydrochloride (3.37g, 0.01 mol) in 50 ml distilled water to a 0.01M solution of the metal (Zn, Cd and Hg) chloride. The resulting mixture was stirred magnetically and refluxed for 3h. The precipitate obtained in each case was filtered, washed with distilled water, dried and finally analyzed. The complex salts conform to the formula $[H\ Vit]^{+2}$ $[MX_4]^{-2}$ with HVit. = protonated thiamine and X = Cl^-.

Preparation of the complexes. The complexes were prepared by adding the labile ligands, $[HVit]^{+2}[MX_4]^{-2}$, [M = Zn, Cd or Hg] (0.01 mol) to a methanolic solution of iron (III) hydroxyl acetate (0.01 mol), refluxed and stirred magnetically for 3h. The resulting precipitate was filtered, washed with methanol and recrystallized from methanol and subsequently dried in vacuum.

Physical measurements. Elemental analyses were carried out at micro-analytical laboratory, University of Ibadan. The metal ions were determined by complexometric titration. The infrared spectra of the ligands and complexes were recorded on a PYE - UNICAM SP 300 spectrophotometer, electronic spectrophotometer, were recorded on a SP 500 spectrophotometer, magnetic susceptibility data were recorded on Gouy's balance using Hg $[Co(NCS)_4]$ as calibrant, and molar conductance on a conductivity bridge with a cell constant 1.0 cm^{-1} mol^{-1}.

Table 1

Analytical and molar conductivity data for the complexes

Complex	Found (Calcd).(%)						Molar conductivity
	C	H	N	Cl	Fe	M	Ω^{-1} cm^2 mol^{-1}
[(Vit) $FeZnCl_5$] $.2H_2O$	22.69	3.95	8.85	33.53	9.00	10.38	
	(22.68)	(3.31)	(8.82)	(33.54)	(8.82)	(10.24)	35.10
[(Vit) FeCd Cl_5] $.2H_2O$	22.32	3.38	8.75	27.33	8.34	19.00	
	(22.91)	(3.02)	(8.91)	(27.46)	(8.21)	(18.52)	42.86
[$HgFe_2(OH)_2$ $(Ac)_4$] $.3H_2O$	15.09	4.58	---	---	17.53	33.13	
	(15.09)	(3.13)	---	---	(17.48)	(31.58)	19.59

Vit, Vitamin B_1 ligand (thiamine); M, Zn (II), Cd(II) or Hg(II); Ac, CH_3COO^-

Table 2

Infrared data for the ligand and complexes (cm^{-1})

	ν(O - H)	ν(N - H)	δ(NH_2) + pyrimidine ring	ν_{asy} (COO) + ν_{sy} (COO)	ν(M - N) + ν(M - O) + ν(M - Cl)	ν(M - M)
$[HVit]^{+2}$ $[MCL_4]^{-2}$	3450	3270	1650	---		---
[(Vit)$FeZnCl_5$]Cl $.2H_2O$	3389	3222	1643	---	600	
					450	250v.w
[(Vit)$FeCdCl_5$]Cl $.2H_2O$	3412	3206	1628	---	600	
					400	240v.w
[$HgFe_2(OH)_2(Ac)_4$] $.3H_2O$	3500	---	---	1600	560(ν.br)	230v.w
	2500(ν.br)	---	---	1400		

M, Zn(II); Cd(II) or Hg(II) from labile ligand $[HVit]^{+2}$ $[MCl_4]^{-2}$

Results and Discussion

Nature and stoichiometry. All the complexes are brown in colour with the exception of mercury, which was dark brown in colour. The analytical data (Table 1)show that thiamine is present in the zinc (II) and cadmium (II) complexes but not that of mercury (II). This may probably be due to sterric factors arising from the sizes of the Hg (II), thiaminium and acetato ligands. The molar conductivity values (Table 1) of ~35 and ~43 Ω^{-1} cm^2 mol^{-1} show the zinc and cadmium complexes as 1:1 electrolyte in dimethyl sulphoxide (DMSO)while a value of ~20 Ω^{-1} cm^2 mol^{-1} shows the non-thiamine coordination mercury complex as a non-electrolyte in DMSO (Geary 1971; Rajavel and Krishnan 1998).

Infrared spectra. The IR results are shown in Table 2. In the labile ligands, two bands observed at 3450 and 3270 cm^{-1} are assigned to ν(O - H) and ν(N - H) vibrations, respectively (Adeyemo *et al* 1983; Ojo 2001).

Two other strong bands at 1650 and 1600 cm^{-1} are assigned for coupling of the pyrimidine ring and δ(NH_2) vibrations, respectively while the band at 1554 cm^{-1} is assigned for pyrimidine ring vibrations. In the complexes, the following changes are observed. The band at 3450 cm^{-1} shifts to 3389 cm^{-1} and 3412 cm^{-1} in the zinc and cadmium complexes, respectively. In the mercury complex, it is replaced by a very broad band at 3500 - 2500 cm^{-1}. The band at 3270 cm^{-1}assigned to ν(N - H) in the ligands shifts to 3222 and 3206 cm^{-1} in the zinc and cadmium complexes, respectively. This band is absent in the mercury complex, thus indicating the absence of thiamine moiety in the complex. The band at 1650 cm^{-1} assigned to the coupled pyrimidine ring and δ(NH_2) vibrations shifts to 1643 an 1628 cm^{-1} in the zinc and cadmium complexes, respectively but it is absent in the mercury complex, which further supports that thiamine is absent in the mercury complex. Two new bands at 1600 and 1400 cm^{-1} absent in the ligands are observed in the mercury complex, and have been assigned to ν_{asy} (COO) and ν_{sy} (COO) vibrations. A weak band at about 300 cm^{-1} which has been assigned to ν(M-M) (metal-metal) vibration is observed in all the complexes (Nakamato and Keif 1967; Ferraro 1971; Adeyemo *et al* 1983b; Onoa *et al* 1999; Bien 1999; Ojo 2001).

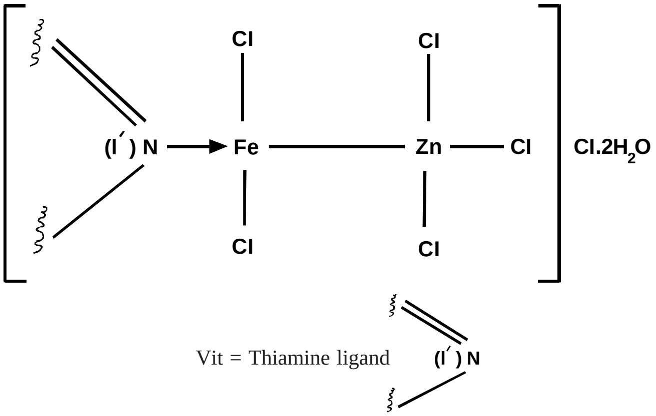


Fig 2. The structure of [(Vit) $FeZnCl_5$] Cl. $2H_2O$.

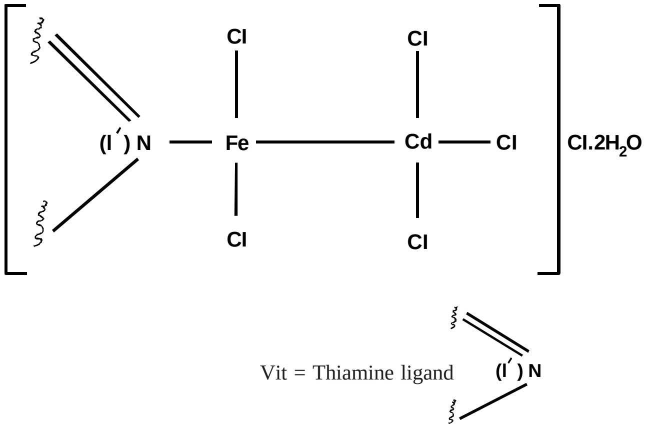


Fig 3. The structure of [(Vit) Fe Cd Cl_5]Cl. $2H_2O$.

Magnetic moment. All the complexes contain iron(III) with the valves of 11.6, 10.7 and 11.5 B.M. for the zinc, cadmium and mercury complexes, respectively. These are exceedingly too high values for systems containing five unpaired electrons. The observation can only be rationalized on the basis of an existing cooperative paramagnetism (ferromagnetism) between the neighbouring iron (III) ions in the crystal lattice arising from the parallel alignment of the magnetic dipoles of the individual ions (Earnshaw 1968; Shriver *et al* 1990).

Electronic spectra. The complexes show no significant absorptions in the visible region. This is consistent with a d^5 tetrahedral electronic configuration which is not expected to exhibit spin forbidden d-d transitions since all tetrahedral complexes are energetically favoured to be high spins. The octahedral, weak, spin-forbidden bands such as ${}^2T_{1g} \leftarrow {}^6A_{1g}$ would have been observed in the visible region if the complexes were not tetrahedral (Purcell and Kotz 1999). Based on the above information , the structures as shown in Fig. 2, 3 and 4 have been proposed for the complexes.

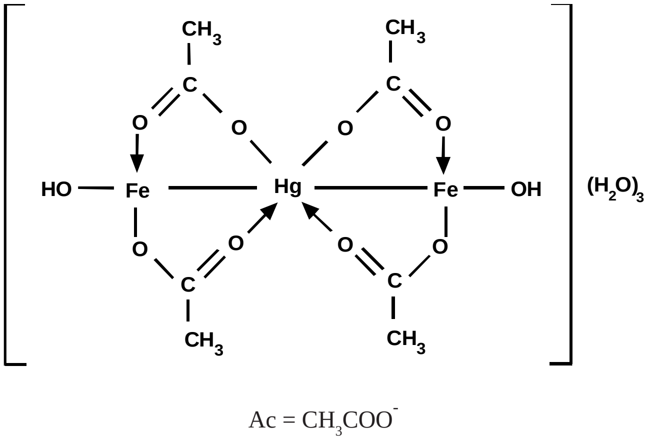


Fig 4. The structure of $[HgFe_2(OH)_2(Ac)_4]$ $.3H_2O$.

Acknowledgment

I am thankful to the Department of Chemistry of the University of Ibadan, Obafemi Awolowo University and Federal University of Technology, Akure, for making some of the facilities available.

References

Adeyemo A, Shamin A 1983a Acetate catalysed interactions of divalent metal ions with vitamin B_1. *Inorg Chim Acta* **78** L21 - L22.

Adeyemo A, Shamin A, Turner A, Akinade K 1983b Studies involving labile vitamin B_1 metal complexes Part 2. IR, NMR studies, structure and binding site determination. *Inorg Chim Acta* **78** 191 - 193.

Adeyemo A 1986 Studies involving labile vitamin B_1 complexes. Part III. Preparation and general mechanism for the formation of complexes of the general formula $M(NH_3)_6$ MX_5. *J Chem Soc Pak* **8**(4) 455 - 459.

Bien M 1999 Studies of antibacterial activity of binuclear rhodium (II) complexes with heterocyclic nitrogen ligands. *J Inorg Bioch* **73** 49 - 55.

Casas J S, Castellano E E, Couce M D, Sanchez A, Sordo J, Varella J M, Zukerman - Schpector J 1995 Vitamin B_1: Chemical interaction with $CdCl_2$ and *in vivo* effects on cadmium toxicity in rats. Crystal structure of $[Cd(thiamine)Cl_3]_2$. $2H_2O$, a complex containing pyrimidine and cadmium - hydroxylethyl bonds. *Inorg Chem* **34** 2430 - 2437.

Earnshaw A 1968 *The Introduction to Magnetochemistry*. Academic Press, London, UK, p 120.

Ferraro J R 1971 *Low Frequency Vibration of Inorganic and Coordination Compounds*. Plenum Press, NewYork, USA, p 70.

Geary W J 1971 The use of conductivity measurement in organic solvent for the characterization of coordination compounds. *Coord Chem Rev* **7**(1) 81 - 122.

Nakamato K, Kieff J A 1967 Frequency assignment made in metal glycine complexes. *J Inorg Nucl Chem* **29** 2561 - 2568.

Ojo J O 2001 Low spin trinuclear complexes of labile vitamin B_1 with cobalt (II). *Pak J Sci Ind Res* **44**(1) 27 - 28.

Onoa G B, Moreno V, Front - Bardia M, Solans X, Perez J M, Alonso C 1999 Structural and cytotoxic study of new Pt (II) and Pd (II) complexes with bi-heterocyclic ligand mepirizole. *J Inorg Bioch* **75** 205 - 212.

Purcell K F, Kotz J C 1977 *Inorganic Chemistry*. W B Saunders Company, Philadelphia, pp 514 - 585.

Rajavel R, Krishnan C N 1998 Studies on vanadyl complexes of schiff based derived from 2 - aminobenzaldehyde. *Orient J Chem* **14**(2) 313 - 316.

Shriver D F, Atkins P W, Langford C H 1990 *Inorganic Chemistry*; Oxford University Press, Oxford, USA pp 433 - 464.

Talbert P T, Weaver J A, Hallbright P 1970 Zinc(II) and cobalt(II) halide interactions with vitamin B_1 and certain N-substituted thiazolium salts. *J Inorg Med Chem* **32** 2147 - 2152.

Short Communication

*Pak. J. Sci. Ind. Res. 2003 **46**(6) 436 - 438*

SYNTHESIS OF 3-METHOXY-4'-PRENYLOXY-FURANO (2'', 3'':7, 8) FLAVONE

M Amzad Hossain* and S M Salehuddin

Chemistry Division, Atomic Energy Centre, P O Box No.164, Ramna, Dhaka - 1000, Bangladesh

(Received August 25, 2001; accepted December 28, 2002)

Flavonoids represent a group of phytochemicals exhibiting a wide range of biological activities such as anti-bacterial, antifungal, anti-inflammatory, antimicrobial, anti-cancer and insect antifeedant (Hodek *et al* 2002). A large number of natural products including flavonoids are being reported in the literature every year and their structures need to be confirmed by synthesis. In this paper, the synthesis of 3-methoxy-4'-prenyloxy-furano (2",3" :7,8) flavone (**8**) has been described starting from β-resacetophenone (Clarke 1955) (**1**), which may be used as synthetic markers. β-Resacetophenone (Clark 1955) (**1**) when refluxed with allyl bromide in presence of K_2CO_3 and acetone yielded 4-O-allylresacetophenone (Rangaswaqmi *et al* 1954) (**2**) which on Claisen migration gave 3-C-allylresacetophenone (Baker and Lothin 1935) (**3**).This was subjected to OsO_4/KIO_4 oxidation followed by orthophosphoric acid cyclization to 2-hydroxyfurano(2',3':4,3) acetophenone (Naik *et al* 1975) (**4**). p-Hydroxybenzaldehyde on treatment with prenyl bromide in the presence of K_2CO_3 and acetone gave 4-O-prenyloxybenzaldehyde (**5**). Alkaline condensation of **4** and **5** yield 2'-hydroxy-4-O-prenyloxy-furano(2", 3" :4', 3')chalcone (**6**). Compound **6** on treatment with H_2O_2 furnished 3-hydroxy-4'-O-prenyloxy-furano(2", 3" :7, 8)flavone (**7**) which upon methylation using dimethyl sulphate, K_2CO_3 and acetone afforded 3-methoxy-4'-O-prenyloxy-furano(2", 3" :7, 8) flavone (**8**).

Melting points were determined on an electrothermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra were recorded on KBr discs on a Pye-Unicam SP3-300 IR spectrophotometer (ν_{max} in cm^{-1}), ^{1}H-NMR spectra were recorded on a Perkin-Elmer R-32 (90 MHz) spectrophotometer in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ values) and UV spectra were recorded on LKB 4053 Ultrospeck spectrophotometer in methanol (λ_{max} in nm). TLC was performed using silica gel GF_{254}. Satisfactory elemental analysis were obtained for all the compounds and structures are in accord with the UV, IR and ^{1}H-NMR data. Mass spectra were recorded on VG 7070E analytical mass spectrometer.

*4-O-Allylresacetophenone (**2**).* β-Resacetophenone (Clarke 1955) (10 g) in acetone (50 ml) was refluxed with allyl bromide (7.5 g) and anhydrous potassium carbonate (40 g) for 6 h. Inorganic salts were filtered off and washed with acetone. Acetone was removed by distillation. The residue was taken up in ether and extracted with 5% aq. Na_2CO_3 solution and then with 5% NaOH solution. Sodium hydroxide extract was acidified and again extracted with ether (2x50 ml), dried over anhydrous Na_2SO_4 and concentrated when a dark coloured oil (12 ml) was obtained, b.p. 156-157°C (9 mm) [Rangaswaqmi *et al* 1954, b.p. 156-157°C].

*3-C-Allylresacetophenone (**3**).* The above 4-O-allylresacetophenone (Rangaswaqmi *et al* 1954) was (4 g) heated in an oil-bath, cautiously. Rearrangement occurred at 180°C with evolution of heat and the test tube was raised for a few min. Then the temperature was maintained at 210-215°C for 2 h, when a pink coloured solid was obtained. The crude mixture was subjected to column chromatography over silica gel using benzene as eluent. Earlier fractions gave some oil and then pure 3-C-allylresacetophenone was obtained as colourless needles (1.3 g), m.p. 132-133°C (Baker and Lothin 1935, m.p. 131°C).

*2-Hydroxy-furano(2',3':4,3)acetophenone (**4**).* 3-C-Allylresacetophenone (Baker and Lothin 1935) (1 g) was dissolved in ethyl acetate (400 ml), an equal volume of water and osmium tetroxide (200 mg) was added. The mixture was stirred on a magnetic stirrer for 1.5 h during which period potassium periodate (6 g) was added in small quantities and the mixture was stirred for two more hours. The ethyl acetate layer was separated and the aqueous solution was further extracted with ethyl acetate (2x25 ml). The combined ethyl acetate extract was washed well with water, dried over anhydrous Na_2SO_4 and the solvent was distilled off. The residue obtained as dark coloured oil was heated on a water-bath with orthophosphoric acid (40 ml) for 20 min and then poured over crushed ice. The solid that separated was taken up in ether and the ether solution was washed successively with 5% Na_2CO_3 solution, water and dried (Na_2SO_4). The solvent was distilled off and the residue was taken up in benzene and passed through a column of neutral alumina when colourless flakes (230 mg) were obtained. m.p. 85°C (Naik *et al* 1975), m.p. 86°C); (M^+, 176); UV : 235, 275, 325; IR : 3440, 1630, 1585, 1500, 1440, 1375; ^{1}H-NMR: 2.45 (s, 3H, - $COCH_3$), 6.98 (d, 1H, J = 2 Hz, H-4'), 7.05 (d, 1H, J = 9 Hz, H-5), 7.55 (d, 1H, J = 2 Hz, H-5'), 7.65 (d, 1H, J = 9 Hz, H-6), 13.90 (s, 1H, - OH); [Anal. Calc. for $C_{10}H_8O_3$: C, 68.2 ; H, 4.5. Found: C, 67.9; H, 4.9%].

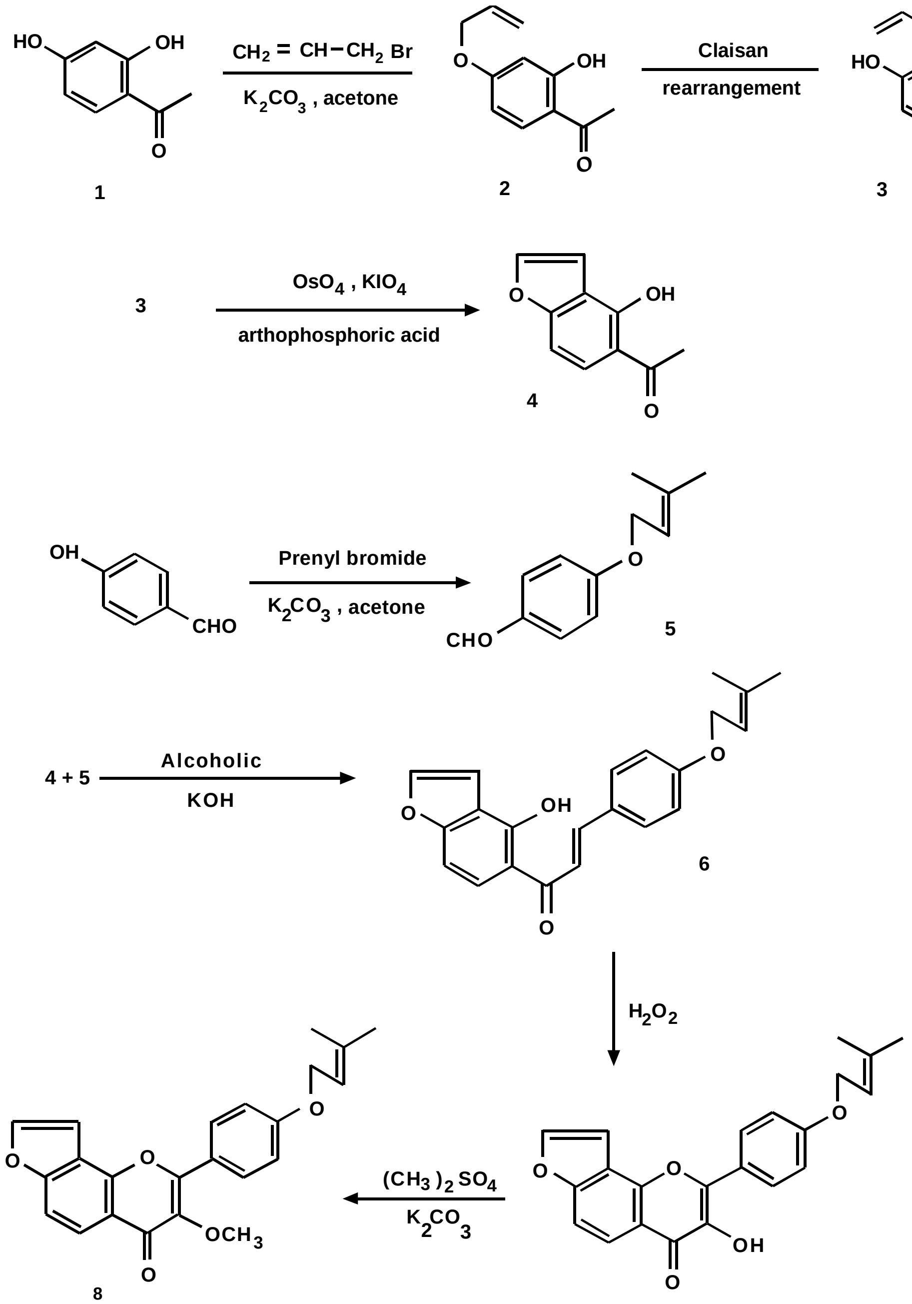
*4-O-Prenyloxybenzaldehyde (**5**).* A solution of p-hydroxybenzaldehyde (10 g) in acetone (50 ml) was refluxed with prenyl bromide (12.5 g) and anhydrous potassium carbonate (30 g) for 4 h. Acetone was distilled off and water was added to the residue. It was extracted with ether and ether solution was then extracted with 5% aq. NaOH. Aq. NaOH extract was

---

*Author for correspondence

acidified and extracted with ether. Ether extract on column chromatography with petroleum spirit gave an oily liquid which on cooling gave colourless needles (6 g), m.p. 61°C; (M^+, 190); IR **:** 2980, 1640, 1500, 1375, 1330, 1250, 1190, 1130, 1065, 1000, 800, 605 cm^{-1}; ^{1}H-NMR : 1.72 [s, 6H, $>C(CH_3)_2$], 4.48 (d, 2H, J = 7 Hz, $-O-C\underline{H}_2-CH=$), 5.43 (t, 1H, $-O-CH_2-C\underline{H}=$), 6.73 (d, 2H, J = 9 Hz, H-3 and 5), 7.55 (d, 1H, J = 9 Hz, H-2 and H-6), 9.40 (s, 1H, - CHO); [Anal. Calc. for $C_{12}H_{14}O_2$: C, 75.7 ; H, 7.4 . Found : C, 75.9; H, 7.5%].

*2'-Hydroxy-4-prenyloxy-furano(2",3":4',3') chalcone (**6**).* A mixture of 2-hydroxy-furano(2',3':4,3) acetophenone (**4**, 1 g) and 4-prenyloxybenzaldehyde (**5**, 0.824 g) in ethanolic solution of KOH (50%, 10 ml) was kept at room temperature for about 75 h. The reaction mixture was diluted with ice-cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. It was crystallized from benzene-petroleum spirit as yellow needles (400 mg), m.p. 102-104°C; (M^+, 348); R_f 0.64 (benzene-acetone-ethyl acetate ; 4:9:1); UV: 250, 275, 320 ; IR : 3450, 1645, 1600, 1590, 1470, 1420, 1375, 1325; ^{1}H-NMR : 1.74 [s, 6H, $>C(CH_3)_2$], 4.42 (d, 2H, J = 7 Hz, $-O-C\underline{H}_2-CH=$), 5.51(t, 1H, $-O-CH_2-C\underline{H}=$), 6.79



Scheme 1

(d, 2H, J = 9 Hz, H-3 and 5), 6.99 (d, 2H, J = 9Hz, H-5' and H-6'), 7.18 (d, 1H, J = 2 Hz, H-4"), 7.43 (d, 1H, J = 9Hz, H-α), 7.58 (d, 1H, J = 9 Hz, H-2 and H - 6), 7.81 (d, 1H, J = 2Hz, H-5"), 8.03 (d, 1H, J = 9 Hz, Hβ), 12.71 (s, 1H, - OH); [Anal. Calc. for $C_{22}H_{20}O_4$: C, 75.8 ; H, 5.7 .Found: C, 75.9 ; H, 5.8%].

