REPORT OF THE RESEARCH PROJECT ON

EVALUATION OF ENVIRONMENTAL $N0_2$, $C0_2$, BENZENE AND LEAD EXPOSURES OF KOLKATA POPULATION BY BIOLOGICAL MONITORING TECHNIQUES.

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ACKNO WLEDGEM ENTS

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EVALUATION OF ENVIRONMENTAL NO_z , CO_z , BENZENE AND LEAD EXPOSURES OF KOLKATA POPULATION BY BIOLOGICAL MONITORING TECHNIQUES

INTRODUCTION

Pollution of urban environment has assumed extremely serious proportion in most of the major cities of the country. A survey by Central Pollution Control Board has identified 23 Indian cities to be critically polluted. Amongst them the air pollution of Kolkata is most alarming [1]. With the development of unplanned urbanization & industrialization, steady rise of vehicular population associated with lesser proportion of road -space and extremely high density of residential population, the problem of environment pollution in Kolkata is extremely critical. Environmental monitoring at different parts of the city indicates a steady rise of almost all types of air-pollutants, specially of the concentrations of Nitrogen dioxide (NO2) , Carbon dioxide (CO2) , Lead and Benzene due to emissions from different factories / industries, automobiles, fossil fuel burning, domestic combustion sources etc. As for example, recent air-monitoring survey conducted by West Bengal Pollution Control Board detected average concentration of Benzene level in ambient air being above the WHO guideline standard for air quality (5-20 ug/m^3) and also observed an increasing trend in the level of Oxides of Nitrogen (NOx) concentrations at different areas of Kolkata-city [2] .

The health effects of the air pollutants cover a wide spectrum of biological response , the more severe effects such as chronic illness / death manifested in a relatively small proportion of the population , while many more individuals in an exposed community responded with altered physiologic or pollutant burden [3] .

Oxides of Nitrogen (NOx) may be present at significant concentration in both ambient & indoor air . Main sources of emissions are combustion processes like vehicular emissions, fossil fuel power stations & domestic combustion processes and mostly emitted in the form of NO and some as NO2 . But in air , chemical reactions oxidise most of NO (a relatively non-irritating gas) to $N0_2$, which has much higher toxicity . $N0_2$ being a strong oxidant , oxidizes unsaturated lipids with peroxidases and also membrane proteins , resulting in the loss of cell permeability control . Exposure to $N0_2$ may result in dysfunction of lung defence mechanism , leading to enhanced susceptibility to resp. infection and increased airway resistance . At the NO2 exposure levels of 0.5 ppm , at which adverse effects have been detected , pathological changes have included the destruction of cilia , alveolar tissue disruption and obstruction of respiratory bronchioles . At higher levels of exposure, NO2 is known to cause serious respiratory effects including bronchospasm & pulmonary oedema .

Like NO2 , CO2 is also now recognized as being potentially toxic at low concentrations in consequence of cellular membrane effects and biochemical alterations such as increased PCO2 , increased concentration of bicarbonate ions , acidosis etc. Long term exposure to levels of CO2 between 0.5-1% may involve increased calcium deposition in body tissues including kidney associated with other altered tissue responses. In high concentrations, it may cause asphyxia , narcosis and unconsciousness .

Although use of Benzene (a widely used solvent) has declined in recent years , specially in developed countries , exposure data suggest widespread environmental exposure in developing countries due to its frequent use in chemical , printing , rubber , paint & petroleum industries and also in artisan work , shoe-manufacturing , pumping & handling of gasoline etc . Environmental exposure is also extensive , because Benzene constitutes 5-10% of unleaded gasoline by weight and presents in both inhaled & environmental tobacco-smoke [4] . The major systemic effect associated with chronic Benzene exposure in humans is depression of bone-marrow resulting in pancytopenia , sometimes progressing to aplastic anaemia . A number of case studies and epidemiologic investigations have established a casual association between inhalation exposure to benzene and leukaemia in humans . Apart from its carcinogenicity, Benzene has also genotoxicity , developmental toxicity, toxicity to reproductive system , immune system and other organs. Understanding and preventing the threat of Benzene to human health is one of the most important environmental issues at present facing National & International authorities .

Among all the toxic metals found in air , the potential public health hazard caused by Lead is unique & exceptional compared to others , because of the fact that humankind uses vast quantities of this metal and it is highly widespread throughout the environment . Lead adversely affects almost all organs and organ systems , with subcellular changes and neuro-developmental effects appearing to be the most sensitive .

However no such data are available regarding absorption or body burden due to exposure of NO_2, CO_2 ,Benzene and Lead among citizens of Kolkata . The traditional & most often used method for exposure estimation is measurement of pollutants in the ambient air i.e. Environmental monitoring . On the other hand , Biological monitoring has some distinct advantages than air measurements , mainly because it detects the absorbed exposure in human beings . Concentrations of pollutants in ambient air are not necessarily very closely related to the amounts absorbed . Hence environmental monitoring and biological monitoring are best used in combination for the estimation of exposure and body burden . Biological monitoring studies would also offer better estimates of internal exposure to human beings than environmental monitoring studies only , because biological parameters (biomarkers) related to internal exposure are more directly associated with the potential systemic adverse health effects . In view of this , it was decided to monitor biologically the ambient NO_2, CO_2 , Benzene and Lead exposures of adult Kolkata citizens by measuring their respective biomarkers like urinary hydroxyproline , blood bicarbonate , urinary creatinine & pH levels , urinary phenol and blood -lead levels with the following objectives.