3-Hydroxy-4'-prenyloxy-furano(2",3":7,8) flavone (7). To the above hydroxychalcone (**6**, 1 g) in pyridine (10 ml) and NaOH (20%, in 20 ml) kept at 60 - 70°C. H_2O_2 (30%, 30 ml) was added with stirring during 15 min. The reaction mixture was acidified 20 min and the solid that separated was filtered. The solid was dissolved in benzene and crystallised from petroleum ether as yellow needles (0.34g) , m.p. 124-127°C ; (M^+, 362) ; R_f 0.74 (benzene-acetone-n-hexane: 4:3:1) ; UV: 225, 255, 355 ; IR : 3470, 2980, 2875, 1643, 1595, 1510, 1472, 1375, 1365 cm^{-1} ; ^{1}H-NMR: 1.69 [s, 6H, $>C(CH_3)_2$], 4.44 (d, 2H, J = 7 Hz, - O - C$\underline{H}_2$- CH =), 5.54 (t, 1H, - O- CH_2- C$\underline{H}$=), 6.72 (d, 2H, J = 9 Hz, H-3 and 5), 6.95 (d, 2H, J = 9Hz, H-5' and H-6'), 7.12 (d, 1H, J = 2 Hz, H-4"), 7.58 (d, 1H, J = 9 Hz, H-2 and H-6), 7.81 (d, 1H, J = 2 Hz, H-5")**,** 13.21 (s, 1H, - OH); [Anal. Calc. for $C_{22}H_{18}O_5$: C, 72.9 ; H, 4.9 .Found: C, 72.5 ; H, 4.5%].

3-Methoxy-4'-prenyloxy-furano(2",3":7,8) flavone (8). A mixture of **7** (1.40g), dimethyl sulphate (0.228g) and anhydrous K_2CO_3 (10g) in acetone (25 ml) was refluxed for 2 h. Acetone was removed by distillation, water was added to the residue and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The product purified by preparative TLC over silica gel GF_{254} using methanol-chloroform (10:1) as developing solvent. It was crystallized from methanol to give yellow crystals (0.68g), m.p 147 - 149°C ; R_f 0.66 (methanol-chloroform; 10: 1), (M^+, 376), UV : 232, 255, 364; IR : 1645, 1605, 1590, 1470, 1372, 1365, 1147 cm^{-1}; ^{1}HNMR : 1.71 [s, 6H, $>C(CH_3)_2$], 3.98 (s, 3H, - OCH_3), 4.41 (d, 2H, J = 7 Hz, - O- C$\underline{H}_2$- CH =), 5.55(t, 1H, - O- CH_2- C$\underline{H}$ =), 6.73 (d, 2H, J = 9 Hz, H-3 and 5), 6.93 (d, 2H, J = 9Hz, H-5' and H-6'), 7.15 (d, 1H, J = 2 Hz, H-4"), 7.59 (d, 1H, J = 9 Hz, H-2 and H-6), 7.84 (d, 1H, J = 2Hz, H-5"). [Anal. Calc. for $C_{23}H_{20}O_5$: C, 73.4; H, 5.3 .Found: C, 73.6 ; H, 5.5%].

The compounds **1** (β-resacetophenone), **2** (4-O-allylresacetophenone), **3** (3-C-allylresacetophenone) and **4** (2-hydroxyfurano (2', 3':4,3) have been prepared by following literature procedures (Clarke 1955; Rangaswaqmi *et a1* 1954; Baker and Lothin 1935; Niak *et al* 1975). The formation of these products has been confirmed by comparing their melting points with the reported values (Clarke 1955; Rangaswaqmi *et a1* 1954; Baker and Lothin 1935; Niak *et al* 1975). p-Hydroxybenzaldehyde on treatment with prenyl bromide in the presence of K_2CO_3 and acetone gave 4-O-prenyloxybenzaldehyde **5**. The formation of which was ascertained by spectral studies. IR spectrum of **5** showed 1640 cm^{-1} indicating the presence of keto group in conjugation. The compound **4** on cross-aldol condensation with **5** afforded the compound **6** after dehydration of the initial product. The IR spectrum of compound **6** showed absorption frequencies at 3450, 1645 cm^{-1} indicating the presence of a hydroxyl, a conjugated carbonyl groups and the absorption peaks at 1600 and 1590 cm^{-1} .This indicated the presence of unsymmetric ethylenic double bond and aromatic rings respectively. The singlet for methyl protons of acetyl group disappeared while two new doublets at δ7.43 and 8.03 appeared showing the presence of two vinylic protons (α and β protons). The elemental analysis for C and H showed satisfactory results (within + 0.4%). The cyclized product **7** was obtained by H_2O_2/pyridine/NaOH treatment of its precusor **6**. The formation of **7** was confirmed by comparing its spectral data and elemental analysis. IR spectra of compound **7** showed 3470 cm^{-1} (phenolic - OH), 1643 cm^{-1} (C=O) and 1595 cm^{-1} (double bond/ aromatic ring). In the ^{1}H-NMR spectrum two doublets at δ7.43 and 8.03 for vinylic protons disappeared. The title compound **8** was finally obtained by methylation of its precursor.

Acknowledgement

Authors are grateful to Dr. J. Palige, Department Number-6, Institute of Nuclear Chemistry and Technology, Warsaw, Poland for ^{1}H-NMR, mass and elemental analyses.

***Key words*:** Synthesis, Chalcone, Flavone

References

Baker W, Lothin O M 1935 Flavonoids and phenolic glycoside from *Salvin officinalis. J Chem Soc* **7** 628 - 631.

Clarke H T 1955 *Organic Synthesis*, Collective Vol.III, 761.

Hodek P, Trefil P, Stiborova M 2002 Flavonoids - potent and versatile biologically active compounds interacting with cytochromes P450 *Chemmico - Biological Interactions* **139** 1 - 21.

Naik H B, Mankiwala S C, Ankiwala, Thakor V M 1975 Attempted synthesis of 3,3'- linked flavonoids. *J Indian Chem Soc*, **18** 52 - 54.

Rangaswaqmi S, Narayanaswami S, Sesshadri T R 1954 A new flavonoids coumarins from *Murraya exotica* L. *J Chem Soc*, **26** 1871 - 1874.

Biological Sciences

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 439 - 442*

VARIATION OF HEAVY METAL CONCENTRATIONS IN WATER AND FRESHWATER FISH IN NIGER DELTA WATERS - A CASE STUDY OF BENIN RIVER

M Okuo James* and P O Okolo

Department of Chemistry, University of Benin, Benin City, Nigeria

(Received January 3, 2002; accepted February 25, 2003)

Levels of Cd, Cr, Fe, Pb and Zn were determined in water and fish samples from three different locations in the Benin river. The sampling points were chosen such that Gbokoda, a village between Koko and Ogheye where a flow station (Olague flow station or crude oil well) is situated serves as a pollution point source and Koko as a baseline concentration point. Three species of fish each, that are top feeder, *Tilapia mariae* (which is herbivorous and feeds mainly on floating phytoplankton), middle feeder, *Pseudotolithus elongatus* (that is ominivorous) and bottom feeder, *Chrysichthys nigrodigitatus* (also ominivorous) were used for the study. The mean wet weight of the species sampled at the different locations ranged between 385.17 - 417.44g. The maximum concentration levels observed in water samples for Cd, Cr, Fe, Pb and Zn were 3.50×10^{-4} g/l, 1.24×10^{-3} g/l, 3.10×10^{-3} g/l and 1.50×10^{-3} g/l, respectively. The mean concentration levels determined for the various species of fish are: for Cd, *Tilapia mariae* 7.30×10^{-5}, *Pseudotolitus elongatus* 8.67×10^{-4} and *Chrysichthys nigrodigitatus* 1.581×10^{-4}, for Fe, *Tilapia mariae* 5.500×10^{-3}, *Pseudotolithus elongatus* 4.700×10^{-3} and *Chrysichthys nigrodigitatus* 3.9133×10^{-3}, for Pb, *Tilapia mariae* 4.4240×10^{-3}, *Pseudotolithus elongatus* 3.4100×10^{-3} and *Chrysichthys nigrodigitatus* 9.6730×10^{-3} for Zn, *Tilapia mariae* 5.467×10^{-3}, *Pseudotolithus elongatus* 5.067×10^{-3} and *Chrysichthys nigrodigitatus* 8.833×10^{-3}. (All values are g/g of fish)

Key words: Heavy metal, Fresh water fish, Benin river, Herbivorous, Omnivorous.

Introduction

Heavy metals have water bodies in both natural and anthropogenic origin and they will cause long-term damage to the aquatic environment.

The levels of heavy metals on freshwater fish and aquatic organisms reported by (Comparetto and Jester 1981; Hart 1982; Luoma 1983; Ndiokwere 1983). The concentration of these heavy metals in an organism's environment and its rate of ingestion and excretion. The concentration of harmful substances especially hydrophobic compounds are higher in sediments and biological tissues than in water itself (Florence and Batley 1980). It is likely that some of these hydrophobic compounds can form chelates with heavy metals.

Many aquatic organisms are able to concentrate these metals to a high level which become hazardous to health. Preston *et al* (1972), suggested that some aquatic organism may provide a useful means of monitoring elemental concentration in surface waters and their impact on the aquatic environment. One objective of this study is to determine the concentration of some heavy metal Cd, Cr, Fe, Pb and Zn in water and fish samples from the Benin river. A second objective is to determine the concentration of these heavy metals at different depths using three species that are top, middle and bottom feeders.

The Benin river runs through an area of dense oil activities including exploration and drilling of crude oil by Chevron, Shell companies, Nigeria Limited. These heavy metals are known to be associated with oil-drilling operations and several oil spills resulting from these activities have been reported in this area of study. Effluent from these activities is discharged either directly into the river on into creeks which drain into the river. The Benin river finally runs into the Atlantic Ocean at Ogheye a distance of 42km from Koko, one of the sampling station.

Through the liver of fish is known to concentrate more metals than any other part (De Goeji *et al* 1974). We chose to focus on muscle tissue which is highly consumed by man. Heavy metals are known to be deleterious to humans, therefore, man is exposed to a health hazard when large quantities of contaminated fishes are consumed.

Method. Sample collection and preparation: The fish and water samples were collected from three sampling locations on Benin river *viz* Ogheye, Gbokoda and Koko. The water sampling was done twice a month for a period of 6 months (Fig 1).

The water samples were collected using 'grab sampling method' (APHA 1985). The samples were stored in 2.5 litre plastic containers which were previously washed with 2% v/v HNO_3 and rinsed thoroughly with distilled water. A two

---

* Author for correspondence

litre sample was collected at each point and immediately preserved with conc. HNO_3 at about 1.5ml/l. The fish species were identified by their scientific names with the help of Zoology Department, University of Benin, Benin City (Table 1). The fish tissues were neatly cut out using a clean dissecting stainless steel knife and forceps and sealed in small polyethylene bags, which had been previously rinsed with 1M HNO_3 and distilled water. Care was taken to prevent contamination by cleaning the dissecting tools thoroughly after each use. The tissues were then placed in a watch glass and dried at 105˚C to constant weight. About 5g (dry weight) each of the fish samples were accurately weighed into a digestion flask. A mixture of concentrated HNO_3 and $HClO_4$ (2:1) was added and heated to dryness. The resultant residue was dissolved in 10ml (1:1) H_2SO_4 and diluted to 100ml with distilled water. The solution was used for heavy metal analyses using a Hitachi 180 - 170 Zeeman Atomic Absorption Spectrophotometer. All the chemicals and reagents used were of analytical grade.

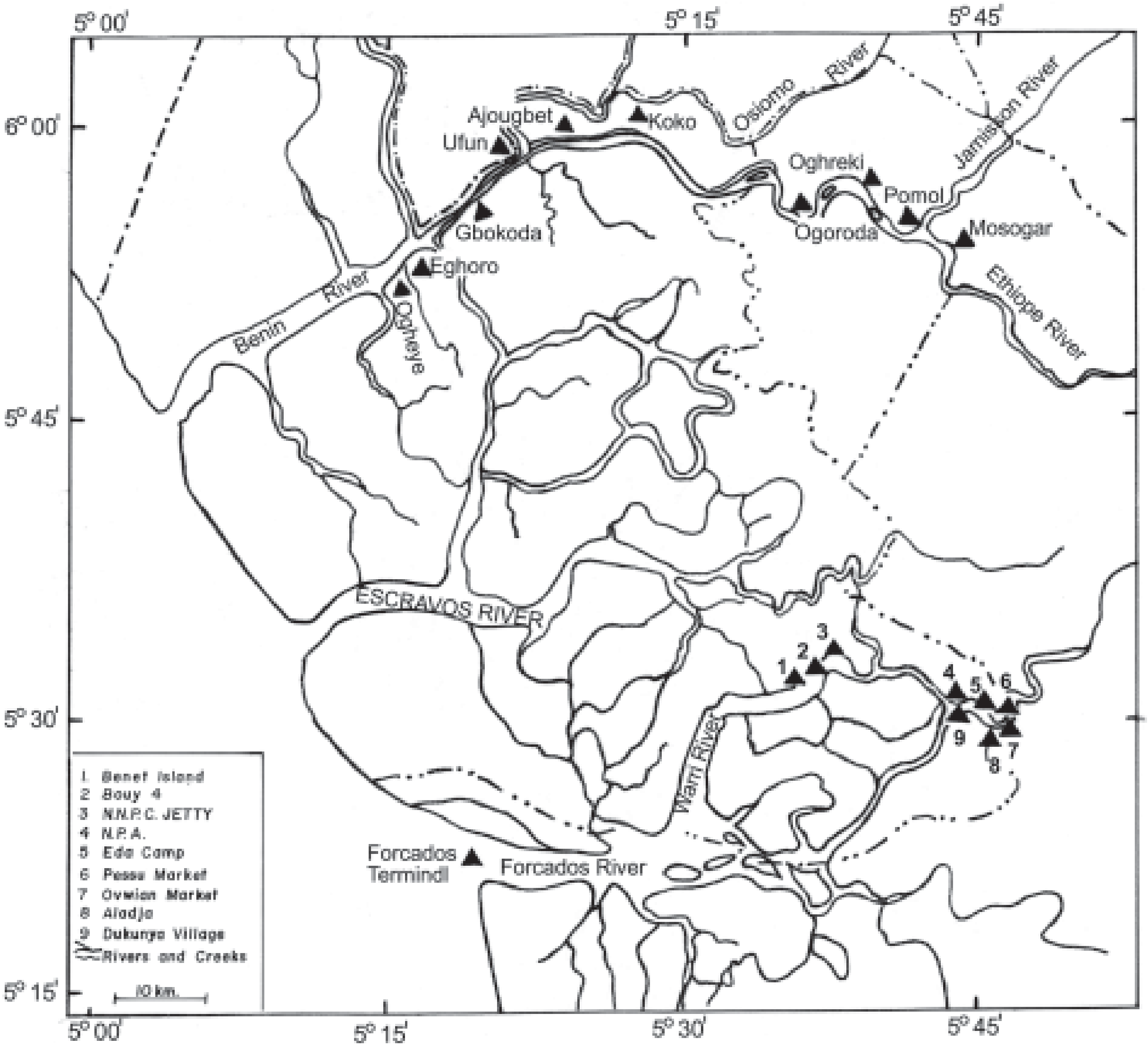
Table 1

Fish samples collected for analysis.

Sampling point	Scientific name	Number of samples
Koko	*Tilpia mariae* (Top feeder)	12 samples for each species
Gbokoda	*Pseudotolithus elongatus* (middle feeder)	
Ogheye	*Chrysichthys nigrodigitatus* (bottom feeder)	

Results and Discussion

The concentration levels of heavy metals in water samples at different locations is presented in Table 2, while the mean



A map showing Benin river and the sampling locations

Table 2

Mean levels of heavy metal concentration in water samples at different locations

Location	Number of samples (n)	Parameters g/l				
		Cd	Cr	Fe	Pb	Zn
Koko	12	$2.00 \times 10^{-5} \pm 5.00 \times 10^{-6}$	BDL	BDL	$1.00 \times 10^{-4} \pm 2.00 \times 10^{-5}$	BDL
Gbokoda	12	$3.50 \times 10^{-4} \pm 1.10 \times 10^{-4}$	$5.00 \times 10^{-4} \pm 3.00 \times 10^{-5}$	$3.10 \times 10^{-5} \pm 6.00 \times 10^{-4}$	$6.30 \times 10^{-4} \pm 2.40 \times 10^{-5}$	$1.51 \times 10^{-4} \pm 7.00 \times 10^{-5}$
Ogheye	12	$1.20 \times 10^{-4} \pm 2.00 \times 10^{-5}$	$1.24 \times 10^{-4} \pm 1.10 \times 10^{-5}$	$7.40 \times 10^{-4} \pm 2.00 \times 10^{-6}$	$6.20 \times 10^{-4} \pm 5.50 \times 10^{-5}$	$6.90 \times 10^{-4} \pm 4.10 \times 10^{-5}$

BDL, Below detection limit of instrument

Table 3

Mean levels of heavy metals in the three species of fish at different sampling location.

Location	Species of fish	Parameters x 10^{-4} g/g of fish				
		Cd	Cr	Fe	Pb	Zn
Koko	*Tilapia mariae*	BDL	BDL	2.920	0.032	BDL
	Pseudotolithus elongatus	0.140	BDL	2.220	0.023	
	Chrysichthys nigrodigitatus	0.040	BDL	BDL	0.320	
Gbokoda	*Tilapia mariae*	0.040	BDL	4.420	0.880	0.500
	Pseudotolithus elongatus	0.140		3.620	0.640	0.630
	Chrysichthys nigrodigitatus	0.160		2.840	0.880	0.630
Ogheye	*Tilapia mariae*	0.180	BDL	9.160	0.360	1.140
	Pseudotolithus elongatus	0.120		8.770	0.360	0.890
	Chrysichthys nigrodigitatus	0.310		8.900	0.390	2.020

Table 4

Mean values of heavy metals in the three species of fish.

Species	Parameters x 10^{-3} g/g fish			
	Cd	Fe	Pb	Zn
Tilapia mariae	0.0733 ± 0.0950	5.5000 ± 2.3690	0.4240 ± 0.4276	0.5467 ± 0.5714
Pseudotolighus elongatus	0.0867 ± 0.0757	4.8700 ± 4.3820	0.3410 ± 0.4904	0.5067 ± 0.4577
Chrysichthys nigrodigitatus	0.1581 ± 0.1528	3.9130 ± 4.5461	0.3673 ± 0.4340	0.8833 ± 4.1891

values of the heavy metals in the three species of fish at each location and the entire body of the river are presented in Tables 3 and 4.

The range of concentrations found in water samples are: $2.00 \times 10^{-5} - 3.50 \times 10^{-4}$ g/l, Cd, $5.00 \times 10^{-5} - 1.24 \times 10^{-3}$ g/l, Cr, $7.40 \times 10^{-4} - 3.10 \times 10^{-3}$ g/l, Fe, $1.00 \times 10^{-4} - 6.00 \times 10^{-4}$ g/l, Zn. This showed that heavy metals were present in considerable amounts. This is so because of the discharge of heavy metals in the environment from industry which has been increased by human activities and urban storm water discharge. Effluents from a petroleum refinery sited on the surrounding ecosystem of the river are known to contain among other heavy metals, lead, cadmium and chromium (Ndiokwere 1983). Also, plywood and timber (saw mill industry) is sited along the course of the river. Copper-chromium arsenate is used as a timber preservative by timber and saw mill industries to prevent fungal attack (Hunton and Symon 1986). All these might contribute to the level of Cd, Cr, and Pb found in the water samples.

The concentration of all the heavy metals determined were highest at Gbokoda (Table 2), a Sampling location where Olague crude oil well is situated and this provides an indication of the difference between baseline point and pollution source. This probably suggest Gbokoda as the pollution point and pollution source. This probably suggest Gbokoda as the pollution point of the river.

There are differences in the bio-concentration of these metals by the different species of fish. The *Chrysichthys* *nigrodigitatus* specie, the bottom feeder tends to bio-accumulate more of Cd and Zn with concentrations of 1.58 x 10^{-4} g and 8.83 x 10^{-4} g/g of fish, respectively. The highest concentration of 5.50 x 10^{-3} g/g and 4.240 x 10^{-5} g/g of fish for Fe and Pb, respectively were determined for *Tilapia mariae*, the top feeder. The high concentrations may be as a result of exposure to, and feeding in contaminated fresh water sediments. Other human activities such as the washing of clothes and motor vehicles at various sites on the bank of this river, can possibly contribute to its pollution by heavy metals. Cr was not detected in any of the fish samples. The concentration of Cr in the fish samples might be below the detection limits of 0.005 μg of the Hitachi 180 - 170 Zeeman Atomic Absorption Spectrophotometer.

The average size of the fish samples from the different locations were approximately the same. The degree to which the differences in the fish sizes influence the bio-accumulative behaviour of the fish species cannot be correlated with the difference in the heavy metal concentration levels, though not investigated. The high levels of heavy metals determined in all the fish samples might be due to local contamination of the river.

Conclusion

Conclusively, the bio-concentration of heavy metals in biota such as fish is an indicator of the pollution of water bodies by heavy metals. This is apparent in the elevated levels of metals observed in the fish samples than that obtained for the water samples. The heavy metals pollutant levels in the fish samples were in the decreasing order Fe > Zn > Pb and Cd.

Acknowledgement

The authors are grateful to the Department of Chemistry, University of Benin, Benin City, for funding this research work. We also acknowledge Mrs Ukwade, P.O., for putting this work in print.

References

APHA, AWWA, WPCF 1985 *Standard Methods for the Examination of Water and Waste Water*. Published by American Public Health Association, 1015, Fifteenth Street NW, Washington DC, USA, 20005, 16th ed, P 17.

Comparetto G M, Jester W A 1981 Arsenic activation, analysis of fresh water fish through the precipitation of elemental arsenic. Abstract presented at *The Int. Conf. On Modern Trends in Activation Analysis*, 6th, Toronto, Canada, 9 - 13 June.

De-Goeji J J M, Guinn V P, Young D B, Mearns A J 1974 *Neutron Activation Analysis - Trace Element Studies of Dover Sole Liver and Marine Sediments. In comparative Studies of Food and Environmental Contamination*, Vienna, Austria, IAEA, SM - 275/15, pp 189 - 200.

Florence T M, Bately G E 1980 Chemical, speciation of natural waters. CRC. *Critical Reviews of Analytical Chemistry* **9** 219 - 296.

Hart B T 1982 *Australian Water Quality Criteria for Heavy Metals, Australian Water Resources Council Technical Paper No **79***. Australian Government Publishing Service.

Hutton M, Symon C 1986 The quantities of cadmium, lead, mercury and arsenic entering the U.K. environment from human activities. *The Science of the Total Environment* **57** 129 - 150.

Luoma S N 1983 *Bio-availability of Trace Metals to Aquatic Organism - a Review of the Science of the Total Environment.* **28** 1 - 22.

Ndiokwere C L 1983 Arsenic, gold and mercury concentration levels in freshwater fish by neutron activation analysis. *Environmental Pollution (Series B)* **6** 263 - 269.

Preston A, Jefferies D F, Dutton J W R, Harvey B R, Steele A K 1972 British Isles Coastal Waters - The concentrations of selected heavy metals in sea water, suspended matter and biological indicators pilot survey. *Environmental Pollution,* **3** 69 - 82.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 443 - 446*

STABILITY OF RUST RESISTANCE AND YIELD POTENTIAL OF SOME ICARDA BREAD WHEAT LINES IN PAKISTAN

Syed Jawad Ahmad Shah [a]****, A J Khan*** [a]***, F Azam*** [a]***, J I Mirza*** [b] ***and Atiq ur Rehman*** [b]

[a]*Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan*

[b]*Crop Diseases Research Institute (CDRI), National Agricultural Research Center, Islamabad, Pakistan*

(Received January 17, 2002; accepted March 24, 2003)

Thirty bread wheat lines resistant to Yellow rust (Yr) were selected after careful screening from two ICARDA nurseries during 1998 - 1999, Rabi season at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar under severe disease pressure. In the following crop cycle, these selections were again field evaluated for stability and effectiveness of Yr resistance at multilocations while their yield potential was ascertained at Tarnab in two different trials with Tatara as commercial check. Results revealed that uniformity was found in the potential behavior of 23 lines (77%) in both the cropping seasons against Yr. This included some high yielding (up to 7067 kg / ha) and low yielding lines (up to 4333 kg / ha) when compared with the check (6089 kg / ha). Yield potential of some high yielding lines with stable Yr resistance should be further evaluated over sites and seasons for wide adaptability, under national uniform testing in order to select and deploy future varieties to combat Yr for acquiring food security in Pakistan.

Key words: Yellow rust, Bread wheat, Yield potential.

Introduction

Large-scale cultivation of bread wheat varieties with genetic uniformity of rust resistance was one of the major causes of 1994 - 1995 Yr epidemic in northern Pakistan, where losses were up to 40% (Saari *et al* 1995). Inqilab-91 was swiftly spread throughout Pakistan after the defeat of Yellow rust resistance gene Yr 9 in Pirsabak - 85 and Pak - 81, which were extensively grown in the Northwest Frontier Province and barani areas of Punjab. At present, almost 80% of the area under wheat cultivation is occupied by this single variety, posing a high risk of crop loss due to change in races of Yr (Anonymous 2000). Therefore, a constant search for new and stable Yr resistance sources with high yield potential is imperative for the development of improved rust resistant cultivars. This paper reports two years results (1998 - 1999 and 1999 - 2000) of stability of Yr resistance in some selected wheat lines from ICARDA germplasm and their yield potential at Tarnab during 1999 - 2000.

Materials and Methods

Field experiments were conducted to select Yr resistant germplasm at NIFA during 1998 - 1999 from two ICARDA bread wheat nurseries, *viz,* Semi Arid Wheat Screening Nursery (SAWSN) and Wheat Observation Nursery for Drought (WON-D), which were composed of 174 and 91entries, respectively. In each nursery, every entry was planted in strips of small adjacent plots having 2 rows/plot of 2.5 m length and 0.3 m apart. A super susceptible wheat variety (Local White) was sown around each nursery as spreader and also to act as the adult plant susceptible check. Nurseries and spreader were inoculated two to three times in early March using prevailing Yr races obtained from CDRI, Murree. This was done after sunset using a turbo - air sprayer at growth stage 34 - 37 (Zadoks *et al* 1974). Rust severity and response data was recorded on flag leaves after flowering was almost complete and when Local White had severity more than 50%. Severity estimates were based on the Modified Cobb Scale (Paterson *et al* 1948), while host response to infection was scored according to (Singh 1993) and converted to Coefficient of Infection Scale developed by Stubbs *et al* (1986).

Stability of resistance in thirty Yr resistant sources selected during 1998 - 1999 were further filed and evaluated in the following crop cycle (1999 - 2000) at Rawalpindi, Islamabad, Chackwall, Nawshara and Peshawar in the CDRI National Wheat Disease Screening Nursery (NWDSN). Each entry was planted in a single 1m row, 0.3 m apart. Two rows of rust susceptible spreader consisting of Local White, Moroco and Sonora were planted around the nursery. In addition, a row of susceptible check (Local White) was also planted at the 5th and then every 25th subsequent row. Artificial rust inoculation and Yr data was recorded in the same way as mentioned above. Thirty selected lines were also evaluated for yield potential during 1999 - 2000 in two different trials of 15 selections each.

---

*Author for correspondence

These were laid out at NIFA in a Randomized Complete Block (RCB) design with three replications, with Tatara as check. Each entry was planted on 4.8 m^2 plot with 4 rows, 4 m long and 0.3 m apart. Both trials were sown on October 10, 1999 with seed rate of 100 kg / ha. Recommended doses of fertilizer were applied and normal agronomic practices were carried out during the growing season. Each entry was harvested at maturity and threshed separately to determine grain yield/plot, which was converted to kg/ha and analyzed statistically according to Gomez and Gomez (1984).

Results and Discussion

Recorded data with brief description are given below:

Stability of Yr resistance. Response of thirty ICARDA bread wheat lines along with susceptible check (Local White) to Yr during two crop cycles in Pakistan is presented in Table 1. During 1998-1999, 27 lines were found to be resistant, while the remaining three displayed moderate susceptibility to Yr. Coefficients of infection for these two classes were < 3 and < 9, respectively. Coefficients of infection values < 3 indicated

Table 1

Yellow rust response of some ICARDA bread wheat lines during two crop cycles in Pakistan

S.No.	Wheat Lines	Nursery number	Coefficient of infection (1998 - 1999)[a]	Average coefficient of infection (1999 - 2000)[b]	Terminal reaction [c]
1	BWL - 2001	SAWSN - 15	< 3	< 3	5MRMS
2	BWL - 2002	SAWSN - 18	< 3	< 3	TRMR
3	BWL - 2003	SAWSN - 23	< 3	< 3	TS
4	BWL - 2004	SAWSN - 25	< 5	< 3	TMSS
5	BWL - 2005	SAWSN - 29	< 5	< 3	TRMR
6	BWL - 2006	SAWSN - 62	< 3	< 5	20MSS
7	BWL - 2007	SAWSN - 64	< 3	< 3	TR
8	BWL - 2008	SAWSN - 72	< 9	< 3	5MRMS
9	BWL - 2009	SAWSN - 119	< 3	< 3	TS
10	BWL - 2010	SAWSN - 124	< 3	< 3	TR
11	BWL - 2011	SAWSN - 135	< 3	< 3	TR
12	BWL - 2012	SAWSN - 136	< 3	< 3	TR
13	BWL - 2013	SAWSN - 144	< 3	< 3	TMS
14	BWL - 2014	SAWSN - 157	< 3	< 5	20MS
15	BWL - 2015	SAWSN - 165	< 3	< 3	5MSS
16	Local White	Check	100	60	60S
17	BWL - 2016	WON-D - 1	< 3	< 8	40MSS
18	BWL - 2017	WON-D - 2	< 3	< 3	TR
19	BWL - 2018	WON-D - 9	< 3	< 3	TR
20	BWL - 2019	WON-D - 10	< 3	> 10	40S
21	BWL - 2020	WON-D - 11	< 3	< 3	TMSS
22	BWL - 2021	WON-D - 15	< 3	< 3	10S
23	BWL - 2022	WON-D - 19	< 3	< 3	TR
24	BWL - 2023	WON-D - 39	< 3	< 3	TR
25	BWL - 2024	WON-D - 43	< 3	< 3	TR
26	BWL - 2025	WON-D - 48	< 3	< 3	TR
27	BWL - 2026	WON-D - 64	< 3	< 3	TR
28	BWL - 2027	WON-D - 81	< 3	< 3	TMR
29	BWL - 2028	WON-D - 82	< 3	< 3	TR
30	BWL - 2029	WON-D - 87	< 3	< 3	TR
31	BWL - 2030	WON-D - 89	< 3	< 3	TR
32	Local White	Check	60	60	60S

[a], Based on NIFA Yr data; [b], Means of Coefficients of Infection values computed for five locations of Yr data in Pakistan; [c], Maximum Potential reaction during 1999 - 2000.

Table 2
Yield potential of Yr resistant lines selected from ICARDA germplasm in two experiments at NIFA during 1999 - 2000 rabi season

S.No.	Wheat lines	Grain yield (kg / ha)	Increase decrease over check (%)
		EXPERIMENT 1	
1	BWL - 2001	4889	(-) 17.91
2	BWL - 2002	5289	(-) 11.19
3	BWL - 2003	5422	(-) 8.96
4	BWL - 2004	5889	(-) 1.12
5	BWL - 2005	6444	(+) 8.19
6	BWL - 2006	6133	(+) 2.97
7	BWL - 2007	4333	(-) 27.24
8	BWL - 2008	6022	(+) 1.10
9	BWL - 2009	7067	(+) 18.53
10	BWL - 2010	5644	(-) 5.23
11	BWL - 2011	5222	(-) 12.32
12	BWL - 2012	5222	(-) 12.32
13	BWL - 2013	5600	(-) 5.97
14	BWL - 2014	5667	(-) 4.85
15	BWL - 2015	5444	(-) 8.59
16	Tatara	5956	-
	LSD (0.05)	1424.12	
		EXPERIMENT 2	
17	BWL - 2016	5778	(-) 7.13
18	BWL - 2017	5556	(-) 10.70
19	BWL - 2018	5111	(-) 17.85
20	BWL - 2019	5556	(-) 10.70
21	BWL - 2020	6267	(+) 0.72
22	BWL - 2021	5378	(-) 13.56
23	BWL - 2022	5333	(-) 14.28
24	BWL - 2023	6444	(+) 3.56
25	BWL - 2024	6178	(-) 0.70
26	BWL - 2025	4978	(-) 19.99
27	BWL - 2026	5200	(-) 16.42
28	BWL - 2027	5511	(-) 11.42
29	BWL - 2028	6444	(+) 3.56
30	BWL - 2029	5556	(-) 10.70
31	BWL - 2030	5333	(-) 14.28
32	Tatara	6222	-
	LSD (0.05)	1467.99	

that the genotypes possess adequate resistance, while < 10 showed partial susceptibility according to Saari and Wilcoxson (1974). In the multilocation screening which was carried out in the following season (1999 - 2000), number of resistant wheat lines was reduced to 26, three expressed moderate susceptibility, and for one genotype rust developed in an out of control fashion with ACI > 10, demonstrating high susceptibility. Inconsistency was recorded in the Coefficients of Infection values of seven genotypes in both years. During the 1998-1999 season, three lines (BWL - 2004, BWL - 2005 and BWL - 2008) had < 9 Coefficient of Infection which were reduced to < 3 in the following year. Similarly, Coefficients of Infection of four lines (BWL - 2006, BWL - 2014, BWL - 2016 and BWL - 2019) were < 3 in 1998 - 1999, but higher during 1999 - 2000, varying between < 5 to > 10. Based on the inconsistent performance of these seven genotypes to Yr, it was concluded that variability in the environment might be responsible, but this needs further study.

Out of the 30 selected genotypes, 23 were found stable for Yr resistance and produced similar resistant (ACI < 3) behavior in the multilocation screening when compared with their original response recorded during 1998 - 1999 (Table 1). In addition, the terminal Yr reaction of these 23 genotypes was desirable because rust severity was negligible to maximum of 5%,while the highest infection type was MR to MS. Furthermore, these results showed that the selection efficiency for rust resistant sources remained about 77%.

Assessment of yield potential. Yield potential of 30 Yr resistant wheat lines evaluated at Tarnab in two trials during 1999 - 2000 are presented along with commercial check (Tatara) in Table 2. Variability in yield potential was observed, but statistically no significant difference was found among the lines when compared with checks in their respective trials. However, seven lines (BWL - 2005, BWL - 2006, BWL - 2008, BWL - 2009, BWL - 2020, BWL - 2023 and BWL - 2028) produced up to 19% more grain yield than the commercial check. Twenty-three lines had lower yield than the check.

Out of the 23 Yr stable lines, only four (BWL - 2009, BWL - 2020, BWL - 2023 and BWL - 2028) produced higher grain yield than Tatara, which ranged from 45 - 1104 kg / ha (Table 2). Further evaluation of this material under national uniform testing for wide adaptability may result in to one or more Yr resistant cultivars. In addition, very useful material was found in this study although low yielding showed stability of resistance to Yr over sites/seasons and can be used as candidate sources of resistance by wheat breeding programs in Pakistan.

References

Anonymous 2000 *Report on National Wheat Disease Screening Nursery 1999-2000*. Crop Disease Research Institute, Pakistan Agricultural Research Council, Islamabad, Pakistan pp 42.

Gomez K A, Gomez A A 1984 *Statistical Procedure for Agri Re-*

search 2nd ed. John Willey & Sons, Inc. USA pp 188 - 192.

Peterson R E, Campbell A B, Hannah A E 1948 A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Can J Res, Sect. C.* **26** 496 - 500.

Saari E E, Wilcoxson R D 1974 Plant disease situation of high yielding dwarf wheat in Asia and Africa. *Annual Rev. of Phytopathology.* **12** 49 - 67.

Saari E E, Hashmi N I, Kisana N S 1995 *Wheat and Pakistan, an update* (Yr 95 doc.) pp 3.

Singh R P 1993 Resistance to leaf rust in 26 Mexican wheat cultivars. *Crop Sci* **33** 633 - 637.

Stubbs RW, Prescott M, Saari E E, Dubin H J 1986 *Cereal Diseases Methodology Manual.* Centro International de Mejoramieto de Maize Y Trigo (CIMMYT). pp 46.

Zadoks J C, Chang T T, Konzak C F 1974 A decimal code for the growth stages of cereals. *Weed Res* **14** 415 - 421.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 447 - 451*

LEAF PHENOLICS OF DIFFERENT VARIETIES OF TROPICAL RAPESEED AT VARIOUS GROWING STAGES

M Ashraf Chaudry*[*], *Nizakat Bibi, Amal Badshah, Misal Khan and Zahid Ali

Nuclear Institute for Food and Agriculture, Tarnab, G.T. Road Peshawar, Peshawar, Pakistan

(Received October 5, 2002; accepted May 19, 2003)

Three species of rapeseed viz RM-9-7 (*Brassica napus*), BM-1 (*Brassica juncea*) and peela raya (*Brassica carinata*) were grown using normal agronomic practices. The leaves of three species were harvested after 20,40,60,80,100 and 120 days of sowing for analysis of different polyphenols after extraction in water and methanol by spectrophotometric methods. The results revealed that maximum concentration of sinapine, total phenols, leucoanthocyanidine and procyanidine were highest after 80 days of sowing in all species except the leucoanthocyanidine content of BM-I and peela raya species where maximum concentration was recorded after 100 days of sowing. Concentrations of methanol extractable phenolics were higher than water extractable phenolics in all species. Maximum values for methanol soluble sinapine (0.243%), total phenols (0.203%), leucoanthocyanidine (0.812 Δ A 550/g) and procyanidine (Δ A 550/g) were found in RM-9-7, BM-1 and peela raya, respectively. It may be concluded that for optimum phenolics concentration in the extract, the leaves of these species should be harvested after 80 days.

Key words: Methanol extractable phenolics, Rapeseed leaves, Growing stages, Sinapine.

Introduction

Rapeseed is among world's most important oilseed crops and is used for the production of high quality edible oil and a feed grade meal. Rapeseed meal has reasonable amino acid but its utilization as a source of protein in human nutrition is limited due to the presence of glucosinolates, phenolic compounds, phytates and hull (Fereidoon & Naezk 1992). The role of these harmful compounds (anti-nutrients) as protective agents against fungi and other pathogens has been reviewed (Butler 1982). Mature grains of mold resistant sorghum cultivars have much higher concentration of flavan - 4 - ols than mold susceptible cultivars (Jambunathan *et al* 1986). The concentration of flavan - 4 - ols in mature sorghum seed should give an indication about the expected reaction of the sorghum cultivars to grain mold in field and could be a very important aid in screening cultivars for grain mold resistance or susceptibility (Jambunathan *et al* 1990). Studies have also shown the major role of phenolic compounds in defence mechanism of plant tissues in response to infections or injuries (Legrand 1983 & Manibhusharaura *et al* 1988). As certain phenolics are bitter tasting and have the ability to precipitate plant and animal proteins, they have been considered as defence compounds against animal predators and microbes (Butler *et al* 1982). Bird resistant sorghum contains condensed tannins (proanthocyanidine, oligomers of flavanols) that are thought to account for their bird repellent properties. The extracted tannin content and the bird repellency of the extracts change considerably during the process of seed maturation and reach a maximum early in the maturation process. The mature seed is usually reported to decline and the decrease in assayable proanthocyanidine of sorghum on maturation has been ascribed to increase polymerization (Bullard *et al* 1981). The decrease in astringency/phenolics is connected with polymerization leading to an increased proportion of higher molecules in fully ripened seeds/fruits (Sattar *et al* 1992). The objective of the present study was to determine the exact stage of maximum synthesis of different phenolics during the leaf-growth of rapeseed for extract preparation to be used as an insect repellent in future.

Materials and Methods

Three newly evolved species viz. RM-9-7 (*Brassica napus*), BM-1 (*Brassica juncea*) and peela raya *(Brassica carinata)* of rapeseed were sown at the experimental fields using the normal agronomic protocol. The leaves of each cultivar were harvested at 20, 40, 60, 80, 100 and 120 days after sowing for the estimation of various polyphenols. The leaves were washed to remove dust and dirt, and dried with tissue paper. For the estimation of the polyphenols (sinapine, total phenols, procyanidine and leucoanthocyanidine), the samples were extracted in water and methanol. An aqueous extract (1:10, w/v) was prepared by boiling ground sample with water (g / 10 ml) for 30 min. The extract was then filtered and the volume was made up to the required dilution with distilled water. For methanol extract (1:10, w/v) ground tissues were boiled with methanol for 10 min and after decanting off the supernatant liquid, the residue was re-extracted for four successive times

---

[*] Author for correspondence

Table 1

Changes in leaf sinapine content (%) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.206^{1}	0.203^{m}	0.195^{n}	0.201^{F}	0.274^{j}	0.242^{o}	0.329^{e}	0.282^{C}
40	0.221^{j}	0.216^{k}	0.203^{m}	0.213^{E}	0.285^{g}	0.251^{m}	0.356^{a}	0.297^{B}
60	0.268^{c}	0.220^{j}	0.246^{e}	0.245^{B}	0.314^{f}	0.248^{n}	0.274^{j}	0.279^{D}
80	0.291^{a}	0.252^{d}	0.270^{b}	0.271^{A}	0.351^{b}	0.335^{c}	0.332^{d}	0.339^{A}
100	0.246^{e}	0.235^{f}	0.232^{g}	0.238^{C}	0.124^{p}	0.278^{i}	0.280^{h}	0.227^{E}
120	0.223^{i}	0.229^{h}	0.235^{f}	0.229^{D}	0.099^{q}	0.262^{k}	0.258^{l}	0.207^{F}
Mean	0.243^{A}	0.226^{C}	0.230^{B}	-	0.241^{C}	0.269^{B}	0.305^{A}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different (P < 0.05).

and all fractions were combined to 100 ml with methanol (Sattar *et al* 1992). All the samples were analyzed for extractable total phenols using Folin-Ciocalteau-phenol reagent, which contains sodium molybdate and sodium tungstate 2.5% and 10%, respectively (Titto 1980). The sinapine and procyanidine content were assayed according to Blair and Reichert (1984). The concentration of sinapine in the methanol extracts was calculated using the formula C = A / EL, where C = concentration in mole/1, A = absorbance at 330 nm, E = extinction coefficient (21390) at 330 nm and L = path length of the spectroscopic cell. The sinapine content was determined by this procedure includes all sinapic acid esters plus free sinapic acid. Procyanidine was determined using HCl / formic acid (1:1) mixture as a complexing reagent. With a solvent of 1-butanol and concentrated HCl, anthocyanidine, formed from flavan - 4 - ols was measured at 550 nm (Dryer *et al* 1981), because flavan - 4 - ols are readily converted to anthocyanidine in acidic solvents at room temperature (Jambunathan *et al* 1986). On heating, the unstable anthocyanidine formed from flavan - 4 - ols are completely destroyed. However, under these conditions flavan - 3 - ol oligomers are converted into anthocyanidine, the resulting absorbance was measured at 550 nm (Subramanian *et al* 1983). The data were subjected to statistical analysis using analysis of variance and the least significant difference (LSD) computed. The means were separated using DMR Test (Steel & Torrie 1980).

Results and Discussion

The major polyphenol of rapeseed is sinapine which constitutes more than 98% of the total phenolic substances (Krygier *et al* 1982; Bibi *et al* 1991; Bibi *et al* 1993). The leaves of variety RM - 9 - 7 contained significantly more mean methanol extractable sinapine than the other two varieties except peela raya where the maximum sinapine contents reached after 40 days as shown in Table 1. The methanol extractable sinapine after 20 days of sowing ranged from 0.195 to 0.206%, which reached to a maximum level of 0.291, 0.252 and 0.270% in variety RM - 9 - 7, BM - 1 and peela raya, respectively after 80 days of sowing. The same trend was followed by water extractable sinapine in all the varieties. After 80 days, the sinapine content (methanol and water extractable) started decreasing and reached minimum after 120 days of sowing. The water extractable sinapine contents were generally more than methanol extractable fraction in all varieties. The interaction analysis showed significant effect (P < 0.05) of species and growing days on sinapine contents. Bibi *et al* (1991) found that the varieties and their fractions of tropical rapeseed varied in different phenolic contents in respect of extractants used. The sinapine content ranged from 0.590 to 0.820%, 0.640 to 0.950% and 0.220 to 0.500% in seed, cotyledons and hulls respectively in all varieties. Considerable changes in the assayable amount and bird repellency of tannin during sorghum seed maturation has been reported by Butler (1982). Maximum tannin content reaches in early maturation stages followed by a decline to different levels for different cultivars (Bullard *et al* 1981 & Sattar *et al* 1992).

Total phenol contents (both methanol and water-extractants) reached their maximum after 80 days of growth followed by decline that could be due to increase in polymerization at maturity stage as revealed in Table 2. Among varieties, the leaves of peela raya had a maximum mean total phenols (methanol extractable) followed by BM-1 (0.137%) and RM-9-7 (0.132%). Rapeseed leaves generally contained more methanol extractable total phenols than water extractable and the mean values of total phenols ranged from 0.132 to 0.203%

Table 2
Changes in leaf total phenol content (%) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.121^{cde}	0.126^{bcde}	0.239^{a}	0.162^{B}	0.021^{fg}	0.005^{g}	0.157^{bc}	0.061^{A}
40	0.153^{bcd}	0.159^{bcd}	0.269^{a}	0.194^{AB}	0.086^{de}	0.031^{efg}	0.188^{b}	0.101^{C}
60	0.161^{bcd}	0.180^{bc}	0.289^{a}	0.210^{A}	0.155^{bc}	0.105^{cd}	0.200^{b}	0.153^{B}
80	0.182^{b}	0.240^{a}	0.257^{a}	0.226^{A}	0.197^{b}	0.259^{a}	0.282^{a}	0.246^{A}
100	0.170^{bc}	0.092^{ef}	0.105^{def}	0.122^{C}	0.076^{def}	N.D	N.D	0.025^{E}
120	0.006^{g}	0.024^{g}	0.235^{fg}	0.030^{D}	N.D	N.D	N.D	-
Mean	0.132^{B}	0.137^{B}	0.203^{A}	-	0.089^{B}	0.067^{C}	0.138^{A}	-

ND; Not determined, 1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

Table 3
Changes in leaf leucoanthocyanidine (Δ A 550/g) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.400^{def}	0.360^{ef}	0.180^{g}	0.313	0.150^{gh}	0.380^{d}	0.500^{c}	0.343^{B}
40	0.410^{de}	0.390^{def}	0.227^{fg}	0.342	0.260^{ef}	0.860^{a}	0.600^{b}	0.573^{A}
60	0.440^{de}	1.020^{a}	0.573^{cd}	0.678	0.240^{efg}	0.240^{efg}	0.180^{fgh}	0.220^{C}
80	0.787^{b}	1.000^{a}	0.540^{cde}	0.776	0.300^{de}	0.200^{efgh}	0.100^{hi}	0.200^{C}
100	0.500^{de}	1.110^{a}	0.690^{bc}	0.767	0.220^{efg}	0.140^{gh}	0.140^{gh}	0.167^{D}
120	0.450^{de}	0.990^{a}	0.499^{de}	0.646	0.180^{fgh}	0.020^{i}	0.020^{i}	0.073^{E}
Mean	0.498^{B}	0.812^{A}	0.452^{B}	-	0.225^{B}	0.307^{A}	0.257^{B}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

and 0.067 to 0.138%, respectively. A significant increase in polyphenol content of germinated mungbean seeds after 120h has been attributed to fresh synthesis or polymerization of existing polyphenols or degradation of high molecular weight insoluble polymers into smaller molecular weight soluble polymers that give colour to the estimating reagent (Charlene *et al* 1985). The total phenol concentration in apples is reported to stay at relatively constant level during storage (Coseteng & Lee 1987), whereas sinapine, catechin, total phenols and leucoanthocyanidine in persimmon have been reported to change in concentration during storage (Bibi *et al* 2001) and solar drying (Chaudry *et al* 1998).

The leucoanthocyanidine were more extractable in methanol ranging from 0.400 to 0.787, 0.360 to 1.110 and 0.180 to 0.690 Δ A 550 /g in RM - 9 - 7. Peela raya and BM-1 leaves as compared to water extractable with values ranging from 0.150 to 0.300, 0.380 to 0.860 and 0.343 to 0.573 ΔA 550 /g for leaves of same varieties, respectively, during 120 days of growth period as reported in Table 3. The leucoanthocyanidine contents were maximum (0.79 Δ A 550 /g) after 80 days of sowing in RM - 9 - 7 leaves while for varieties peela raya and BM-1, the highest values of 1.110 and 0.690 Δ A 550 /g, respectively after 100 days of sowing. In case of water extractant, the leucoanthocyanidine content reached its maximum value of 0.3 Δ A 550 /g after 80 days of sowing for RM-9-7 leaves while for varieties peela raya and BM-1, the leucoanthocyanidine contents were maximum (0.86 and 0.60 Δ A 550 /g, respectively) after 40 days of sowing followed by decline during further growth. For all the three varieties, the methanol extractable procyanidine content were 0.025 - 2.570, 0.140 - 4.140 and 0.160 - 2.460 ΔA 550 /g as compared to water soluble procyanidine content with values of 0.060 - 0.620, 0.120 - 1.520 and 0.086 - 0.280 ΔA 550 /g for RM - 9- 7, peela raya and BM-1 leaves, respectively. In case

Table 4
Changes in leaf procyanidine (Δ A 550/g) in three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.02430^{hi}	0.01367^{i}	0.1567^{gh}	0.649	0.0583^{l}	0.1200^{jk}	0.0799^{kl}	0.086^{E}
40	0.1833^{hi}	0.2143^{fg}	0.3000^{f}	0.196	0.1220^{jk}	0.1843^{i}	0.1200^{jk}	0.142^{D}
60	2.520^{c}	3.687^{b}	2.227^{d}	2.811	0.0797^{kl}	0.5833^{f}	0.1600^{ij}	0.274^{C}
80	2.467^{c}	4.080^{a}	2.420^{c}	2.989	0.6200^{f}	1.100^{c}	0.2867^{h}	0.669^{B}
100	1.853^{e}	2.287^{d}	1.883^{e}	2.008	0.2100^{i}	1.523^{a}	0.7867^{d}	0.840^{A}
120	1.823^{e}	2.443^{c}	1.790^{e}	2.019	0.4000^{g}	1.341^{b}	0.7067^{e}	0.816^{A}
Mean	1.460^{B}	2.121^{A}	1.463^{B}	-	0.248^{C}	0.808^{A}	0.357^{B}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

of methanol soluble procyanidine content, the leaves of RM - 9 - 7 had maximum value of 2.57 ΔA 550 /g after 60 days of sowing, while leaves of peela raya and BM-1 showed maximum values of 4.14 and 2.46 (Δ A 550 /g), respectively after 80 days of sowing followed by decrease upto 120 days. The water soluble procyanidine content of RM-9-7 leaves showed maximum value of 0.62 Δ A 550 /g after 80 days of sowing and for leaves of peela raya and BM-1, the highest values were 1.52 and 0.80 Δ A 550 /g, respectively after 100 days of sowing followed by decrease in procyanidine content for all the leaves of the three varities due to some interconversion of the phenolic compounds during further growing period Table 4.

It has been reported that differences existed in the quantities of condensed tannins including procyanidine and simple phenols both as function of maturation and cultivar in peaches (Samuel and Callakan 1990). Deposition of these compounds began in the early stages of development in all cultivars evaluated and increased to maximize between the first and second swell in fruit growth (Amiot *et al* 1992). This is followed by diminution of phenolics during ripening and the greatest differences seen between cultivars were those related to oleuropin and verbascoside.

Conclusion

It can be concluded from the results that maximum phenolics content were found after 80 days. However, a generalization regarding the number of days to maximize phenolics contents of all kind and all applicable extractants and for all the species of rapeseed may not be possible as in some cases different phenolics compounds are maximum at different growth stages in different species and different extractants. The character seems to be genetically controlled and for each species it will have to be determined separately.

Acknowledgement

The authors are thankful to the Director, NIFA for providing facilities of the present research work.

References

Amiot M J, Taechini M, Aubext S, Nicolas J 1992 Phenolic composition and browning susceptibility of various apples cultivars at maturity. *J Food Sci* **57** 958 - 962.

Bibi N, Sattar A, Chaudry M A 1991 Phenolic constituents in major fractions of tropical rapeseed varieties. *Die Nahrang* **35** 1053 - 1059.

Bibi N, Sattar A, Chaudry M A 1993 Variation of phenolic compounds in rapeseed varieties grown at Peshawar. *Pak J Sci Ind Res* **36**(5) 195 - 198.

Bibi N, Chaudry M A, Khan F, Ali Z, Sattar A 2001 Phenolics and physicochemical characteristics of persimmon during post-harvest storage. *Nahrung* **45** 82 - 86.

Blair R, Reichert R D 1984 Carbohydrates and phenolic constituents in a comprehensive range of rapeseed and canola fractions. *J Sci Food Agric* **35** 29 - 35.

Bullard R W, York J O, Kilburu S R 1981 Polyphenolic changes in ripening bird resistant sorghum. *J Agric Food Chem* **29** 973.

Butler L G 1982 Relative degree of polymerization of sorghum tannin during seed development and maturation. *J Agric Food Chem* **30** 1090 - 1094.

Butler L J, Pearson A W, Fenwick G R 1982 Problems which limit the use of rapeseed meal as a protein source in poultry. *J Sci Food Agric* **33** 866 - 875.

Charlene F B, Antonio C L, Evelyn Mae F M 1985 Polyphenols in mungbean: determination and removal. *J Agric Food Chem* **33** 1006 - 1009.

Chaudry M A, Bibi N, Khan F, Sattar A 1998 Phenolics and quality of solar cabinet dried persimmon during storage. *Italian J Food Sci* **10** 269 - 274.

Coseteng M Y, Lee C Y 1987 Changes in polyphenol concentration in relation to degree of browning in apple. *J Food Sci* **52** 985 - 989.

Dryer D L, Reese J C, Jones K C 1981 Aphid feeding deterrents in sorghum bioassay isolation and characterization. *J Chem Ecol* **7** 273 - 284.

Fereidoon S, Naezk M 1992 An overview of the phenolics of canola and rapeseed. *JAOCS* **69** 917 - 924.

Jambunathan R, Butler L G, Bandyopalhyay R, Mughogho L K 1986 Polyphenol concentration in grain leaf and callus tissues of mold suspeptible and mold resistant sorghum cultivars. *J Agric Food Chem* **34** 425 - 429.

Jambunathan R, Milind S K, Ranajit B 1990 Flavan - 4 - ols concentration in mold susceptible and mold resistant sorghum cultivars at different stages of grain development. *Agric Food Chem* **38** 545 - 548.

Krygier K, Sosulki F, Hogge L 1982 Free esterified and insoluble-bound phenolic acid extraction and purification. *J Agri Food Chem* **30** 330 - 334.

Legrand M 1983 *Biochemical Plant Pathology*. Phenylpropanoid metabolism and its regulations in disease. J A Callow (Ed.). John Wiley & Sons Ltd. New York, USA, pp 367 - 371.

Manibhusharaura K, Zuber M, Matsuyama N 1988 Phenol metabolism and plant disease resistance. *Acta Phytopathologica Entomologica Hungarica* **23** 103 - 108.

Peterson M S, Johnson A U 1977 Tannin. In: *Encyclopedia of Food Science and Technology*, Academic Press, Westport, Connecticut, USA, Vol **3,** pp 732 - 734.

Samuel D S, Callakan A 1990 Variability in the quantities of condensed tannins and other major phenol, in peach fruit during maturation. *J Food Sci* **55** 1585 - 1587.

Sattar A, Bibi N, Chaudry M A 1992 Phenolic compounds in persimmon during maturation and on tree ripening. *Die Nahrung* **36**(5) 467 - 472.

Steel R G D, Torrie J H 1980 *Principles and Procedures of Statistics*. Mc Graw Hill. Book Co. Inc. New York, USA, pp 137.

Subramanian, V, Butler L B, Jambunathan R, Prasada-Rao K E 1983 Some agronomic and biochemical characters of brown sorghum and their possible role in bird resistance. *J Agric Food Chem* **31** 1303 - 1307.

Titto R J 1980 Methods for the analysis of certain phenolics *J. Agric Food Chem* **33** 213 - 217.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 452 - 456*

LEVELS OF CADMIUM, CHROMIUM AND LEAD IN DUMPSITES SOIL, EARTHWORM (*LYBRODRILUS VIOLACEOUS*), HOUSEFLY (*MUSCA DOMESTICA*) AND DRAGON FLY (*LIBELLULA LUCTOSA*)

***A A Adeniyi* [a]*, *A B Idowu* [b] *and O O Okedeyi* [a]**

[a]*Department of Chemical Sciences, Lagos State University, Ojo, PMB 1087, Apapa, Lagos, Nigeria*

[b]*Department of Biological Sciences, University of Agriculture, PMB 2240, Abeokuta, Nigeria*

(Received December 27, 2001; accepted June 28, 2003)

Chemical analyses of cadmium, chromium and lead in dumpsites soil, earthworm *(Lybrodrilus violaceous)*, housefly (*Musca domestica*) and in indigenous dragonfly (*Libellula luctosa*) were performed by atomic absorption spectrophotometry to estimate the degree of metal pollution in two Lagos dumpsites located at Iba Housing Estate (dumpsite A) and Soluos along LASU - Isheri road (dumpsite B). Soil pH and moisture content were also determined. Chromium was not detected (ND) in most of the samples except in the soil samples whose mean and standard deviation (SD) were 0.43 ± 0.37 µg/g and 0.23 ± 0.37 µg/g, respectively for dumpsites A and B, and the earthworm samples harvested from dumpsite B (1.00 ± 1.41 µg/g). The cadmium levels were 4.00 ± 3.16 µg/g and 7.50 ± 6.37 µg/g for earthworm; 2.86 ± 1.43 µg/g and 4.29 ± 3.74 µg/g for housefly, 0.75 ± 1.26 µg/g and 1.25 ± 0.95 µg/g for dragonfly, respectively for dumpsites A and B. However, the concentration of lead in the invertebrates were, 130.00 ± 112.58 µg/g and 105.75 ± 94.44 µg/g for earthworm; 145.71 ± 101.87 µg/g and 225.71± 79.31 µg/g for housefly; 165.00 ± 69.78 µg/g and 85.00 ± 69.73 µg/g for dragonfly respectively for dumpsites A and B. Cadmium and lead levels were found to be higher in the invertebrates harvested from the dumpsites than those collected from the non-dumpsites. The non-dumpsite values for cadmium were 1.24 ± 0.94 µg/g, 0.45 ± 0.56 µg/g and 0.38 ± 0.14 µg/g for earthworm, housefly and dragonfly, respectively. Similarly, the non-dumpsite lead levels for earthworm, housefly and dragonfly were 23.12 ± 10.11 µg/g, 20.75 ± 11.85 µg/g and 33.62 ± 14.95 µg/g, respectively.

Key words: Heavy metals, Pollution, Dumpsites, Cadmium, Chromium, Lead, Earthworm, Housefly, Dragonfly.

Introduction

All trace metals are natural constituents of soils and enter the food chain mainly through uptake from soils (Rain 1995; Freedman 1996; Jinadasa *et al* 1997; Starr and Taggart 1998).The dumping of waste on soils has been found to increase their heavy metals content (Harrop *et al* 1990; Adeniyi 1996; Spurgeon and Hopkin 1996). The disposal of wastes pose a major environmental problem in heavily populated cities, especially in developing countries (Main 1995). This result in urban pollution and unsanitary conditions (Alloway and Ayres 1994; Van der Watt *et al* 1997). Organisms habiting contaminated soils take up heavy metals (John and Morgan 1990; Khan and Weiss 1993; Garate *et al* 1993; Pize and Josen 1995; Dudka *et al* 1996; Abdul-Rida 1996; Krivolutsky 1996; Marinussen *et al* 1997; Nuortev and Elberg 1999).Heavy metals uptake by earthworms and other soil animals is known to be influenced by a number of factors such as soil metal concentration, soil pH, soil texture and soil organic matter as well as the balance between uptake and egestion by the organisms (Crawford *et al* 1996; Smolders *et al* 1998). Insects are also known to take up metals through feeding in a contaminated habitat (Peters 1988; Rain 1995). The use of invertebrates as indicators of heavy metals pollution is of interest in cities like Lagos where dumpsites are now in the heart of residential/ industrial areas. The documented adverse health effects of cadmium and lead have led to public concern over soil contamination with these metals (Naqvi and Howell 1993; Spurgeon and Hopkin 1996; Dudka *et al* 1996).

The objective of the study is to evaluate the levels of cadmium, chromium and lead in dumpsite soil, earthwortm, housefly and dragonfly. The out come is expected to provide baseline data that will assist the appropriate agencies in the formulation and enforcement of a sustainable environmental action plan for waste management in Nigeria's sprawling cities.

Materials and Methods

Sampling. Samples were collected randomly from two dumpsites (A and B). Dumpsite A is located at Iba Housing Estate, Ojo, while dumpsite B is located opposite Soluos Hotel

---

*Author for correspondence; E-mail: lekeadeniyi@yahoo.com

Table 1

Heavy metals in the soil and animals samples

Metals		Mean levels µg / g ± SD				$F_{(0.05)}$
		Soils	Earthworm	Housefly	Dragonfly	
Cd	*a)	1.01 ± 0.63	4.00 ± 3.16	2.86 ± 1.43	0.75 ± 1.26	0.92
	b)	0.61 ± 0.26	7.50 ± 6.37	4.29 ± 3.74	1.25 ± 0.95	1.92
	c)	0.21 ± 0.04	1.24 ± 0.94	0.45 ± 0.56	0.38 ± 0.14	4.09**
Cr	a)	0.43 ± 0.37	ND	ND	ND	2.48
	b)	0.23 ± 0.37	1.00 ± 1.41	ND	ND	3.13
	c)	ND	ND	ND	ND	-
Pb	a)	13.00 ± 6.30	130.00 ± 112.58	145.71 ± 101.87	165.00 ± 69.78	1.97
	b)	14.00 ± 13.56	105.75 ± 94.44	225.71 ± 79.31	85.00 ± 69.73	1.26
	c)	4.13 ± 2.35	23.12 ± 10.11	20.75 ± 11.85	33.62 ± 14.95	6.35*

*a, Dumpsite A; b, Dumpsite B; c, Control site. **, Significant difference between the metal levels in the soil and animal samples at $p < 0.05$; SD, Standard deviation; ND, Not detected.

along LASU - Isheri Road. While control samples were collected from a non-dumpsite (within the premises of Lagos State University, Ojo) far from the dumpsites. Samples of soil and animals were collected between July-October 1999 and July-October 2000. The wastes in dumpsite A are essentially domestic as the dumpsite is located within a residential estate, while that of dumpsite B are predominantly agricultural / industrial waste materials trucked to the site. In both sites, the wastes are burnt in continually smoldering fires that emitted foul smoke and gases leaving residual wastes.

The soil samples were collected from the two dumpsites and a non-dumpsite (control site) with the aid of locally made soil auger (screw down and pull) from the soil surface (0-15 cm, Adeniyi 1996). Earthworms *(Lybrodrilus violaceous)* were collected from the respective sites by digging the soil to about 15 cm depth and the soil hand-picked for worms. The collected earthworms were washed with distilled water and identified. Similarly, housefly *(Musca domestica)* and dragonfly *(Libellula luctosa)* were collected with the aid of a sweep net. After collection the insects were sorted out and identified using morphological structures. Only adult insects and matured earthworm with clitellium were used. The earthworm and insects were then dried in the oven between 50 - 60°C for four days (Garate *et al* 1993; Idowu 1994; Mackay *et al* 1997).

Instrumentation. The determination of the heavy metals were performed with the use of a Perkin Elmer and Oak Brown Atomic Absorption Spectrophotometer. The instrument's settings and operational conditions were done in accordance with the manufacturer's specifications. The instrument was calibrated with analytical grade standard solutions (1 mg/dm^3) in replicate.

Physico-chemical analysis. The animal samples were prepared for analysis by following the methods described by Nuorteva and Elberg (1999), Pize and Josen (1995) using 0.5g of dried sample in 10 ml conc. HNO_3.

The soil pH and moisture content were determined according to Adeniyi *et al* (1993). The heavy metals were extracted from the soil samples for analysis using 5 g of sieved air-dried samples with 2N HNO_3 (Adeniyi 1996; Abdul-Rida 1996).

Statistical analysis. ANOVA was used to estimate statistically significant levels of metals at 95% confidence level (Pentecost 1999).

Results and Discussion

Chromium was not detected (ND) in most of the samples (Table 1), earthworms harvested from dumpsite B had a chromium level of 1.00 ± 1.41 µg/g; soil samples had 0.43 ± 0.37 µg/g and 0.23 ± 0.37 µg/g for sites A and B, respectively (Fig 1). However, chromium was not detected in the housefly and dragonfly samples (dumpsites and non-dumpsite) and in the earthworms harvested from dumpsite A (Table 1).

This trend had been observed by earliers (Pize and Josen 1995; Adeniyi 1996; Marinussen 1997). These differences were however, statistically non-significant at 95% confidence level.

The cadmium and lead concentration (Table 1) in the earthworm, housefly and dragonfly samples were higher than in soils in both the dumpsites and control site. Fig 2 and 3 and may be taken as an indication of bio-accumulation of metals by the animals (Peters 1988; Khan and Weis 1993; Abdul-Rida 1996; Nuorteva and Elberg 1999). The contamination of these animals by cadmium and lead (Table 1) is of concern because

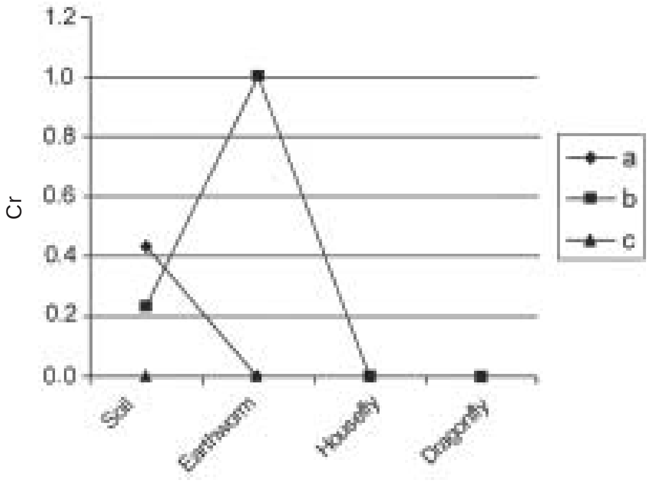


Fig 1. Concentration of chromium in the soil and animal samples collected from the dumpsites (A and B) and control site (C).

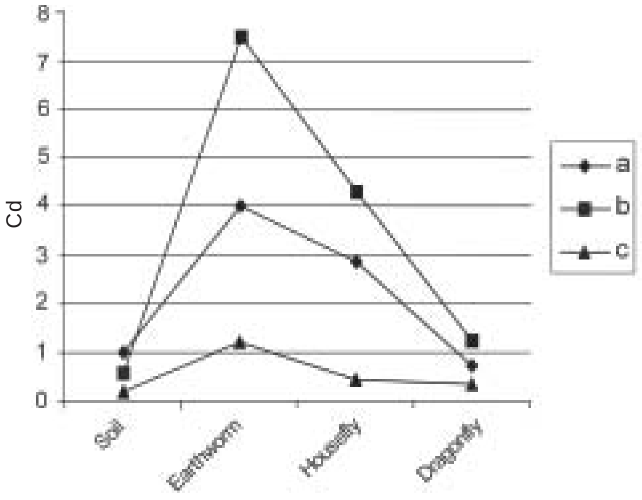


Fig 2. Concentration of cadmium in the soil and animal samples collected from the dumpsites (A and B) and control site (C).

they are important links in the complex food web (Spurgeon and Hopkin 1996; Starr and Taggart 1998).

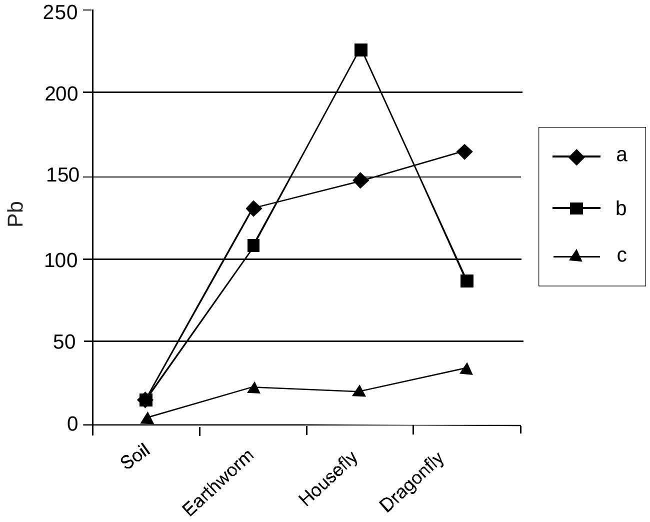


Fig 3. Concentration of Lead in the soil and animal samples collected from the dumpsites (A and B) and control site (C).

Table 2

Some characteristics of the sampled soils

Parameters	a	b	c
pH	8.94 ± 0.20	10.14 ± 0.20	7.21 ± 0.38
Moisture content (%)	35.15 ± 6.24	16.34 ± 4.23	44.47 ± 3.46
Texture*	SL	SL	SCL

SL, Sandy loam; SCL, sandy clay loam; a, dumpsite A; b, dumpsite B; c, non-dumpsite (Control site).

In dumpsite B (Table 1), where the waste materials were predominantly agricultural / industrial, the Pb concentration in the housefly samples was higher than for dumpsite A and control samples. Earlier studies have shown that cadmium and lead concentrations in earthworms, housefly and dragonfly varied widely depending on the nature of the sites (Marino *et al* 1995; Crawford *et al* 1996). This observation is equally true for the present study (Fig 2 and 3). It should be noted however, that the chromium, cadmium and lead levels observed for the non-dumpsite samples were generally lower than for the dumpsites values. The differences in the levels of chromium, cadmium and lead in the dumpsites A and B soil, and animal samples are non-significant at 95% confidence level. This is an indication that the metals detected in the soil and animal samples collected from the two dumpsites is point source (that is form the waste materials dumped in the respective sites). (Pize and Josen 1995; Freedman 1996; Krivolutsky 1996; Bagatto and Shorthouse 1996). The levels of these metals in the animals and soil samples are of concern since these metals are known to bioaccumulate as they journey through the environmental and biological reservoirs (Harrop *et al* 1990; Wang and Demshar 1992; Garate *et al* 1993; Rain 1993; Bartsh *et al* 1999). Moreover, these animals are important links in the complex food web (Peters 1988; Freedman 1996; Spurgeon and Hopkin 1996).

Table 2 shows characteristics of the sampled soils. The soil pH value of 8.94 ± 0.20, 10.14 ± 0.20 and 7.21 ± 0.38 for dumpsites A, B and control site, respectively agreed with the trend reported before for Lagos dumpsite soils (Adeniyi *et al* 1993; Adeniyi 1996). While the soil moisture content were 35.15 ± 6.24%, 16.34 ± 4.23% and 44.47 ± 3.46%, respectively for dumpsites A, B and control site. These values are expected for tropical soils with sandy loam (SL) and sandy clay loam (SCL) texture (Adeniyi and Oyedeji 2001).

References

Abdul-Rida M M A 1996 Concentration and growth of earthworms and plant in polluted (Cd, Cu, Fe, Pd and Zn) and non-polluted soils: Interactions between soil-earthworm. *Soil Biology and Biochemistry* **28** 1029 - 1035.

Adeniyi A A 1996 Determination of cadmium, copper, iron, lead, manganese and zinc in waterleaf *(Talinum triangulare)* in dumpsites. *Environmental International* **22** 259 - 262.

Adeniyi A A, Fashola J, Ekanem O A 1993 Comparative evaluation of heavy metals in Lagos dumpsites. *Nig J Rev Sci* **1** 65 - 69.

Adeniyi A A, Oyedeji O O 2001 Determination of Total Petroleum Hydrocarbons (TPH), organic carbon and heavy metals in soils adjoining to The National Arts Theatre water front and Abegede Creek Ijora, Lagos. *Fresenius Environmental Bulletin* **12** 873 - 876.

Alloway B J, Ayres D C 1994 *Chemical Principles of Environmental Pollution.* Chapman and Hall, Melbourne, Australia pp 16 - 42.

Bagatto G, Shorthouse J D 1996 Accumulation of Cu and Ni and successive stages of *Lymantria dispar* (L.) (Lymantriidae: Lepidoptera) near Ore Smelters at Sudbury, Ontario, Canada. *Environmental pollution* **92** 7 - 12.

Bartsch M R, Cope W G, Rada R G 1999 Effects of cadmium-spiked sediments on cadmium accumulation and bioturbation by nymphs of the burrowing mayfly *Hexagenia bilineata. Water Air, Soil-Pollution,* **190** 277 - 292.

Crawford L A, Lepp N W, Hodkindson I D 1996 Accumulation and Egestion of dietary copper and cadmium by the grasshopper *Locusta migratoria* R &F (Orthoptera: Acrididae). *Environmental Pollution* **92** 241 - 245.

Dudka S, Piotrowska M, Terelak H 1996 Transfer of cadmium, lead and zinc from Industrially contaminated soil to crop plants: A field study. *Environmental Pollution* **94** 181 - 188.

Freedman B 1996 *Environmental Ecology.* The impacts of pollution and other stresses on ecosystem structure and function, Academic Press, New York, USA, pp 54 - 62.

Garate A, Ramos I, Manzanares M, Lucena J J 1993 Cadmium uptake and distribution in three cultivars of *Lactuca* sp *Bull Environ Contam Toxicol* **50** 709 - 716.

Harrop D O, Mumby K, Pepper B, Nolan J 1990 Heavy metals levels in the near vicinity to roads in a North London Borough. *Sci Total Environ* **93** 543 - 546.

Idowu A B 1994 Structural and physiological studies on the repellant secretion of *Zonocerus variegatus* (L.) Ph.D thesis, University of Ibadan, Ibadan, Nigeria.

Jinadasa K B P N, Milham P J, Hawkins C A, Carnish P S, Williams P A, Kaldor C J, Conroy J P 1997 Survey of cadmium levels in vegetables and soils of greater Sydney, Australia. *J Environ Qual* **226** 924 - 933.

John E M, Morgan D J 1990 The distribution of Cd, Cu, Pb, Zn and Ca in the tissues of earthworms-*Ludricus vebellum*. *Oecologia* **84** 430 - 432.

Khan A T, Weis J A 1993 Bioaccumulation of heavy metals in two populations of Mummichog *(Fundus heteroclitus), Bull Environ Contam Toxicol* **51** 1 - 5.

Krivolutsky D A 1996 An intergrated bioindication system applied to soil pollution assessments: From earthworm to ecosystems. In: *Proceedings of the NATO Advanced Research Workshop on New Approaches to The Development of Bioindicator Systems for Soil Pollution.* (24 - 28 April 1993) Bouche M B Straalen N M (eds) Moscow, pp 141 - 153.

Mackay W P, Mena R, Gardea J, Pinagatore N 1997 Lack of accumulation of heavy metals in an arthropod community in the Northern Chihahuan Desert. *J of The Kansas Entomological Soc* **70** 329 - 334.

Main H 1995 The effects of urbanisation on rural environments in African In: *People and Environment in Africa.* Binns T (ed). John Wiley, New York, USA, pp 47 - 60.

Marino F, Ligero A, Diaz-Cosin D J 1995 Variations in heavy metals concentration in different earthworm species in five mines of Galacia (NW Spain). *Miscel Lanea Zoologica* **17** 75 - 85.

Marinussen M P, Zeaseat J C, Van der-Hann F A M 1997 Copper accumulation in the earthworm *Dendrobaena veneta* in a heavy metal (Cu, Pb, Zn) contaminated site compared to Cu accumulation in laboratory experiments. *Environ Pollut* **96** 227 - 233.

Naqvi S M, Howell R D 1993 Cadmium and lead uptake by red swap crayfish *(Procambrus clarkii)* of Lousiana. *Bull Environ Contam Toxicol* **51** 296 - 302.

Nuorteva P, Elberg K 1999 Levels of cadmium and some other metals in insects. *Proceedings of the XXIV Nordic Congress of Entomology,* Tartu, Estonia, pp 125 - 137.

Pentecost A 1999 *Analysing Environmental Samples.* Longman, London, UK, pp 85 - 90.

Peters M T 1988 *Insects and Human Society.* Van Nostrand Reinhold, New York, USA, pp 27 - 57.

Pize V, Josen G 1995 The influence of traffic pollution on earthworm and their heavy metal content on an urban ecosystem. *Podobiologia* **3** 422 - 453.

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

Smolders E, Lambregts R M, Mclaughlin M J, Tiller K G 1998 Effect of soil solution chloride on cadmium availability to Swiss Chard. *J Environ Qual* **27** 426 - 431 (1998).

Spurgeon D J, Hopkin S P 1996 Risk assessment of the threat of secondary poisoning by metal to predators of earthworms in the vicinity of a primary smelting works. *Science of the Tot Environ* **187** 167 - 183.

Starr C, Taggart R 1998 *Biology - The Unity and Diversity of Life*. Wadsworth, London, UK, pp 882 - 889.

Van der Watt H V H, Summer M E, Canbera M L 1994 Bioavailability of copper, manganese and zinc in poultry litter *J Environ Qual* **23** 43 - 49.

Wang S T, Demshar A P 1992 Determination of lead in dried blood spot specimens by Zeeman effect background corrected atomic absorption. *Analyst* **117** 959 - 961.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 457 - 461*

AVAILABLE AND UNAVAILABLE CARBOHYDRATE CONTENT OF BLACK GRAM (*VIGNA MUNGO*) AND CHICK-PEA (*CICER ARIETINUM*) AS AFFECTED BY SOAKING AND COOKING PROCESSES

Zia-ur-Rehman,* M Rashid and A M Salariya

Biotechnology and Food Research Centre PCSIR Laboratories Complex, Ferozepur Road, Lahore-54600 Pakistan

(Received July 8, 2002; accepted July 7, 2003)

The effects of soaking (Tap water, sodium bicarbonate solution) and cooking in tap water were investigated on available and unavailable carbohydrate contents and starch digestibility of black grams and chick-peas. Available carbohydrates including total soluble sugars, reducing sugars, non-reducing sugars and starch contents of these two legumes decreased to various extents as a result of soaking and cooking. From 3.43 - 25.63% total soluble sugars and 4.26 - 22.70% starch contents were lost on soaking black grams and chick-peas in tap water and sodium bicarbonate solution. Maximum amounts of total soluble sugars (28.43 - 59.64%) and starch contents (29.93 - 67.40%) were lost on cooking the water and alkali soaked legumes. However, these losses were comparatively less in case of water soaking process. Soaking and cooking processes also brought about some changes in the profile of unavailable carbohydrates of black grams and chick-peas. Soaking in sodium bicarbonate solution led to an appreciable increase of hemicellulose (42.50 - 54.31%) and NDF (28.69 - 30.68%) but not in legumes soaked in tap water. However, cooking process caused reduction in NDF (19.25 - 41.04%), ADF (5.48 - 25.31%), cellulose (12.88 - 28.42%) and hemicellulose (31.86 - 59.37%). Lignin contents of these legumes increased to some extents on cooking whereas it remained unchanged as a result of soaking . Starch digestibility of black grams and chick peas was markedly improved after cooking. However, no appreciable improvement in starch digestibility was observed after soaking these legumes in tap water or alkaline solution.

Key words: Black grams, Chick-peas, Soaking, Cooking, Carbohydrates, Starch.

Introduction

Available and unavailable carbohydrates play an important role in human health. Available carbohydrates serve as a source of energy for human body whereas the importance of unavailable carbohydrates in normal and therapeutic diets has been well acknowledged in literature (Spiller 1986; Morrow 1991; El-Bayoumy *et al* 1997). Unavailable carbohydrates which are commonly known as dietary fibre, mainly consist of cellulose, hemicellulose, lignin and pectin. These unavailable carbohydrates components exert different physiological effects on human health. Unavailable carbohydrates particularly pectin lower serum cholesterol help to reduce the risk of heart attack (Kelsey 1978). Presence of unavailable carbohydrates in diets is also helpful to prevent appendicitis, colon cancer and constipation in human due to absorption of water from the digestive track (Awan 1993; Hu Frank *et al* 2000;). In diabetics, legumes are reported to reduce the level of glucose in human blood due to the presence of unavailable carbohydrates (Brand *et al* 1990).

Available and unavailable carbohydrates are present in various food legumes. Food legumes are usually cooked after soaking in water prior to consumption. Cooking process is known to improve the nutritional quality of legumes whereas most of the macro and micro-nutrients are lost during soaking and cooking process (De-Leon *et al* 1992; Addy *et al* 1995). However, very little information is available in literature regarding available and unavailable carbohydrates in raw and cooked legumes. Therefore, present study was undertaken to investigate the effect of soaking and cooking on available and unavailable carbohydrate in black grams (*Vigna mungo*) and chick-peas (*Cicer arietinum*). Starch digestibility of these legumes was also studied after soaking and cooking processes.

Materials and Methods

Black grams and chick-peas were obtained from Ayub Agricultural Research Institute, Resalewala, Faisalabad (Pakistan). These legumes were cleaned to remove broken seeds, dust and other foreign materials and then subjected to soaking treatments prior to cooking.

Soaking treatment. A 50g sample of legumes was soaked in 250ml of tap water (pH - 7.0) and sodium bicarbonate solution (1% w/v, pH - 9.5) separately at 30˚C and 100˚C for 1-2h. The

---

*Author for correpondence; E-mail: pcsir@brain.net.pk

soaking solution was drained off, rinsed twice with distilled water and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc. Philadelphia, PA) at 55°C for 24h. Presoaked legumes were cooked in a pressure cooker as given below.

Pressure cooking. Presoaked legumes were placed in one liter beakers containing tap water (5ml/g). Tops of the beakers were covered with aluminium foil. After cooking in a pressure cooker at 15 lbs/inch^2 for 15 min, excess water was drained off and then dried in a hot air oven at 55°C for 24h. Raw and processed legumes were ground in a Wiley Mill to pass through a 40 mesh sieve.

Chemical analysis. The total water soluble sugars were extracted according to the method of Cerning & Guilbot (1973). Starch was extracted from the sugar free pellet by the method of Clegg (1956). Quantitative determination of total soluble sugars and starch was carried out according to the method of Yemm and Willis (1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi 1945), and non-reducing sugars were estimated by calculating the difference between total soluble sugars and reducing sugars. Unavailable carbohydrates including neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin contents were estimated according to the method of Van Soest & Wine (1967) and Mc Queen & Nicholson (1979). In accordance with ealier researchers, a preliminary overnight incubation with bacterial alpha amylase was employed. This methodology had already been used by earlier workers for estimation of dietary fibre components in legumes (Vidal-Valverde & Frias 1991). *In vitro* starch digestibility was determined after digestion with pancreatic α amylase in 0.1M phosphate buffer at 37°C for 2h. (Costas 1982). All determination were carried out in triplicate and standard deviation (SD) were calculated according to the method of Steel & Torrie (1980). Duncans multiple range test was used to determine significant differences ($P < 0.05$).

Results and Discussion

Table 1 summarizes the available carbohydrates contents of raw and soaked black grams and chick-peas.Total soluble sugars, reducing sugars, non-reducing sugars and starch contents in black grams and chick peas were 9.64 and 9.83%, 0.78 and 0.83%, 8.86 & 9.00% and 42.95 and 44.33% respectively. Results in Table 1 show that soaking of black grams and chick-peas decreased the quantity of availabale carbohydrates i.e. total soluble sugars, reducing sugars, non-reducing sugars. When the soaking temperature and time was increased, the extent of decrease in all these carbohydrates became more pronounced in these legumes. Sodium bicarbonate solution, as soaking solution, the extent of loss was significantly ($P < 0.05$) higher than that observed when these legumes were soaked in tap water. It is apparent from Table 1 that water soaking process caused reductions in total soluble sugars from 4.46 - 19.29% from black grams and 5.90 - 21.56% from chick-peas whereas 14.41 - 34.33% and 15.36 - 32.96% total soluble sugars contents were reduced from black grams and chick-peas respectively, on soaking in sodium bicarbonate solution. Reduction in reducing and non-reducing sugars from these legumes on soaking in tap water and sodium bicarbonate solution were also observed. Starch contents of black grams and chick-peas were reduced from 6.86 - 14.94% and 5.07-11.45% respectively, due to water soaking process. However, starch contents were further reduced from 14.43 - 29.68% and 13.28 - 24.92% from black grams and chick-peas respectively, as a result of soaking these legumes in alkaline solution of sodium bicarbonate. In fact, reduction in the levels of available carbohydrates with these treatments occurred mainly because of their solubility in simple water and sodium bicarbonate solution which has already been observed by earlier workers in case of other dry beans (Silva and Braga 1982; Sudesh *et al* 1986). Generally, legume starch is composed of soluble and insoluble portions and the soluble portion might have been extracted out and consequently caused significant reduction in starch contents.

Available carbohydrates contents of black grams and chick-peas were further decreased as a result of cooking (Table 2). Cooking process caused reduction in total soluble sugars 28.43 - 59.64% and starch contents 30.50 - 67.40% from black grams whereas, 28.88 - 55.55% total soluble sugars and 29.93 - 63.40% starch contents were lost from chick-peas. It is evident from these findings that when soaking in water or sodium bicarbonate solution was combined with cooking, the extent of losses of available carbohydrates from these legumes was significantly ($P < 0.05$) higher in comparison to that of simple soaking treatment. This is understandable again based on the fact that, in boiling water during cooking, the solubility of sugars will comparatively be much higher than at ordinary temperature. Contrary to these observations, Rao & Belavady (1978) reported that cooking brought about a significant increase in soluble sugars. This could be explained by the fact that cooking water was not discarded in that study whereas soaking and cooking water was rejected and beans alone were analyzed for various carbohydrates components during the present study.

Table 3 summarizes the contents of unavailable carbohydrates contents of raw, soaked and cooked black grams and chick-peas. NDF, ADF, cellulose, hemicellulose and lignin contents in raw black grams and chick peas were 24.41 and 25.58%, 11.67 and 9.44%, 9.78 and 8.29%, 12.74 and 16.14%

Table 1

Effect of soaking on available carbohydrates contents (%)* of black grams and chick-peas

Soaking conditions			Black grams				Chick-peas			
Soaking solution	Temp. (°C)	Time (hr)	Reducing sugars	Non reducing sugars	Total soluble sugars	Starch	Reducing sugars	Non reducing sugar	Total soluble sugars	Starch
Raw	-	-	0.78^{a} ± 0.02	8.86^{a} ± 0.36	9.64^{a} ± 0.66	42.95^{a} ± 1.7	0.83^{a} ± 0.02	9.00^{a} ± 0.22	9.83^{a} ± 1.12	44.33^{a} ± 1.72
Tap water	30	1	0.75^{a} ± 0.02	8.46^{a} ± 0.29	9.21^{a} ± 0.51	40.00^{a} ± 1.3	0.78^{a} ± 0.04	8.47^{a} ± 0.19	9.25^{a} ± 1.08	42.08^{a} ± 1.84
	30	2	0.49^{a} ± 0.03	7.73^{b} ± 0.22	8.22^{b} ± 0.44	37.00^{b} ± 1.5	0.57^{b} ± 0.03	7.77^{b} ± 0.18	8.34^{b} ± 1.07	40.11^{b} ± 1.34
	100	1	0.60^{a} ± 0.02	7.89^{a} ± 0.37	8.49^{a} ± 0.40	39.12^{a} ± 1.8	0.76^{a} ± 0.05	8.42^{a} ± 0.17	9.18^{a} ± 0.98	42.22^{a} ± 1.28
	100	2	0.38^{b} ± 0.05	7.40^{b} ± 0.41	7.78^{b} ± 0.32	35.50^{b} ± 1.4	0.51^{b} ± 0.04	7.20^{b} ± 0.22	7.71^{c} ± 0.92	39.25^{b} ± 1.62
1.0%Sodium bicarbonate solution	30	1	0.50^{b} ± 0.04	7.75^{a} ± 0.52	8.25^{b} ± 0.32	36.75^{b} ± 1.14	0.60^{b} ± 0.04	7.72^{a} ± 0.20	8.32^{c} ± 0.89	38.44^{b} ± 1.80
	30	2	0.30^{c} ± 0.02	6.62^{b} ± 0.44	6.92^{c} ± 0.28	35.00^{b} ± 1.2	0.40^{c} ± 0.05	7.40^{b} ± 0.24	7.80^{c} ± 0.55	36.66^{c} ± 1.26
	100	1	0.60^{a} ± 0.04	7.27^{a} ± 0.43	7.87^{b} ± 0.21	34.88^{b} ± 1.1	0.65^{b} ± 0.04	7.20^{a} ± 0.25	7.85^{a} ± 0.48	36.05^{c} ± 1.22
	100	2	0.33^{c} ± 0.05	6.00^{b} ± 0.38	6.33^{c} ± 0.29	30.20^{c} ± 1.0	0.28^{c} ± 0.02	6.00^{c} ± 0.07	6.28^{c} ± 0.44	33.28^{c} ± 1.30

*; Mean values ± S.D., n = 3 (dry weight basis). Mean values within a column with the different superscripts are a,b,c significantly different at $P < 0.05$

Table 2

Effect of cooking process on available carbohydrates contents (%)* of soaked black grams and chick-peas

Soaking conditions			Black Grams				Chick-peas			
Soaking solution	Temp. (°C)	Time (hr)	Reducing sugars	Non reducing sugars	Total soluble sugars	Starch	Reducing sugars	Non reducing sugars	Total soluble sugars	Starch
Raw	-	-	0.78^{c} ± 0.05	8.86^{a} ± 0.35	9.64^{a} ± 0.45	42.95^{a} ± 1.65	0.83^{a} ± 0.06	9.00^{a} ± 0.82	9.83^{a} ± 1.75	44.33^{a} ± 1.05
Tap water	30	2	0.35^{b} ± 0.06	6.55^{b} ± 0.27	6.90^{b} ± 0.33	29.85^{b} ± 1.79	0.42^{b} ± 0.05	6.80^{b} ± 0.72	7.22^{b} ± 1.11	31.06^{b} ± 0.57
	100	2	0.27^{c} ± 0.01	5.73^{b} ± 0.29	6.00^{b} ± 0.32	27.11^{b} ± 1.00	0.38^{b} ± 0.06	5.93^{b} ± 0.55	6.31^{c} ± 0.92	30.15^{b} ± 0.61
1.0%Sodium bicarbonate	30	2	0.23^{c} ± 0.02	4.00^{c} ± 0.22	4.23^{c} ± 0.24	19.65^{c} ± 1.25	0.30^{c} ± 0.04	5.25^{b} ± 0.34	5.55^{c} ± 0.82	20.56^{c} ± 0.32
solution	100	2	0.14^{d} ± 0.01	3.75^{c} ± 0.22	3.83^{c} ± 0.19	14.00^{d} ± 1.35	0.22^{d} ± 0.02	4.00^{c} ± 0.32	4.22^{c} ± 0.42	16.22^{c} ± 0.40

*; Mean Values ± S.D., n = 3 (dry weight basis). Mean values within a column with the different superscripts a,b,c,d are significantly different at $P < 0.05$.

and 1.89 & 1.15% respectively. Soaking of black grams and chick-peas caused some changes in unavailable carbohydrates profile i.e. NDF, ADF, cellulose, hemicellulose and lignin. Soaking of these legumes in tap water did not significantly alter NDF, hemicellulose and lignin contents on dry matter basis. A slight but significant increase in ADF, (4.28% black grams, 3.49% chick-peas) and cellulose (5.01% black grams, 3.98% chick- peas) was observed (Table 3). Soaking in 1% sodium bicarbonate solution caused a sharp increase in hemicellulose (54.31% black grams, 42.50% chick-peas), cellulose increase slightly (6.44% black grams, 6.15% chick-peas) and lignin contents remained unchanged. As a result of increase in hemicellulose and cellulose, NDF and ADF contents of black grams and chick-peas also increased by 30.68 & 28.69% and 4.88 & 4.98% respectively. Earlier workers also reported, significant increase in hemicellulose during soaking of lentils in sodium bicarbonate solution at room temperature for 9h. (Vidal-Valverde *et al* 1992).

Cooking of presoaked black grams and chick-peas also brought about some changes in unavailable carbohydrates. Reduction in the amounts of cellulose from 12.88 - 28.42% and hemicellulose from 31.86 - 59.37% was observed from black grams whereas 15.56 - 33.05% cellulose and 33.02 - 50.24% hemicellulose contents were reduced from chick-peas on cooking (Table 3). However, lignin contents were distinctly increased on cooking these legumes. It is apparent from these findings that the reduction in hemicellulose contents were almost two times more than those for cellulose contents. Similarly, hemicellulose and cellulose contents were greatly reduced on cooking the alkali soaked legumes compared to water soaked legumes. Reduction in cellulose and hemicellulose could be attributed to chemical degradation of cellulose in glucose, hemicellulose into arabinose, xylose and galactose as a result of cooking (Robinson & Lawler 1986). These results are in consistent with the findings of Vidal-Valverde & Frias (1991), who found that hemicellulose contents in kidney beans were

Table 3

Unavailable carbohydrates contents (%)* in raw, soaked and cooked black grams and chick-peas

	Black grams					Chick-peas				
Treatments	NDF	ADF	Cellulose	Hemicellulose	Lignin	NDF	ADF	Cellulose	Hemicellulose	Lignin
Raw	$24.41^{a}\pm1.26$	$11.67^{a}\pm0.76$	$9.78^{a}\pm0.88$	$12.74^{a}\pm1.01$	$1.89^{a}\pm0.36$	$25.58^{a}\pm1.09$	$9.44^{a}\pm0.70$	$8.29^{a}\pm0.35$	$16.14^{a}\pm0.81$	$1.15^{a}\pm0.25$
Soaking process (100˚C-2h)										
Tap water	$24.95^{a}\pm1.22$	$12.17^{a}\pm0.66$	$10.27^{a}\pm0.70$	$12.78^{a}\pm0.83$	$1.90^{a}\pm0.22$	$25.93^{a}\pm1.18$	$9.77^{a}\pm0.60$	$8.62^{a}\pm0.52$	$16.16^{a}\pm0.73$	$1.15^{a}\pm0.22$
Sodium bicarbonate solution (1% w/v)	$31.90^{b}\pm1.21$	$12.24^{a}\pm0.60$	$10.41^{a}\pm0.61$	$19.66^{b}\pm0.52$	$1.83^{a}\pm0.32$	$32.92^{b}\pm1.01$	$9.92^{b}\pm0.42$	$8.80^{b}\pm0.50$	$23.00^{b}\pm0.49$	$1.12^{b}\pm0.25$
Cooking process after soaking in										
Tap Water	$19.71^{c}\pm1.30$	$11.03^{b}\pm0.73$	$8.52^{b}\pm0.44$	$8.68^{c}\pm0.49$	$2.51^{b}\pm0.12$	$19.27^{c}\pm0.46$	$8.46^{c}\pm0.48$	$7.00^{c}\pm0.61$	$10.81^{c}\pm0.52$	$1.46^{b}\pm0.21$
Sodium bicarbonate solution (1% w/v)	$14.78^{d}\pm1.27$	$9.60^{c}\pm0.61$	$7.00^{c}\pm0.40$	$5.18^{d}\pm0.22$	$2.60^{b}\pm0.11$	$15.08^{d}\pm0.15$	$7.05^{c}\pm0.22$	$5.55^{d}\pm0.20$	$8.03^{d}\pm0.18$	$1.50^{b}\pm0.11$

* Mean Values± S.D., n = 3 (dry weight basis). Mean values within a column with the different superscripts a,b,c,d are significantly different at $P < 0.05$

Table 4

Effect of soaking and cooking on starch digestibility (%)* of black grams and chick-peas

Soaking conditions			Black grams		Chick-peas	
Soaking solution	Temp. (˚C)	Time (hr)	Without cooking	Pressure cooking	without cooking	Pressure cooking
Raw	-	-	$37.37^{a} \pm 1.32$	$45.67^{a} \pm 1.45$	$39.00^{a} \pm 1.27$	$46.77^{a} \pm 1.22$
Tap water	30	2	$41.00^{a} \pm 1.66$	$59.88^{b} \pm 1.49$	$42.80^{b} \pm 1.22$	$62.00^{b} \pm 1.74$
	100	2	$43.38^{b} \pm 1.72$	$64.00^{b} \pm 1.28$	$44.97^{b} \pm 1.41$	$65.50^{b} \pm 1.60$
Sodium bicarbonate solution	30	2	$42.22^{b} \pm 1.54$	$72.61^{c} \pm 1.72$	$44.00^{b} \pm 1.08$	$74.11^{c} \pm 1.72$
	100	2	$44.00^{b} \pm 1.29$	$83.00^{c} \pm 1.80$	$45.89^{b} \pm 1.82$	$86.00^{d} \pm 1.46$

* Mean values ± S.D., n = 3 (dry weight basis). Mean values within a column with the different superscripts a,b,c,d are significantly different at $P < 0.05$.

greatly decreased as a result of cooking. It is apparent from Table 3 that NDF contents decreased from 19.25 - 39.45% & 24.66 - 41.04% respectively, on cooking presoaked black grams and chick-peas. Similarly, decrease in ADF contents of black grams and chick-peas were 5.48 - 17.78% and 10.38 - 25.31%, respectively after cooking. These results revealed that reduction in NDF contents was comparatively higher than ADF contents. Data presented in Table 3, also showed that reductions in NDF and ADF contents were markedly higher on cooking the alkali soaked legumes. Reductions in NDF and ADF contents in cooked legumes could be attributed to partial degradation of cellulose and hemicellulose into simple sugars (Robinson and Lawler 1986; Rehman and Shah 1994). These results are in agreement with those obtained by Vidal-Valverde *et al* (1992) who reported distinct decrease in NDF due to a drastic loss in hemicellulose on cooking presoaked lentils.

Besides losses in available and unavailable carbohydrates, starch digestibility was affected significantly ($P < 0.05$) on cooking, whereas it remained almost unchanged after soaking in water or sodium bicarbonate solution as shown in Table 4. Initially, starch digestibility of uncooked black grams and chick-peas was 37.37% & 39.00% which became 45.67% & 46.77% respectively after cooking in a pressure cooker. Maximum increase in starch digestibility was found to be about 120% after cooking alkali soaked black grams and chick peas in a pressure cooker whereas the increase was only 68 - 71% on cooking water soaked legumes in a pressure cooker. Improvement in starch digestibility after cooking could be attributed due to complete hydrolysis of starch under the drastic conditions of heating under pressure. However, improvement in digestibility of starch become about two times more for alkali soaked legumes compared to water soaked legumes on cooking. These results are in agreement with those obtained by Mbofung *et al* (1999) who reported distinct improvement in starch digestibility of cow-peas after cooking. In this study, legumes were markedly resistant to pancreatic amylase attack but cooking led to a dramatic increase in its susceptibility to digest by this enzyme. In fact, cooking improves the digestibility of starch through gelatinization and destruction of anti-nutrients (Yu - Hui 1991).

Conclusion

Available and unavailable carbohydrates contents of black grams and chick-peas were reduced to various extents due to soaking and cooking processes. Soaking temperatures and time significantly affected the rate of extraction of available and unavailable carbohydrates. Sodium bicarbonate solution extracted comparatively more carbohydrates compared to water soaking process. Maximum amount of available and unavailable carbohydrates were reduced as a result of cooking process. Besides losses in available and unavailable carbohydrates, significant improvement in starch digestibility of black grams and chick -peas was observed after cooking. However, no appreciable improvement in starch digestibility of black grams and chick-peas was observed after cooking. However, no sappreciable improvement in starch digestibility was observed after soaking these legumes in tap water and sodium bicarbonate solution.

References

Addy E O H, Salami L I, Ibocli L C, Remawa H S 1995 Effect of Processing on Nutrient Composition and Anti-nutrientive Substances of African Locust Bean and Baobale Seed. *Plant Foods Hum Nutri* **48**(2) 113 - 117.

Awan J A 1993 *Elements of Food and Nutrition* 1st Ed. Press Street, Bazar, Faisalabad, Pakistan, pp 3.

Brand J C, Snow B J, Nobhan G P, Truswell A S 1990 Plasma glucose and insulin responses to traditional Pima Indian Meals. *Am J Clin Nutr* **51** 416 - 420.

Cerning J, Guilbot J 1973 Specific method for the determination of Pentosans in cereals and products. *Cereal Chem* **50** (2) 176 - 184.

Clegg K M 1956 The application of the anthrone reagent to the estimation of starch in cereals. *J Sci Food Agric* **7** 40 - 44.

Costas G B 1982 Physical characteristics, enzymatic digestibility and structure of chemically modified smooth pea and waxy maize starches. *J Agri Food Chem* **30** 925 - 930.

De-Leon L F, Elias L G, Bressani R 1992 Effect of salt solutions on the cooking time, nutritional and sensory characteristics of common beans. *Food Res Int* **25** 131 - 136.

El-Bayoumy K, Chung F L, Richie S J, Reddy B S, Cohen L, Weisburger J, Wynder E L 1997 Dietary control of cancer. *Proc Soc Exp Biol Med* **216** 211 - 233.

Hu F B, Rimm E B, Stampfer M J, Ascherio A, Spiegelman D, Willett W C 2000 Prospective study of major dietary patterns and risk of cornary heart disease in men. *Am J Clin Nutr* **72** 912 - 921.

Kelsey J I 1978 A review of research on effect of fiber intake on man. *Am J Clin Nutr* **31** 142 - 152.

Mbofung C M F, Rigby N, Waldron F 1999 Use of two varieties and hard-to-cook beans (*Phaseolus vulgaris*) in the Processing of Koki (a Steamed Legume Product). *Plant Food Hum Nutri* **54** 131 - 150.

McQueen R A, Nichelson J W G 1979 Modification of the neutral detergent fiber procedure for cereal and vegetables by using alpha amylase. *J Assoc Off Anal Chem* **62** 679 - 681.

Morrow B 1991 The rebirth of legumes.*Food Tech* **45** 96 - 121.

Rao P U, Belavady B 1978 Oligosaccharides in pulses; varietral differences and effects of cooking and germination. *J Agric Food Chem* **26** 316 - 319.

Rehman Z U, Shah W H 1994 Preparation of dietary fiber from mustard seed meal. *Pak J Sci Ind Res* **37** 156 - 159.

Robinson C H, Lawler M R 1986 *Normal and Therapeutic Nutrition*, 7th ed, Callias MacMillion Publishers, London, UK.

Silva H C, Braga C L 1982 Effect of soaking and cooking on the oligosaccharide content of dry beans (*Phaseolus vulgaris*, L. *J Food Sci* **47** 924 - 925.

Somogyi M 1945 A new reagent for the determination of sugars. *J Biol Chem* **160** 61-68.

Spiller G A 1986 *Hand Book of Dietary fibre in Human Nutrition.* CRC Press, Inc., Boca Raton FL, USA.

Steel R G D, Torrie J H 1980 *Principles and Procedures of Statistics.* McGraw Hill, London, UK.

Sudesh J, Usha M, Randhir S 1986 Effect of Processing on available carbohydrates in legumes. *J Agric Food Chem* **34** 417 - 420.

Van Soest P J, Wine R H 1967 Use of detergents in the analysis of fibrous feeds determination plant cell wall constituents. *J Assoc Off Anal Chem* **51** 417 - 420.

Vidal-Valverde C, Frias J 1991 Legumes processing effects on dietary fiber components. *J Food Sci* **56** 1350 - 1352.

Vidal-Valverde C, Frias J, Esteben R 1992 Dietary fiber in processed lentils. *J Food Sci* **57** 1151 - 1163.

Yemm E W, Willis A J 1954 The Estimation of carbohydrates in plant extracts by anthrone. *Biochem J* **57** 508 - 514

Yu-Hui T 1991 Effect of the hard-to-cook defect and processing on protein and starch digestibility of Cow-peas. *Cereal Chem* **68**(4) 413 - 418.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 462 - 464*

Observations on *Rafiqius bodenheimeri* (Steiner 1936) Khan and Hussain 1998 and *Discolaimus lahorensis* Khan, 1998 from Karachi, Sindh

H A Khan*[*] *and S A Khan

PCSIR Laboratories Complex, Off University Road, Karachi - 75280, Pakistan

(Received May 11, 2002; accepted July 26, 2003)

Rafiqius bodenheimeri (Steiner 1936) Khan and Hussain 1998 *Discolaimus lahorensis* are described from Karachi, *R. bodenheimeri* (Steiner 1936) Khan and Hussain 1998 is different in size and shape of post vulval uterine sac which is being 70.2µm in length, whereas, uterine sac is collapsed in *R. saeedi*. Males are present, in *R. bodenheimeri* while males not found in *R. saeedi*. *R. bodenheimeri* is different from *R. amurensis* (Truskova 1971, Siddiqi, Deley and Khan 1992) Khan and Hussain 1998 in body length, shape of spicules and size of gubernaculum.

Key words: Soil, Nematodes, Systematics.

Introduction

During a survey, nematodes were isolated from soil sample identified as *Rafiqius bodenheimeri* (Steiner 1936) Khan and Hussain, 1998 and *Discolaimus lahorensis*. It appeared to be first record of these nematodes from Karachi and described in detail.

Material and Methods

Soil samples were collected from various localities of Karachi and carried to the laboratory. All samples were sieved by Cobb's gravity method (1918) and later improved by Baermann's method. Nematodes were collected under the stereoscopic binocular and killed by gentle heat. Nematodes were processed by slow method to glycerine and mounted on glass slides in a drop of anhydrous glycerine according to Siddiqi (1986). Measurements were taken from preserved specimens. Some specimens were also studied in freshly killed conditions, specimens were deposited in Common Wealth Institute of Parasitology England and are rested in Nematology Laboratory, Food and Marine Resources Research Centre PCSIR Labs. Complex, Karachi.

Genus Rafiqius Khan and Hussain 1998: Diagnosis (Emended) *Acrobelinae*. Body more than 0.5 mm in length; Cephalic probolae was present; axils as a deep cleft boarded by flap, like usually drawn out a set, located laterodorsally on lateral lips. Stoma constituted on chelorhabdion and prorhabdion slightly swollen behind the middle. Lateral field was prominent with five to seven incisures on mid body. Isthmus short and broad, less in length adjacent to body; basal bulb strongly valvate. Post vulval uterine sac was well developed, usually longer than body width. Ovary monodelphic. Female tail was subcylindrical with rounded truncate or notched terminus. Phasmids distinct on tail. Lateral field extending beyond phasmid, but in some specimens stopped at phasmids. Male rare, equal to female. Oesophagus, spicules, gubernaculum and tail were typically cephaloboid type.

Type species:
Rafiqius saeedi (Siddiqi, Deley and Khan 1992)
Syn. *Acrobeloides saeedi* (Siddiqi, Deley and Khan 1992)
Other species: *R. bodenheimeri* (Steiner, 1936)
Thorne 1937, Siddiqi, Deley, Khan 1992, Khan and Hussain 1998 n. Comb.
Syn: *Acrobeloides bodenheimeri* (Steiner 1936) Siddiqi, Deley and Khan 1992. *R. camberensis* Khan and Hussain 1998
Syn: *Acrobeloides camberensis* (Deley, Geraert and Coomans 1990) Siddiqi, Deley and Khan 1992
Syn: *Cephalobus camberensis*, Deley, Geraert and Coomans 1990.

Rafiqius bodenheimeri n. Comb. Measurement. ♀♀ L = 0.60 - 0.64 (0.62 ± 0.77) mm; a = 15.30 - 16.00 (15.60 ± 3.70); b = 4.50 - 4.60 (4.40 ± 3.70); c = 15.70 - 17.00 (16.40 ± 2.70); c^1 = 19.50 - 20.00 (19.70 ± 3.50); V = 66.00 - 70.00 (6.80 ± 7.50). ♂♂ L = 0.64 - 0.71 (0.67 ± 1.10) mm; b = 4.20 - 4.50 (4.30 ± 1.80); c = 23.00 - 24.60 (23.80 ± 3.10); c = 19.50 - 20.00 (19.60 ± 4.40); spicules = 45.60 - 46.20 (44.90 ± 6.40)µm; gubernaculum = 24.60 - 25.30 (24.60 ± 4.20) µm.

Description: Body of the heat relaxed specimens clyindrical narrow at both ends just behind vulva tapering. Cuticle prominently annulated, 2.30 µm at mid body; lateral field distinct, 6.50 - 90.00 µm and have five distinct incisures. Cephalic region expended with six slightly separated lips making a hexagonal shape; labial probolae three, asymmetrical, rounded anteriorly slightly elevated above the head contour; cephalic

---

[*] Author for correspondence

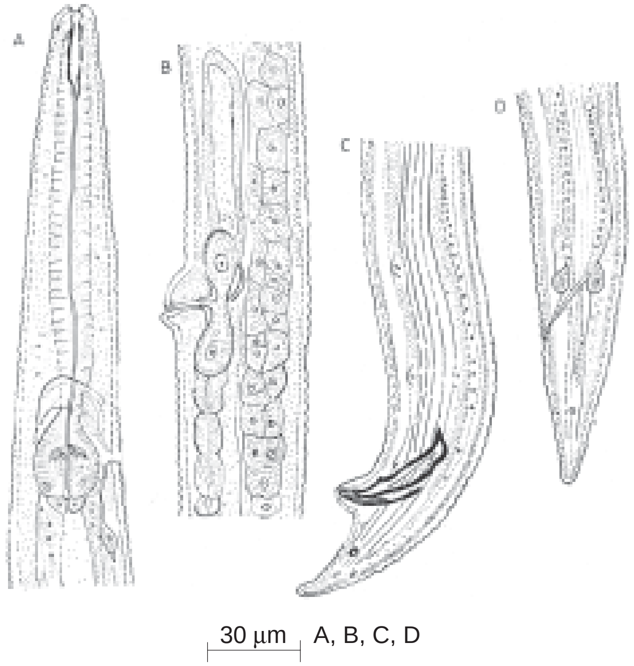


Fig 1. *Rafiqius bodenheimeri*, A. Female head; B. Female reproductive region (Female organs); C. Male tail; D. Female tail.

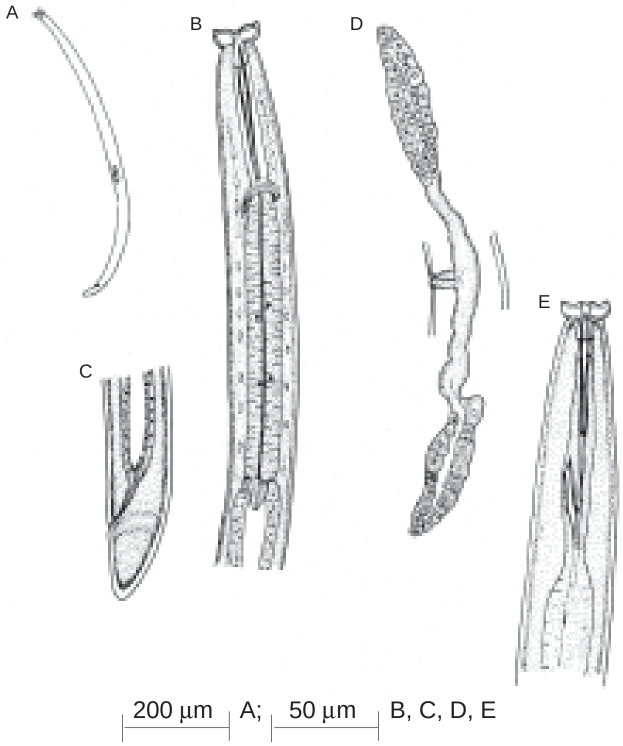


Fig 2. *Discolaimus lahorensis*. A. Entire female; B. Female head; C. Female tail; D. Vulval region; E. Juvenile head.

probolae present in three pairs,each pair separated by flap like tines per cleft. Amphid slit like in middle. Oesophagus 133.00 - 175.00 µm in length; corpus cylindroid, slightly swollen in the middle. Isthmus 23.00 - 26.30(24.60 ± 4.70) µm in length with a large triqutorius valve anterior to its center. Cardia is prominent 4.50 x 18.00 µm; Nerve ring located at the distance of 108.00 µm from anterior region. Excretory pore 117.00 - 119.00 (117.50 ± 9.20) µm from anterior region. Hemizo-nids located just anterior to excretory pore. Vulva, a transversely oval slit; vulval lips prominently raised above the body surface; posterior lip larger than anterior lip and broadly rounded; vagina thick walled, more than 2/5th the body width in length. Two pairs of vaginal glands (One anterior and other posterior). Post vulval uterine sac is prominently 49.00 - 70.20 (59.50 ± 7.30) µm long; uterus, strong tube. Spermathica oval 30.00 - 39.00 (34.10 ± 4.10) µm long and 18.00 - 21.00 (19.40 ± 6.10)µm wide. Ovary relaxed with double flexure 1 - 2 body behind vulva. Anus, a large backwardly directed aperture containing one dorsal and two subventral rectal glands near rectum intestine junction. Tail subscylindroid to conoid rounded terminus. Phasmids located just behind the mid tail at the distance of 20.70 µm from tail; lateral field extending past phasmids.

Male: Similar to female in general body shape and cuticular annulation; lateral field contains five incisures. Testis reflexed in the middle of the body. Spermatogonium round and sufficient in number. Three retal glands were observed near spicular head. Three pairs ventrolateral supplementary papillae anterior to cloacal apertures; four pairs of caudal papillae present, two anterior and two posterior to phasmids. Four papillae are lateral and four other ventro - sublateral in position. Phasmid located just behind the middle of tail. Spicules paired, 43.00 - 46.00 (44.30 ± 6.40)µm in length, head direc-ted antero - ventrally; spicular opening located behind head on dorsal side and posteriorly on ventral side near pointed tip. Gubernaculum straight to slightly arcuate, 20.70 - 24.00 (22.40 ± 3.20)µm long orura prominent, about half of the length of spicule. Cloacal aperture on large ventral elevation of the body; anterior lip pointed, posterior lip large and round. Tail conoid, slightly arcuate ventrally with pointed tip.

Relationship: *Rafiqius bodenheimeri* n. Comb. is recognized by its length, tail, postvulva-uterine sac (body length in *R. saeedi* 0.86 - 1.20 mm; *Rafiqius bodenheimeri*: 0.45 - 0.65 mm). *R.bodenheimeri* also differs from *R.saeedi* (Siddiqi, Deley and Khan 1992) Khan and Husain, 1998 in shape of the post-vulval uterine sac which is well develop and 70.20 µm long while it is collapsed in *R. saeedi*. *R. bodenheimeri* lacks metacarps, whereas, prominent in *R. saeedi*. Males are present in *R. bodenheimeri* while lacking in *R. saeedi*. *R. bodenheimeri* is also different from *R. amurensis* in body length (L = 0.50 - 0.80 mm in *R. bodenheimeri*: L = 0.61 - 0.76 mm in *R. amurensis* (Truskova 1971): Siddiqi *et al* 1992; Khan and Hussain 1998).

Spicules in *R.bodenheimeri* is 16.00 µm and *R. amurensis* = 32.00 µm. A detailed work on biology is necessary to solve some critical points in this connection.

Discolaimus lahorensis Khan 1998: ♀♀ 1.60 - 1.80 (1.30 ± 0.20) mm; a = 39.00 - 40.00 (40.10 ± 0.50); b = 3.60 - 3.90 (3.70 ± 0.20); c = 36 - 44 (40.50 ± 4.50); V = 50.00 - 51.00 (50.50 ± 0.70); odontostyle = 17.00 - 18.30 (17.60 ± 0.30)µm; Odon-tophore = 37.00 - 38.50 (38.00 ± 0.30)µm.

Description: Body stout and curved posteriorly after relaxing by gentle heat. Cuticle contains transverse striations near head and tail. Head not separated from the body; lips typical, somewhat angular, 56.00 µm in breadth with 16 papillae. Amphids of stirrup shape, 8.00 µm in breadth. Odontostyle prominent 17.00 - 18.30 (17.30 ± 0.20)µm long with apertur, 1/2 of its length. Odontophore 37.00 - 38.20 (37.00 ± 0.82)µm in length; guiding ring single; Oesophageal extension starts before the mid point. Cardia oval, 16.00 µm long. Dorsal gland nucleus located more than one width behind the beginning of oesophageal expansion; other gland nuclei inconspicuous. Nerve ring located at 180.00 - 195.00 (187.00 ± 1.00)µm. Gonads amphidelphic; Vulva transverses slit; vagina less than half of corresponding body with ovaries paired and opposed; prerectum more than 13 times of the rectum length. Tail convexconoid with broadly rounded terminus.

Conclusion

Discolaimus lahorensis, Khan (1998) was originally collected from Poagrass, West Regional Laboratories, Lahore. Four samples collected from various localities of Sindh were found positive for *Discolaimus lahorensis*. There is sufficient variation in shape of odontostyle length of oesophagus, shape of the body and is compared to the original description. *Disocolaimus lahorensis*, Khan 1998 except in shape and length of odontostyle and length of oesophagus. These variations may be due to environmental conditions of this province.

References

Bassu C 1991 Frilibende Nematoden aus Kuslenduch and agrenschden Biltopen den deutsehen und danichen Kusten. *Zoologischer Anzeiger* **226** 114 - 148.

Cobb N A 1918 Amended characterization of nemic genera *Cephalobus* and *Acrobeles. J Parasit* II, 108 pp.

DeLey P, Geraert E A, Coomans 1990 Seven Cephalobids from Sanigal (Nematoda: Rhabditida). *J African Zool* 10$: 287 - 304.

Khan H A, Hussain S S 1998 Biosystematics of *Rafiqius saeedi* (Siddiqi, Deley and Khan 1992) *Gen N (Nematoda*: Cephalobidae with observation on its life cycle. *Pak J Zool* **29**(2) 139 - 143.

Khan H A 1998 A new species of *Discolaimus* (Nematoda: Discolaimidae) with the description of two other species from Pakistan. *Bangladesh J Zool* **26**(2) 13 - 18.

Saeed M, Khan S A, Saeed V A, Khan H A 1988 *Cephalobus litoralis:* Biology and tolerance to description. *J Nematol* **20**(2) 327 - 329.

Siddiqi M R 1986 Tylenchida: Parasite of Plant and Insect. Farnhamm Royal, U.K. Common Wealth Agric Bureau, Bureaux, IX 645 pp.

Siddiqi M R, DeLey P, Khan H A 1992 *Acrobeloides saeedi* sp. n. from Pakistan and redescription of *A. bodenheimeri* (Steiner) and *Placodera lobata* Thorne (Nematoda: Cephalobidae). *Afro-Asian J Nematol* **2** 5 - 16.

Steiner G 1936 Opuscula miscellanea nemtologica II. *Proc Helm Soci Wash* **2** 41 - 16.

Throne G 1937 A revision of nematode family Cephalobidae Chitwood and Chitwood 1934. *Proc Helm Soc Wash* **4** 1 - 16.

Truskova G M 1971 A new species of the genus *Acrobeloides* (Nematoda: Cephalobidae). *Zoolgischekii Zhurnal* **50** 434 - 436.

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 465 - 470*

MICROBIAL PRODUCTION OF XYLITOL FROM ACID TREATED CORN COBS

R F Allam

Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Cairo, Egypt

(Received December 12, 2001; accepted September 23, 2003)

The fermentation of xylan hydrolyzate corn cobs by different yeast species revealed the formation of different polyalcohol sugars. Both *Schizosaccharomyces japonicus* and *Kluyveromyces bulgaricus* form xylitol as sole product. Relatively high xylitol pro-duction by *S. japonicus* and *K. bulgaricus* was achieved under static fermentation, among other parameters, initial pH 9 and 8, carbon concentrations 88 - 110 g / l, ammonium chloride 1.0, 2.0 g / l and 10, 15 g / l yeast extract for *S. janponicus* and *K. bulgricus*, respectively were obtained. However, maximal xylitol yields were recorded after 4 days of incubation for *S. japonicus* (18 g / l) and *K. bulgaricus* (16 g / l), respectively.

Key words: Corn cobs, Xylitol, Yeast.

Introduction

Hemicellulose (as xylan) comprises up to 20% of the dry biomass of some lignocellulosic materials, such as corn cobs, with xylose as the major (about 94%) constituent sugar (Jeffries 1983; Welther *et al* 2000; Leathers 2003). The extraction and hydrolysis of xylan component, for example with diluted mineral acid, can be achieved more easily than cellulose hydrolysis and can be regarded as a pretreatment step to enhance subsequent cellulose saccharification (Watson *et al* 1984). The fermentation of D-xylose and other pentose sugars will facilitate the exploitation of plant biomass for the production of xylitol and ethanol (Du Preez *et al* 1986).

Xylitol, a five carbon sugar alcohol, is used as a sweetener in foods and may apply to medical purpose as sugar substitute for the treatment of diabetes (Kitpreechavanich *et al* 1984).

Many yeasts possess xylose reductase which catalyzes the reduction of D-xylose to xylitol as first step in xylose metabolism (Bruinenberg 1986; Kim *et al* 2002). This paper deals with production of xylitol from a cheap carbon source (corn cobs), rich in xylose and outlines some factors affecting its production.

Materials and Methods

Yeast strains. The following yeast strains were examined: *Candida albicans, C. utilis, C. lipolytica* CAIM, *C. lipolytica, C. tropicalis, C. kefyr, Cryptococcus laurentii* Y-2536, *Debaryomyces hansenii, Hansenula polymorpha, Kluyveromyces bulgaricus, Lipomyces lipoferus, Metschnikowia pulcherrima, Nadosenia fluvsence, Pachysolen tannophilus* Y-2460, *Rhodotorula rubra, Saccharomyces cerevisiae, S. cerevisiae* var *eulisaudans, S. diastaticus, S. lipolytica* CAIM 26, *S. lipolytica, S. rouxii* CAIM 21, *S. uvarum, Schizosaccharomyces japonicus, Schizosaccharomyces pombe, Trichosporon cutaneum.*

Xylan corn cobs hydrolyzate: this was prepared according to Whislher (1963).

Medium and fermentation conditions. The organism was routinely maintained on yeast malt agar medium. A loopful of cells taken from mother slant was transferred to a 250-ml Erlenmeyer flask containing 25 ml of inoculum medium of the following composition (g / l): yeast extract, 5; malt extract, 5; NaCl, 1; xylose, 10; pH 5.5 and shaking at 150 rpm for 24 h at 30°C.

Two ml of freshly cultured yeast suspension was inoculated into test tube (20 x 3 cm), each containing 20 ml of a sterilized medium having the following composition (g / l): NH_4Cl, 1; yeast extract, 5; NaCl, 3; $MgSO_4.7H_2O$, 1; K_2HPO_4, 3 and hydrolyzate xylan corn cobs (containing 10 g / l xylose) at pH 5.5 - 6. The tubes were incubated in inclubator at 30°C for 4 days.

Analytical methods. After removal of the yeast cells by centrifugation, the cell free fermentation broth was analyzed according to Somogyi's method (1952) for reducing sugar and the method of Neish (1952) for polyalcohol contents, respectively.

Isolation and identification of xylitol. After cultivation for 4 days, the culture medium was centrifuged at 500 rpm. The culture filtrate was deproteinized by the addition of 25% zinc sulfate, neutralized to pH 7.5 with 5N NaOH, and then centrifuged. The supernatant was concentrated in vacuum to dryness. The residue was extracted with boiling absolute ethanol and the extract was filtered. The paper chromatography was done to detect the presence of reducing sugars (Moore

E-mail: allam01@yahoo.com

Table 1

Screening of some yeasts for xylitol production from hydrolyzed corn cobs

Tested yeasts	Total polyalcohol content (g / l)	Types of detected sugar alcohols			
		Hexitol	Xylitol	Arbitol	Others
Candida albicans	0.20	+	+	+	+
C. utilis	0.27	-	+	-	+ +
C. lipolytica CAIM	0.26	-	+ +	+	-
C. lipolytica	0.13	+	+ +	-	-
C. tropicalis	0.12	-	+	+	+
C. kefyr	0.18	+	+	+	-
Cryptococcus laurentii Y-2536	0.32	+	+	+	+
Debaryomyces hansenii	0.26	+	+ +	+	-
Hansenula polymorpha	0.20	-	+ +	+ + +	+
Kluyveromyces bulgaricus	0.54	-	+ + +	-	-
Lipomyces lipoferus	0.15	+ +	-	-	+ +
Metschnikowia pulcherrima	0.08	+	+	+	-
Nadosenia fluvsence	0.21	-	-	+ +	+
Pachysolen tannophilus Y-2460	0.30	-	+ +	+	+
Rodotorula rubra	0.27	+	+	+	-
Saccharomyces cerevisiae	0.23	+ +	+	-	-
S. cerevisiae var. *eulisaudans*	0.23	-	-	+	+
S. diastaticus	0.24	+ +	+	+	+ +
S. lipolytica CAIM 26	0.23	+	-	+ +	+
S. lipolytica	0.24	+	+	+	+
S. rouxii CAIM 21	0.23	+	+ +	-	+
S. uvarum	0.26	+	+ +	+	+
Schizosaccharomyces japonicus	0.70	+	+ + +	-	-
S. pombe	0.25	-	+ +	+	+
Trichosporon cutaneum	0.23	+	+	+	-

-, negative; +, small amount; + +, medium amount; + + +, large amount.

et al 1960), while xylose and xylitol were analyzed by Shimadzu HPLC (C10) with refractive index detector. The sample (20µl) was injected in shim-pack CLC-NH_2 (6.0 mm i.d.x15 cm) column, the mobile phase acetonitrile / water (7/3) in flow rate: 1.0 ml / min, at 40˚C for 30 min.

Results and Discussion

Screening of yeasts for xylitol formation. The tested strains were found to be able to hydrolyze corn cobs as carbon source and produce xylitol and other sugar alcohols, as shown in Table 1. Among them, *Candida utilis, C. lipolytica* CAIM, *Cryptococcus laurentii* Y-2536, *Debaryomyces hansenii, Hansenula polymorpha, Kluyveromyces bulgaricus, Pachysolen tannophilus* Y-2460, *Rhodotorula rubra, Saccharomyces cerevisiae, S. diastaticus, S. uvarum* and *Schizosaccharomyces japonicus* produced total polyalcohols in a relatively good amount. However, *Kluyveromyces bulgaricus* and *Schizosaccharomyces japonicus* were recorded as the best xylitol producers. These two promising yeasts were therefore, selected for further experimentation.

Table 2

Effect of aeration condition on xylitol production

Yeast	Residual sugar (g / l)		Consumed sugar (%)		Xylitol (g / l)	
	Static	Shaked	Static	Shaked	Static	Shaked
Schizosaccharomyces japonicus	16.40	1.50	51.00	95.59	16.30	4.34
Kluyveromyces bulgaricus	10.70	2.60	68.00	92.00	11.90	1.78

- Initial xylose concentration 33.5 g / l.

Effect of cultivation technique. Both static and submerged cultivation techniques were examined for xylitol production by the promising yeasts. The data given in Table 2 revealed the superiority of the static technique. Under this condition, relatively more amounts of xylitol were formed, in spite of the

Table 3

Effect of pH regulation on xylitol production by *S. japonicus* and *K. bulgaricus*

Initial pH	Final pH	*S. japonicus* Consumed Sugar (%)	*S. japonicus* Xylitol (g / l)	*K. bulgaricus* Consumed sugar (%)	*K. bulgaricus* Xylitol (g / l)
3.00	4.37	48	15.00	43	13.70
4.00	4.57	51	17.60	46	14.80
5.00	5.36	54	19.10	50	15.90
6.00	5.91	57	18.70	54	17.70
7.00	5.84	56	20.30	57	18.70
8.00	6.40	62	20.80	60	19.70
9.00	7.00	61	22.50	58	18.80
10.00	7.71	55	19.80	55	16.50

- Initial reducing sugar 44 g / l.

Table 4

Effect of different nitrogen sources and concentrations on xylitol production by the tested yeasts

Nitrogen source	Nitrogen conc. (g / l)	Consumed sugar % *S. japonicus*	Consumed sugar % *K. bulgaricus*	Xylitol (g / l) *S. japonicus*	Xylitol (g / l) *K. bulgaricus*
NH_4Cl	0.50	57	73	10.51	5.50
	1.00	43	66	14.00	5.30
	2.00	44	70	10.70	9.00
Urea	0.50	67	74	7.50	5.40
	1.00	54	70	7.40	5.00
	2.00	66	73	6.50	4.80
$(NH_4)_2SO_4$	0.50	47	45	12.80	10.00
	1.00	66	50	9.40	8.00
	2.00	70	60	8.60	5.80
NH_4NO_3	0.50	70	76	4.30	0.40
	1.00	73	73	4.20	1.40
	2.00	70	69	4.30	1.70
$NH_4H_2PO_4$	0.50	45	55	11.00	8.20
	1.00	35	60	12.30	6.70
	2.00	40	70	10.00	5.70

- NH_4Cl, NH_4NO_3, $NH_4H_2PO_4$, $(NH_4)_2SO_4$ and urea were used at 88 g / l xylose.

assimilation of relatively low xylose levels. As the yeast assimilates xylitol after accumulation as a carbon source in submerged culture, therefore, it is safe to conclude that under the static culture condition, xylose was favourably metabolized to xylitol. This may be because the yeast shows little ability to consume xylitol under limited aeration (Jeffries 1985; Ahmed 1991; Sanchez *et al* 1997; Zagustina *et al* 2001; Walthers *et al* 2001). Our results are also in agreement with the results of Ligthelm *et al* (1988) who reported that xylitol, ribitol and glycerol were formed in high yields under oxygen limitation conditions by *Pachysolen tannophilus*,*C. shehatae* and *Pichia stipitis*. Faria *et al* (2002) reported that *C. guilliermondii* gave 0.71 g of xylitol/g xylose consumed at limited aeration. Also Walthers *et al* (2001) stated that high xylitol was produced (0.62 g/g) by *Candida tropicalis* ATCC 96745 under semi aerobic conditions.

pH regulation. The optimum initial pH values for xylitol production by *S. japonicus* and *K. bulgaricus* were found to be 9 and 8, respectively (Table 3). These results are in agreement with Kitpreechavanich *et al* (1984) who found that the

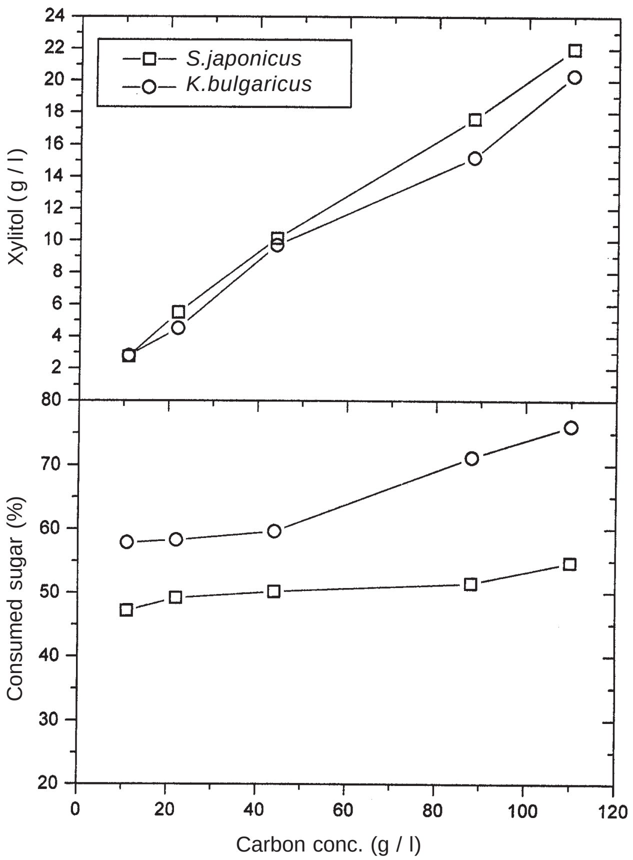


Fig. 1 Effect of hydrolyzed xylan corn cobs concentration on xylitol production.

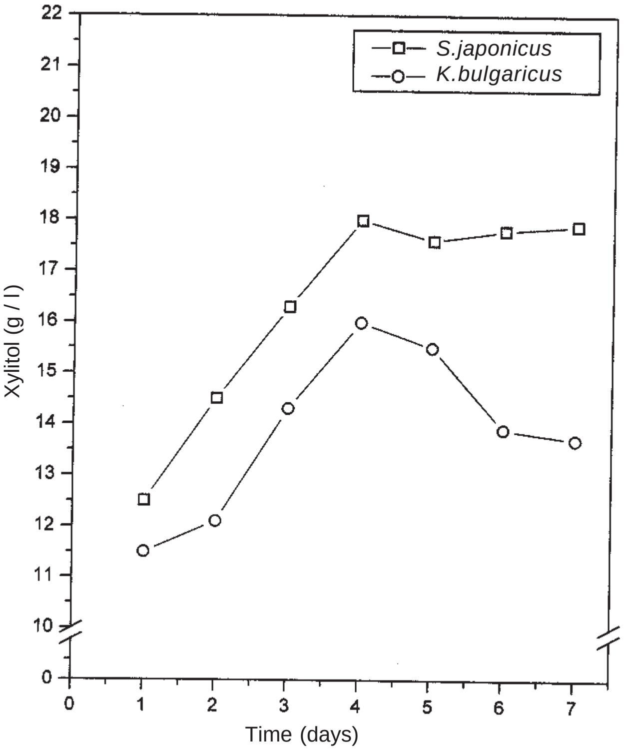


Fig. 2 Time course of xylitol production.

conversion of xylose to xylitol is about 90% at pH 7.5 by *C. pelliculosa*. On the other hand, Du Preez *et al* (1986) reported that *C. shehatae* produced considerable amounts of xylitol at pH 3.5 - 4.5.

Carbon source. The data illustrated in Fig 1 clearly indicates that the formation of xylitol was steadily increased with the increase of the corn cobs. Maximum xylitol outputs were recorded with *S. japonicus* and *K. bulgaricus* at the highest hydrolyzate level namely 110 g / l. Our results are in agreement with the results of Yoshitake *et al* (1973) who reported that the concentration of xylitol increased with the increase of xylose up to 10% in culture medium of *Enterobacter*. Du Preez *et al* (1986) reported that at concentration of 100 g/l they obtained 31.9 g / l xylitol and Zagustina *et al* (2002) reported tht 150 g /l concentration and limited aeration favours the reduction of xylose.

Nitrogen nutrition. Different sources and concentrations of nitrogen were tested in relation to their effect on the production of xylitol from hydrolyzate xylan corn cobs by the experimental organism. As shown in Table 4, the tested ammonium salts and urea except ammonium nitrate are more suitable for xylitol production. The optimum concentration of NH_4Cl seems to be 1.0, 2.0 g / l, wherein relatively high xylitol yields were maintained at this N_2 level in case of *S. japonicus*

Table 5

Effect of yeast extract concentration on xylitol production

Yeast extract conc. (g / l)	Consumed sugar %		Xylitol g/l	
	S. japonicus	*K. bulgaricus*	*S. japonicus*	*K. bulgaricus*
2.50	47.16	63.52	5.86	4.30
5.00	64.00	66.59	8.63	5.70
10.00	67.85	67.61	12.40	5.90
15.00	74.43	66.59	11.60	9.21

- Yeast extract ranged from 2.5 - 15 g / l; - corn cobs hydrolyzate 88 g / l; - pH 9 and 8 for *S. japonicus* and *K. bulgaricus*, respectively.; - 30˚C for 4 days.

and *K. bulgaricus* wherein $(NH_4)_2SO_4$ the concentration of 0.5 g / l favours xylitol production in both *S. japonicus* and *K. bulgaricus*. Holzer and Witt (1960) reported that ammonium salts seem to stimulate the oxidative pentose-phosphate pathway in *S. cerevisiae*.

Effect of organic nitrogen sources on xylitol production. The results in Table 5 reported the superiority of the medium, corn cobs hydrolyzate and containing of different concentrations of yeast extract for xylitol production. Where, relatively higher xylitol yields were obtained both in case of *S. japonicus* and *K. bulgaricus*. However, maximum yields were obtained by *S. japonicus* at 10 g / l yeast extract and that of 15 g / l for *K. bulgaricus*. In agreement with our results, Ahmed (1991) and Hottori and Suzuki (1974) described that 10 g/l gave the optimal concentration, of mannitol and erythritol. Contrary to these results, Hajany (1964) found that at highest concentration, a very poor arbitol production was obtained.

Time course of xylitol production. The time course of xylitol production by the tested yeasts using the most favourable medium composed of (g / l): NH_4Cl, 1; yeast extract, 5; NaCl, 3; $MgSO_4.7H_2O$, 1; KH_2PO_4, 3 and hydrolyzate xylan corn cobs (unpublished data) was studied (Fig 2). The xylitol production reached maximum after 4 days for both *S. japonicus* (18 g / l) and *K. bulgaricus* (16 g / l) at carbon source xylose (88 g / l) from hydrolyzate corn cobs. In agreement with his results Cao *et al* (1994) found that maximum xylitol was obtained after 96 h from 260 g / l xylose.

Assessment of the presence of xylose and xylitol. Both paper chromatography and HPLC were used to analyze the product present in the alcoholic extract.

The spots were visualized on paper chromatogram by spraying with KIO - benzidine reagent. The spots were closely agreed with authentic xylitol for both strains. While, xylose was detected by aniline hydrogen phthalate reagent. The HPLC analysis assessed the presence of xylose and xylitol which were separated at different time intervals of 7 and 13 min, respectively, identical to the authentic samples.

Conclusion

Schizosaccharomyces japonicus and *Kluyveromyces bulgaricus* were the most potent microorganisms to produce xylitol from hydrolyzate xylan corn cobs using static technique at pH 9, 8 for *S. japonicus* and *K. bulgaricus,* respectively. The maximum productivity was reached on using 110 g / l carbon source and ammonium chloride after four days fermentation. Xylose and xylitol were identical with authentic samples when analyzed using HPLC.

References

Ahmed Y M 1991 Biochemical studies on the use of farm by-products for the production of ketosugars and sugar alcohols by microorganisms. Ph.D. Thesis, Faculty of Agric., Cairo University, Egypt.

Bruinenberg P 1986 The NADP(H) redox couple in yeast metabolism. *Anto. Van Leeuwenhoek* **52** 411 - 429.

Cao N J, Tang R, Gong C S, Chem L F 1994 The effect of cell density on the production of xylitol from D-xylose by-yeast. *Appl Biochem Biotechnol* **45 - 46** 515 - 519.

Du Preez J C, Bosch M, Prior B A 1986 Xylose fermentation by *Candida shehatae* and *Pichia stipitis:* Effect of pH, temperature and substrate concentration. *Enzyme Microb Technol* **8** 360 - 364.

Faria L F, Gimenes M A, Nobrega R, Pereira N Jr 2002 Influence of oxygen availability on cell growth and xylitol production by *Candida guilliermondii. Appl. Biochem Biotechnol* **98 - 100** 449 - 458.

Hajany G J 1964 D-Arabitol production by *Endomyces chodati. Appl Microbiol* **12** 87 - 92.

Hattori K, Suzuki T 1974 Production of erythritol by n-alkalne grown yeasts. *Agric Biol Chem* **38** 581 - 586.

Holzer H, Witt I 1960 Bschleunigung des oxidativen pentosephosphatcyclus in Hefezellen durch moniumsalze. *Biochim Biophys Acta* **38** 163 - 164.

Jeffries J E 1983 Utilization of xylose by bacteria, yeasts and fungi. *Adv Biochem Engin Biotechnol* **27** 1 - 32.

Jeffries T W 1985 Emerging technology for fermentation D-xylose. *Trends Biotechnol* **3** 208 - 212.

Kim J H, Han K C, Koh Y N, Ryn, Y W, Sea J H 2002 Optimization of fed batch fermentation of xylitol production by *Candida tropicalis. J Ind Microbiol Biotechnol* **29** 16 - 19.

Kitpreechavanich V, Hayashi M, Nishio N, Nagaishi S 1984 Conversion of D-xylose into xylitol by xylose reductase from *C. pelliculose* coupled with the oxidoreductase system of methanagen strain. *Hu Biotechnol Lett* **6** 651 - 656.

Leathers T D 2003 Bioconversion of maiz residues to value added co-products using yeast like fungi. *FEM Yeast Res* **3** 133 - 140.

Ligthelm M E, Prior B A, Du Preez J C, Brandt V 1988 The oxygen requirement of yeasts for the fermentation of D-xylose and D-glucose to ethanol. *Appl Microbiol Bio-technol* **28** 63 - 68.

Moore W E, Effland M J, Johnson D B, Daughorty M N, Schwerdtfeger E J 1960 Chromatographic analyses of sugar alcohols and glycols. *Appl Microbiol* **8** 169 - 173.

Neish A C 1952 *Analytical Methods for Bacterial Fermentation.* 2nd rev. Natl. Research Council Can., Praire Regional Lab., Saskatoon, Saskatchewan N.R.C. Canada.

Ssnchez S, Bravo V, Castro E, Moya A J, Camacho F 1997 Influence of pH and aeration rate on the fermentation of D-xylose by *Candida shehatae*. *Enzyme Microb Technol* **21**(5) 355 - 360.

Somogyi M 1952 Notes on sugar determination. *J Biol Chem* **195** 19 - 23.

Walthers T, Hensirisak P, Agblervor F A 2001 Model compound studies: Influence of aeration and hemicellulosic sugars on xylitol production by *Candida tropicalis*. *Appl Biochem Biotechnol* **91 - 93** 423 - 435.

Watson N E, Prior B A, Lategan P M 1984 Factors in acid treated bagasse inhibiting ethanol production from D-xylose by *Pachysolen tannophilus*. *Enzyme Microb Technol* **6** 451 - 456.

Whislher R L 1963 *Methods Carbohydrate Chem*., **1** 88 - 90.

Yoshitake J, Ishizaki H, Shimamura M, Imai T 1973 Xylitol production by *Enterobacter* sp. *Agric Biol Chem* **35** 905 - 911.

Zagustina N A, Rodonova N A, Mestechkina N M, Secherbukhin V D, Bezborodov A M 2001 Fermentation of xylitol in *Candida guilliermondii* 2581 culture. *Prikl Biokim Mikrobiol* **37** 573 - 577.

Pak. J. Sci. Ind. Res. 2003 **46**(6) 471 - 472

ANTIBACTERIAL ACTIVITY OF *EUPHORBIA HETEROPHYLLA* LINN (FAMILY - EUPHORBIACEAE)

Falodun A[*a], E O P Agbakwuru[a] and G C Ukoh[b]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*

[b] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*

(Received September 28, 2001; accepted September 9, 2003)

Dried leaves of *Euphorbia heterophylla* were used for extraction with three different solvents namely petroleum ether (60-80°C), butanol and ethanol. An *in vitro* antibacterial activity of the plant extracts were evaluated using the agar-diffusion method. The butanolic extract exhibited marked inhibitory action on the growth of *Escherichia coli*, NCTC 10418, *Staphylococcus aureus* NCTC 6571, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* at 100 mg/ml.

For a very long time, man has been dependent on plants not only as valuable sources of food, drinks and shelter but have effectively used plants for the well being since his creation (Sofowora 1981 & 1982). *Euphorbia heterophylla* L. (spurge weed) is a weak annual weed growing abundantly in Nigeria in semi-humid places, especially in cassava plantation (Trease & Evans 1989). It is commonly used in southern Nigeria as purgative remedy. According to a traditional medical practitioner, the leaves of the plant are popularly used to "wash out" the bowel. For this purpose, an aqueous extract of the leaves is used to prepare food usually yam porridge or is taken alone and purgation ensues after about 4 h (Oksuz *et al* 1994). The antinociceptive activity of the roots of *Euphorbia heterophylla* has also been reported (Vamsidhar *et al* 2000).

A decoction of the leaves is also used by herbal healers to treat stomach disorders and constipation. This study was, therefore, undertaken to investigate the antibacterial activity of the leaves of *Euphorbia heterophylla* against some selected bacteria.

Plant materials. The leaves of *Euphorbia heterophylla* were collected from the main campus of the University of Benin, Edo state, Nigeria for this study. The leaves were sun-dried and pulverized using a mechanical grinder. Ether (60-80°C), butanol and ethanol were used for extraction.

Extraction method. The dry powder of the leaves (950g) was used separately for extraction with 500 ml petroleum ether, 300 ml ethanol and 400 ml butanol using a long glass

Table 1
Antibacterial activity

Extracts	*E. coli*	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Petroleum ether					
50 mg/ml	+	+	+	+	+
100 mg/ml	+	+	+	+	+
150 mg/ml	+	+	+	+	+
200 mg/ml	+	+	+	+	+
DMSO	+	+	+	+	+
Butanolic extract					
50 mg/ml	+	+	+	+	+
100 mg/ml	-	-	-	-	-
150 mg/ml	-	-	-	-	-
200 mg/ml	-	-	-	-	-
DMSO	+	+	+	+	+
Ethanolic extract					
50 mg/ml	+	+	+	+	+
100 mg/ml	+	+	+	+	+
150 mg/ml	+	+	+	+	+
200 mg/ml	+	+	+	+	+
DMSO	+	+	+	+	+

+ ; Indicates presence of growth, - ; Indicates absence of growth.

---

*Author for correspondence

Table 2
Inhibitory effect of butanolic extract of *Euphorbia heterophylla*

Butanolic extracts	Zones of Inhibition (mm)				
	E. coli NCTC 10418	*K. pneumoniae*	*S. aureus* NCTC 6571	*P. aeruginosa*	*B. subtilis*
50 mg/ml	-	-	-	-	-
100 mg/ml	15	16	16	14	15
150 mg/ml	25	20	20	20	25
200 mg/ml	28	25	28	25	28
DMSO	-	-	-	-	-

- ; Growth without inhibition.

column (11 mm in diameter) at room temperature for 72 h. The extracts were concentrated by using rotary evaporator (Rota-vapour Buchi, AG-CH 9230, SWISS).

Test bacteria. The test bacteria used in this study were *Esherichia coli* NCTC 10418, *Klebsiella pneumoniae, Staphyllococcus aureus* NCTC 6571, *Pseudomonas aeruginosa and Bacillus subtillis.* They were obtained from the department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Nigeria.

Antibacterial assay. Molten nutrient agar (25 ml) was poured in each petri dish and allowed to solidify. The antibacterial activity of the different extracts was demonstrated using the method originally described by Bauer *et al* (1966) which is widely used for antibiotic susceptibility testing (Barry & Thornsberry 1985). The overnight cultures of the bacteria diluted to 10^6 which was used to flood each of the five nutrient agar plates. Sterile cork borer (7 mm) was used to make five wells in each of the agar plates of the organism were used to flood each of the five nutrient agar plates, and the excess was poured away into discarded jar. The 7 mm sterile cork borer (6 mm) was used to make five wells in each of the agar plates. The wells were filled with 0.1 ml, 0.2 ml, 0.3 ml and 0.4 ml, respectively, of the different extracts in dimethylsulphoxide, DMSO (control). The fifth well was filled with 0.3 ml of DMSO.

Only the butanolic extract exhibited inhibition of the test bacteriae (Table 1). The butanolic extract exhibited marked inhibitory activity on *Esherichia coli*, NCTC 10418, *Staphylococcus aureus* NCTC 6571, *Klebsiella pneumoniae* and *Pseudomonoas aeruginosa* (Table 2). The minimum inhibitory concentration (MIC) was 100 mg/ml. It was observed that the inhibitory effect increased with concentration as shown in Table 2. The sensitivity of the test organisms to the butanolic extract of *Euphorbia heterophylla* justified the claims by traditional herbalists that it is useful in the treatment of stomach disorders (caused by bacterial infection) in the local community.

Key words: *Euphorbia heterophylla*, Extracts, Antibacterial activity.

References

Barry AL, Thornsberry C 1985 Susceptibility tests: Diffusion test procedures. In: *Manual of Clinical Microbiology,* Lennette EH, Balows A, Hausller WJ, Shodomy H J (Eds), Washington DC, USA, pp 978 - 987.

Bauer AW, Kiby W M, Sherris JC, Turok M 1966 Antibiotic susceptibility testing by a standardised single disc method. *American Clinical Pathology* **5** 493 - 496.

Oksuz S, Roberto R, Gill H C, John MP, Geoffrey C, Ayhen U 1994 Biologically active compounds from Euphorbiaceae II. *Planta Medica* **60** 594.

Sofowora E A 1981 *Man, Plants and Medicine in Africa, Some Fundamental Perspective.* Inaugural lecture series 48, University of Ile-Ife press, Nigeria, pp 4 - 7.

Sofowora E A 1982 In: *Medicinal Plants and Traditional Medicine in Africa.* John Wiley and Sons, New York, USA, pp 1 - 20.

Trease E G, Evans WC 1989 In: *Pharmacognosy*. Balliere Tindall, London, 13th Edition, pp 167 - 235.

Vamsidhar I, Mohammed A H, Nataraj B, Madhusudana Rao C, Ramesh M 2000 Antinociceptive activity of *Euphorbia heterophylla* roots. *Fitoterapia* **71**(5) 562 - 563.

Technology

Pak. J. Sci. Ind. Res. 2003 ***46****(6) 473 - 476*

WRENCH ANALYSIS FOR 3-D MODEL USED IN ROBOTIC END-EFFECTOR

Zulfiqar Ali Soomro

Mechanical Engineering Department, Quaid-e-Awam University of Engineering Sciences & Technology Nawabshah, Pakistan

(Received June 8, 2002; accepted April 18, 2003)

In this paper, wrench analysis of a new proposed 3-D robotic model is discussed and applied. The model is basically used for calculating applied force through known spring stiffnesses and concerned compressive displacements. The wrench is correlated by already determined Jacobian matrix with global displacements. Local displacements are determined practically by applying load vertically in centrer. The global displacements (taken as reference) are calculated by congruence matrix through wrench analysis and shown. The theoretical relationship between global displacements and individual local displacements is also calculated and shown. Besides this practical determination of the wrench analysis is also verified by applying force on any leg of the model.

Key words. Wrench analysis, 3 - D robotic model.

Introduction

To calculate the wrench of any manufactured 3-D model is an extreme work rather than its twist. This is the model, which is used to determine its wrench analysis due to its turning affect from its three directions. An important elastic relationship is obtained of individual contact forces with externally applied global wrench. The wrench equation can be used to solve the forces in any statically indeterminate grasp problem. The significance of this relationship will be emphasized in the subsquent section (Kerr *et al* 1991).

To achieve the desired in-grasp manipulation, some preload has to be applied along some of the contacts in order to produce the effective global wrench 'w'. The present interest is to achieve a practicable solution such that a desired manipulation of objects can be achieved by preloading the minimum number of contacts with minimum possible preloads. It should be noted that the twist is in the axis coordinates that are its translational terms appear before rotational (Ghaffor and Kerr 1992).

δW is an infinitesimal wrench in ray coordinates in the form of δf, δm, and δd that is the infinitesimal twist of the grasped object in ray coordinates (Ghaffor *et al* 2000). The parameters of external infinitesimal wrench and body infinitesimal twist can be given by defining δf as force vector of (δfx, δFy, δFz) and δM as moment vector of (δMx, δMy, δMz) and δd as vector of translational displacements of (δ x, δy, δz).

A grasp with this stiffness matrix provides a restraint along six degrees of freedom when an object is subjected to an external wrench. The grasp arrangement does not have capability to induce fine motion in full dexterity and in particular cannot manipulate the object along z-axis. This can be visualized by substituting relationship of vector of preload magnitude δf and δw (Ghaffor *et al* 2000).

Any twist vector in the twist space under the mapping is a linear combination of these six twist vectors which correspond to be linearly independent. The six wrench vectors define a basis for the wrench space (Klafter *et al* 1989). Any wrench vector in this wrench space under mapping is a linear combination of these six wrench vectors.

Wrench analysis is investigated upon 3-D robotic model comprising of six legs attached with two different diameter platforms by spherical joints (Soomroza 2001). These joints give six degrees of freedom (three translatory and three rotationary) to calculate wrench matrix. A wrench is like torque having direction in x, y, z directions while wrench is the combination of force and moments in x, y, z directions.

Methodology. A 3-D model is used for grasping the object. This 3-D model is used in the end-Effector of the robotic manipulator. It possesses six legs joined by prismatic joints with inside springs as shown in Fig 1. These legs are fitted with two platforms giving six degrees of freedom. These motions are calculated by wrench analysis through applied forces 'F'stiffness [K] and compressive displacements as under.

Wrench Analysis. Since, we know that F=[K]. δd multiplying both sides by [J] we get:

$[J].F = [J].[K].\ \delta d$

or $W = [J].[K].\ \delta d$

But $\delta d = [J^t].\ \delta d$

putting this value, we get

$W = [J]\cdot[K]\cdot [J^t]\cdot \delta D$

$\therefore\ W = [K_g]\cdot \delta D$

or $\delta D = [K_g]^{-1}.W$

δD or δd then can be compared with that of practical obtained.

Where;

$[J]$ = Jacobian Matrix

F = Applied Force

$[K]$ = Stiffness Matrix

δd = Local displacement

δD = Global displacement

$[J^T]$ = Transpose of Jacobian

W = Wrench Analysis

$[K_g]$ = Congruence Matrix

From above equation we get:

i.e. $\delta w = J_g\, [k]\, J^T.\ \delta D_g$

where;

$$\delta w = \begin{bmatrix} Fx \\ Fy \\ Fz \\ Mx \\ My \\ Mz \end{bmatrix} = \begin{bmatrix} 0.00 \\ 0.00 \\ -9.81 \\ 0.00 \\ 0.00 \\ 0.00 \end{bmatrix}$$

and $J_g.[k]J^{T}$’. is called congruence transformation denoted by (K_g).

$$\begin{bmatrix} 0.00 \\ 0.00 \\ -9.81 \\ 0.00 \\ 0.00 \\ 0.00 \end{bmatrix} = \begin{bmatrix} 0.4330 & 0.0000 & -0.5000 & -0.4330 & -0.0000 & 0.5000 \\ 0.2500 & 0.5000 & 0.0000 & -0.2500 & -0.5000 & -0.0000 \\ 0.8660 & 0.8660 & 0.8660 & 0.8660 & 0.8660 & 0.8660 \\ -48.1180 & -96.2371 & -48.1180 & 48.1180 & 96.2371 & 48.1180 \\ 83.3430 & 0.0000 & -83.3430 & -83.3430 & 0.0000 & 83.3430 \\ 0.0000 & 0.0000 & -27.781 & 0.0000 & 0.0000 & -27.781 \end{bmatrix} \begin{bmatrix} 1.4098 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 1.4677 & 0.0000 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.0000 & 1.4335 & 0.0000 & 0.0000 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 3.6021 & 0.0000 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 1.3278 & 0.0000 \\ 0.0000 & 0.0000 & 0.0000 & 0.0000 & 0.0000 & 1.3328 \end{bmatrix}$$

$$\begin{bmatrix} 0.4330 & 0.2500 & 0.8660 & -48.1185 & 83.3438 & 0.0000 \\ 0.0000 & 0.5000 & 0.8660 & -96.2371 & 00.0000 & 0.0000 \\ -0.5000 & 0.0000 & 0.8660 & -48.1185 & -83.3438 & -27.7810 \\ -0.4330 & -0.2500 & 0.8660 & 48.1185 & -83.3438 & 0.0000 \\ -0.0000 & -0.5000 & 0.8660 & 96.2371 & 00.0000 & 0.0000 \\ 0.5000 & -0.0000 & 0.8660 & 48.1185 & 83.3438 & -27.7810 \end{bmatrix} \begin{bmatrix} \delta D_{g1} \\ \delta D_{g2} \\ \delta D_{g3} \\ \delta D_{g4} \\ \delta D_{g5} \\ \delta D_{g6} \end{bmatrix}$$

$$\begin{bmatrix} 0.00 \\ 0.00 \\ -9.81 \\ 0.00 \\ 0.00 \\ 0.00 \end{bmatrix} = 1.0e{+}004\text{x} \begin{bmatrix} 0.0002 & 0.0001 & -0.0001 & -0.0038 & 0.0296 & 0.0001 \\ 0.0001 & 0.0001 & -0.0000 & -0.0195 & 0.0104 & -0.0000 \\ -0.0001 & -0.0000 & 0.0008 & 0.0075 & -0.0166 & -0.0067 \\ -0.0038 & -0.0195 & 0.0075 & 4.3900 & -0.9006 & 0.0135 \\ 0.0296 & 0.0104 & -0.0166 & -0.9006 & 5.4029 & 0.0233 \\ 0.0001 & -0.0000 & -0.0067 & 0.0135 & 0.0233 & 0.2135 \end{bmatrix} \begin{bmatrix} \delta D_{g1} \\ \delta D_{g2} \\ \delta D_{g3} \\ \delta D_{g4} \\ \delta D_{g5} \\ \delta D_{g6} \end{bmatrix}$$

$$\begin{bmatrix} \delta D_{g1} \\ \delta D_{g2} \\ \delta D_{g3} \\ \delta D_{g4} \\ \delta D_{g5} \\ \delta D_{g6} \end{bmatrix} = 1.0e{+}004\text{x} \begin{bmatrix} 0.0002 & 0.0001 & -0.0001 & -0.0038 & 0.0296 & 0.0001 \\ 0.0001 & 0.0001 & -0.0000 & -0.0195 & 0.0104 & -0.0000 \\ -0.0001 & -0.0000 & 0.0008 & 0.0075 & -0.0166 & -0.0067 \\ -0.0038 & -0.0195 & 0.0075 & 4.3900 & -0.9006 & 0.0135 \\ 0.0296 & 0.0104 & -0.0166 & -0.9006 & 5.4029 & 0.0233 \\ 0.0001 & -0.0000 & -0.0067 & 0.0135 & 0.0233 & 0.2135 \end{bmatrix} \begin{bmatrix} 0.00 \\ 0.00 \\ -9.81 \\ 0.00 \\ 0.00 \\ 0.00 \end{bmatrix}$$

$$\begin{bmatrix} \delta D_{g1} \\ \delta D_{g2} \\ \delta D_{g3} \\ \delta D_{g4} \\ \delta D_{g5} \\ \delta D_{g6} \end{bmatrix} = \begin{bmatrix} -0.6131 \\ 3.3722 \\ -1.9119 \\ 0.0165 \\ -0.0048 \\ -0.0596 \end{bmatrix}$$

But we also know from equation that $\delta d_1 = [J^T_g].\ \delta D_g$

$$\begin{bmatrix} \delta d_{11} \\ \delta d_{12} \\ \delta d_{13} \\ \delta d_{14} \\ \delta d_{15} \\ \delta d_{16} \end{bmatrix} \begin{bmatrix} 0.4330 & 0.2500 & 0.8660 & -48.1185 & 83.3438 & 0.0000 \\ 0.0000 & 0.5000 & 0.8660 & -96.2371 & 00.0000 & 0.0000 \\ -0.5000 & 0.0000 & 0.8660 & -48.1185 & -83.3438 & -27.7812 \\ -0.4330 & -0.2500 & 0.8660 & 48.1185 & -83.3438 & 0.0000 \\ -0.0000 & -0.5000 & 0.8660 & 96.2371 & 00.0000 & 0.0000 \\ 0.5000 & -0.0000 & 0.8660 & 48.1185 & 83.3438 & -27.7812 \end{bmatrix} \begin{bmatrix} -0.6131 \\ 3.0656 \\ -1.9119 \\ 0.0251 \\ -0.0144 \\ -0.0948 \end{bmatrix} \begin{bmatrix} -2.2697 \\ -1.5543 \\ -0.0865 \\ -1.0418 \\ -1.7572 \\ 0.0865 \end{bmatrix} \text{mm}$$

Putting these values of 'δD_g' on applying load of 9.81 N centrally in above equation

i.e. $w = [K_g] \cdot \delta D_g$

$$\begin{bmatrix} Fx \\ Fy \\ Fz \\ Mx \\ My \\ Mz \end{bmatrix} = 1.0e + 004 \times \begin{bmatrix} 0.0002 & 0.0001 & -0.0001 & -0.0038 & 0.0296 & 0.0001 \\ 0.0001 & 0.0001 & -0.0000 & -0.0195 & 0.0104 & -0.0000 \\ -0.0001 & -0.0000 & 0.0008 & 0.0075 & -0.0166 & -0.0067 \\ -0.0038 & -0.0195 & 0.0075 & 4.3900 & -0.9006 & 0.0135 \\ 0.0296 & 0.0104 & -0.0166 & -0.9006 & 5.4029 & 0.0233 \\ 0.0001 & -0.0000 & -0.0067 & 0.0135 & 0.0233 & 0.2135 \end{bmatrix}$$

$$\begin{bmatrix} -0.6131 \\ 3.3722 \\ -1.9119 \\ 0.0165 \\ -0.0048 \\ -0.0596 \end{bmatrix} = \begin{bmatrix} 0.3591 \\ 0.1642 \\ -10.0248 \\ -20.0884 \\ 66.0294 \\ 0.2422 \end{bmatrix} \text{ in 'N' for forces and N - mm for moments}$$

Practical value of Wrench (δW). We can also determine the values of wrench practically by applying on un-known force.

Suppose, we are applying un-known force to any leg of the model. On doing this action, we observe some observations as 2.75 mm on scale as local co-ordinate (δd_{LI}).

For finding the first value of force (F_1), we use the following formula:

$F_1 = [k] \cdot \delta d_{L1}$

$F_1 = [1.4098] \cdot (2.75) = 3.877$ N

Put above value in equation

i.e.

$\delta W = [J_g] \cdot [F]$

$$\begin{bmatrix} Fx \\ Fy \\ Fz \\ Mx \\ My \\ Mz \end{bmatrix} = \begin{bmatrix} 0.4330 & 0.0000 & -0.5000 & -0.4330 & -0.0000 & 0.5000 \\ 0.2500 & 0.5000 & 0.0000 & -0.2500 & -0.5000 & -0.000 \\ 0.8660 & 0.8660 & 0.8660 & 0.8660 & 0.8660 & 0.8660 \\ -48.1180 & -96.2370 & -48.1180 & -48.1180 & 96.2371 & 48.118 \\ 83.3430 & 0.0000 & -83.3430 & -83.3430 & 00.0000 & 83.343 \\ 0.0000 & 0.0000 & -27.7810 & 0.0000 & 00.0000 & -27.781 \end{bmatrix} \begin{bmatrix} 3.877 \\ 0.000 \\ 0.000 \\ 0.000 \\ 0.000 \\ 0.000 \end{bmatrix}$$

$$\begin{bmatrix} Fx \\ Fy \\ Fz \\ Mx \\ My \\ Mz \end{bmatrix} = \begin{bmatrix} 0.1711 \\ 0.0988 \\ 0.3422 \\ -19.0160 \\ 32.9367 \\ 0.0000 \end{bmatrix} \text{ N for forces and N - mm for moments}$$

From above are required values of Force (Fx, Fy, Fz) and Torque (Mx, My, Mz) in shape of wrench (δw). We have also considered the value of stiffness (K_{av1}) Leg (1) as 1.4098 N/mm.

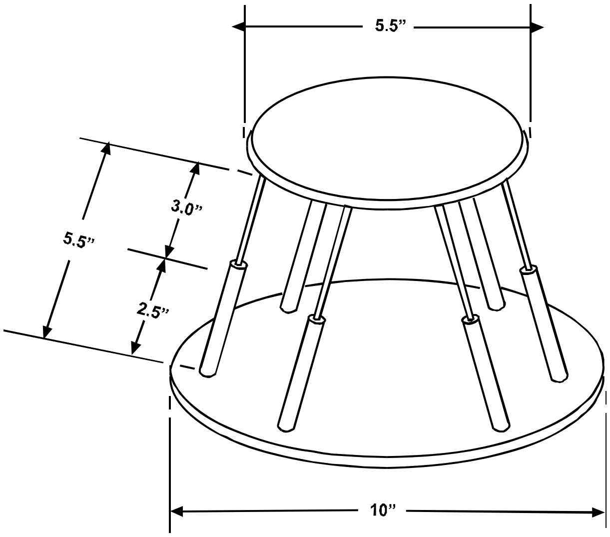


Fig 1. Front view of proposed 3-D Robotic Model.

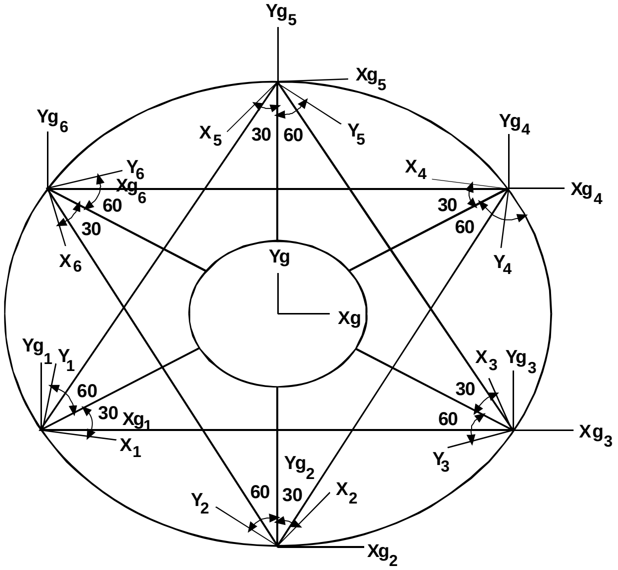


Fig 2. Correlation of Local and Global Displacements.

Results and Discussion

The structural design of the model is described briefly as above is shown in Fig 1. In Fig 2, brief transformation of local displacements into global displacements is shown. These local and global displacements are correlated and calculated by using wrench analysis in above sections. However, load was applied vertically and centrally downward. Parallel to z-axis initially. Thus, local displacements were determined experimentally as in Table 1. Then global displacements are calculated by using the relationship through wrench analysis as in Table 2. Thus, theoretical results of local displacements from global displacement by congruence matrix of wrench analysis are achieved. A practical wrench analysis in shape of wrench matrix has been calculated practically in above sections. Also, wrench

Table 1
Experimental readings

S.No.	Load applied (W) Centrally in Newton (N)	Displacement (δd) measured along each leg in mm					
		δd_1	δd_2	δd_3	δd_4	δd_5	δd_6
1	(0, 0, - 9.81, 0, 0, 0)	- 1.50	- 1.50	- 1.30	- 1.40	- 1.30	-1.20
2	(0, 0, - 19.62, 0, 0, 0)	- 1.74	- 1.81	- 1.53	- 1.72	- 1.69	-1.65
3	(0, 0, - 29.43, 0, 0, 0)	- 1.91	- 1.89	- 1.82	- 1.79	- 1.75	-1.73

Table 2
Theoretical results

S.No.	Load applied (W) centrally in Newton (N)	Global displacement (δD) determined along each leg in mm (using relation $\delta D = [Kg]^{-1} \cdot W$)	Local displacement (δd) calculated along each leg in mm (using equation $\delta d = [J^t]. \delta D$)
1	(0, 0, - 9. 81, 0, 0, 0)	(- 0.6131, 3.3722, -1.9119) 0.0165, - 0.0048, - 0.0596)	(- 2.2697, - 1.5543, - 0.0865 - 1.0418, - 1.7572, 0.0865)
2	(0, 0, - 19.62, 0, 0, 0)	(- 1.2263, 6.7444, - 3.8238 0.0329, - 0.0096, - 0.1192)	(- 4.5395, - 3.1086, - 0.1731 - 2.0836, - 3.5145, 0.1731)
3	(0, 0, - 29.43, 0, 0, 0)	(- 4.5395, - 3.1086, - 0.1731) - 2.0836, - 3.5145, 0.1731)	(- 6.8092, - 4.6629, - 0.2596) - 3.1254, - 5.2717, 0.2596)

matrix depending upon elements of forces and moments in x,y,z directions have also been determined in above sections. In this way global displacements are converted into local displacements by already calculated Jacobian matrix in Table 2.

In matrix from, wrench analysis is shown by six elements. First three elements represent force analysis in X, Y and Z directions. While last three elements of matrix represent the moments in concerned directions.

Conclusion

The wrench analysis is applied and calculated on a new 3-D model shown in Fig 1. It can be fitted into robotic End-Effector. For this, concerned wrench equations are discussed. The relative factors are calculated and investigated practically as well as theoretically. Thus, unknown applied forces and concerned moments in x, y, and z-axes are calculated. The global displacements are determined by congruence and wrench analysis. Then local displacements are obtained by correlation of global displacements through jacobian (J). Besides wrench analysis, this 3-D Robotic model is also used for calculating the force and local or global displacements apart from its stiffness.

References

Ghaffor A, Kerr D R 1992 “In-grasp robotic fine motion with frictionless elastic point contacts”, *J Mech Eng* SCI Pro 1 March E Part C 41 - 47.

Ghaffoor A, Dai J S, Duffy J 2002 “Fine motion control based on constraint criteria under pre-loading configurations” Journal of Robotic systems by John Wiley & Sons, Inc., USA **17** (4) 171 - 185.

Kerr D R, Sanger D J S, Duffy, J 1991 “Redundant grasps, redundant manipulators, and their dual relationship.” *J Robotic Systems* RS 459.

Ghaffoor A, Dai J S, Duffy J, *Grasp stiffness matrix for soft finger contact model in robotic applications”* 2000 ASME Design Engineering Technical Conference, September 10 - 13, 2000 Baltimore, Maryland, USA.

Klafter R D, Tachnielewski, Michael Negin, 1989 *Robotics Engineering, An Integrated Approach, Prentice Hall, Ist ed.*

Soomro Z A, “Design Modeling & Simulation of Robotic End-Effector For In-grasp Fine Manipulation Using Parallel compliant Mechanism” Msc-26 Thesis, College of Electrical and Mechanical Engineering National University of Science and Technology Rawalpindi, Pakistan-2001.

CONTENTS OF VOL. 46

Vol. 46(No.1) January - Feburary, 2003

PHYSICAL SCIENCES

SHORT COMMUNICATION

BIOLOGICAL SCIENCES

SHORT COMMUNICATION

TECHNOLOGY

Vol. 46(No.2) March - April, 2003

PHYSICAL SCIENCES

BIOLOGICAL SCIENCES

SHORT COMMUNICATION

TECHNOLOGY

Vol. 46(No.3) May - June, 2003

PHYSICAL SCIENCES

SHORT COMMUNICATION

BIOLOGICAL SCIENCES

SHORT COMMUNICATIONS

TECHNOLOGY

REVIEW ARTICLE

Vol. 46(No.4) July - August, 2003

PHYSICAL SCIENCES

SHORT COMMUNICATIONS

BIOLOGICAL SCIENCES

SHORT COMMUNICATION

TECHNOLOGY

Vol. 46(No.5) September - October, 2003

PHYSICAL SCIENCES

BIOLOGICAL SCIENCES

TECHNOLOGY

Vol. 46(No.6) November - December, 2003

PHYSICAL SCIENCES

SHORT COMMUNICATIONS

BIOLOGICAL SCIENCES

SHORT COMMUNICATION

TECHNOLOGY

AUTHOR INDEX TO VOLUME 46

SUBJECT INDEX TO VOLUME 46

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
PCSIR - Scientific Information Centre
PCSIR Laboratories Campus, Karachi - 75280, Pakistan
Ph:92 - 021 - 8151739 - 43, Fax:92 - 021 - 8151738, E-mail: pcsirsys@super.net.pk and pcsir-sic@cyber.net.pk

EXCHANGE FORM

We wish to receive Pakistan Journal of Scientific and Industrial Research in exchange of :

Name of Journal: ____________________
Frequency: ____________________
Subjects Covered: ____________________
Institution: ____________________
Address: ____________________
____
____

Signature: ____________________
Name: ____________________
Designation: ____________________
Date: ____________________
E-mail: ____________________
Fax: ____________________
Phone: ____________________

PAKISTAN JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
PCSIR - Scientific Information Centre
PCSIR Laboratories Campus, Karachi - 75280, Pakistan
Ph:92 - 021 - 8151739 - 43, Fax:92 - 021 - 8151738, E-mail: pcsirsys@super.net.pk and pcsir-sic@cyber.net.pk

SUBSCRIPTION FORM

I / we wish to subscribe to ‘Pakistan Journal of Scientific and Industrial Research’. The filled in proforma is being returned for compliance.

Subscriber’s data:
Name: ____________________
Address: ____________________
____
____
E-mail: ____________________
Fax: ____________________
Phone: ____________________

Signature: ____________________
Order No. (if any): ____________________

Tick the relevant box: ☐ Send invoice ☐ Bill later on ☐ Cheque for ....enclosed

Subscription Rates: Local: Rs. 350/ = per copy; Rs. 2000/ = per volume
Foreign: US$ 70/ = per copy; US$ 400/ = per volume

Payment should be made through cross cheque in favour of Pakistan Journal of Scientific and Industrial Research and mailed to the Director PCSIR - Scientific Information Centre, Pakistan

INSTRUCTIONS TO AUTHORS

Pakistan Journal of Scientific and Industrial Research publishes original research in all scientific and technological fields in the form of **research articles, short communications** and critical **reviews**. All manuscripts are evaluated by subject experts. However, authenticity of the contents will be the sole responsibility of the authors.

The papers should be submitted with a covering letter signed by all the authors, with their names, addresses and highest qualifications, indicating the corresponding author and certifying that the paper has neither been, nor will be sent elsewhere for publication. Names and addresses of four experts of the relevant field including two foreign referees should also be provided. In case a paper is withdrawn after registration, the author/s will be billed for service charges.

Three legible double spaced typed copies of the manuscript are to be submitted containing, in order, **Running Title** (not more than 60 characters), **Title** of the article, full names, addresses, telephone numbers, fax numbers, e-mail addresses of all the **Authors, Abstract** (not more than 150 words), three or more **Key Words, Introduction, Materials and Methods, Results and Discussion, Illustrations** and **References**.

All **illustrations** including figures, drawings and photographs should be of good quality, suitably labelled, captioned, numbered and referred to in the text alongwith **tables** and **references**. The **drawings** should be in black ink, on art or tracing paper, drawn to fit in single or double columns (8 or 16.5 cm) on reduction. Letterings and symbols should be of sufficient size so as to be clearly legible on reduction. Use IUPAC rules for **units** and their **abbreviations**.

Three sets of **original coloured or black & white photographs(s)**, mounted on light weight white card board, should be provided. In case of coloured photographs(s), 50% printing expenses would have to be borne by the author(s).

References should be cited in **the text** by the last name of the author (both authors if only two, the first author and *et al* when more than two) followed by the year, in chronological order.

All **references** in the bibliography should be listed in alphabetical order of the authors' last names followed by date of publication and other complete details as given in the examples.

EXAMPLES

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.

Two hard copies of the revised paper should be sent along with an electronic copy in Page Maker 6, 6.5, MS Word, Excel, Word Perfect 5.1, IMB compitable. Responsibility for any error/omission in the paper lies with the author.

The **manuscripts** should be addressed to:

Executive Editor, Pakistan Journal of Scientific and Industrial Research, Scientific Information Centre, PCSIR Laboratories Campus, Karachi-75280, Pakistan.
Phone: (92-021) 8151739, 8151741-3,
Fax: (92-021)-8151738
E-mail: pcsirsys@super.net.pk and pcsir-sic@cyber.net.pk