OBJECTIVES

I. To assess the environmental Nitrogen dioxide (NO_2) exposure of study population by measuring urinary hydroxyproline and hydroxyproline to creatinine ratio .
II. To evaluate the internal exposure of environmental Carbon dioxide(CO_2) by measuring the blood bicarbonate concentration and pH of urine.
III. To measure the urinary phenol and phenol to creatinine ratio, as the biological marker of environmental benzene exposure.

IV. To determine the blood lead levels of the population , as one of the biological markers of environmental lead (Pb) exposure. V. To study the impact of environmental exposure of NO2, CO2, Benzene and Lead on the health status of study population by comparing the results with that of control (i.e. rural) population .

METHODOLOGY

The methodology adopted in this present investigation on "Evaluation of environmental NO2, CO2, Benzene and Lead exposures of Kolkata population by biological monitoring techniques", is furnished below in brief:

A) Sampling:

845 adult male individuals were selected through simple random technique from four different areas, of which three areas were from city of Kolkata and one from rural areas.

Study-subjects:

A total of 593 individuals were investigated as "Study-subjects" from three city-areas as follows:-

ZONE-1: 190 individuals from different urban residential areas of Kolkata, viz. Garia, Jadavpur, Kasba etc.

ZONE-2: 195 individuals from different urban commercial areas of Kolkata, viz. Bowbazar, College-Square, Tangra-Topsia etc .

ZONE-3: 208 individuals from different urban industrial areas of Kolkata viz. Dumdum, Cossipure, Belgachhia etc . **Control-subjects:**

ZONE-4: A total of 252 adult individuals were investigated as "Control-subjects" from different rural areas of District Nadia and 24 PGS (South)., West Bengal, where the air pollution sources are considered to be limited.

Considering that adult male population is exposed to the environmental pollutants more in duration & dose than their female counterparts and child population, only adult male individuals were interviewed & examined to get the data on different parameters.

B) Data Collection:

An extensive questionnaire survey was undertaken documenting age, education, family income, duration & place of stay, dietary habits, addiction, occupation, residential status, personal health complaints etc. to get data on socioeconomic and present health status. All the study and control subjects were clinically examined and urine & blood samples were collected for estimation, to get data on different clinical & biological parameters

C) *Biological Measurements:*

- Hydroxyproline , the biomarker of NO2 exposure , was estimated from the urine samples spectrophotometrically by the method as described by "Matsuki and Kasuga"[5]

- Urinary Phenol was measured colorimetrically by antipyrine colour reaction method[6]

- Urinary Creatinine was determined by using the Kits from "Glaxo- India Ltd ".

- pH of urine was detected with the help of Digital slop-corrected pH-Meter .

- Blood - Bicarbonate was estimated enzymatically by using the Kits from "Randox Laboratories Ltd".

- Blood - Lead estimations were performed by Atomic Absorption Spectrophotometry method.

Data thus collected, were compiled and statistically analysed by using Student's "f'test.

Analysis of Data regarding the socioeconomic status , clinical status and biochemical status of the subjects , surveyed in this particular research study on "Evaluation of environmental Nitrogen di-oxide , Carbon di-oxide , Benzene and Lead exposures of Kolkata population by biological monitoring techniques" , reveals the following :

SOCIOECONOMIC STATUS:

Education:

Rural subjects (Zone-4) were found to be more illiterate (31%), whereas the illiteracy level among the subjects of Zone-1 , Zone-2 and Zone-3 of Kolkata were observed to be 2%, 15%, & 6% respectively (Fig. 1) . In contrast to rural population the percentage of graduates were found higher in the subject-population of Kolkata , i.e. 60% in Zone-1, 27% in Zone-2 and 29% in Zone- 3. The drop out rate at mid-school level was found to be highest in rural areas (31%) in comparison to that of different zones of city- areas (7%,11%,& 21% in Zones 1, 2 & 3 respectively).

Income status:

Analysis of family income of the individuals in different zones revealed that urban population was economically more sound than rural population and out of them the financial status of the sample population from Zone-1 was found to be better than that of Zones 2 & 3 in Kolkata (Fig.2).

Residential status :

The residential status of the study & control subjects , in relation to type of houses , ventilation facilities and drainage facilities , is depicted in Figures 3 , 4 & 5 . In rural areas (Zone-4), most of the individuals were residing at "Kutcha" type of houses (62%), having adequate ventilation facilities (73%), but with inadequate drainage facilities (68%). In all three Zones of Kolkata , the housing status of the study subject revealed that most of the houses were of "Pucca" type(94% in Zone-1, 81% in Zone-2 & 73% in Zone-3), with adequate ventilation facilities in

82%, 63% & 76% of those pucca-houses in Zone 1,2 & 3 respectively. The drainage facilities in urban areas were found to be adequate in 62%, 67% & 86% of houses in Zone 1,2 & 3 respectively .

Addiction:

The percentage of smokers and alcohol-addicted individuals in urban sample population were observed to be higher in comparison to rural samples , as evident from Fig. 6 & 7 . But Khaini-addicted individuals were found to be highest in numbers in Zone 4 (39%) and least in Zone 1 (5%), as depicted in Fig. 8.

CLINICAL STATUS:

Though the average height & weight of the control subjects (Zone-4) were found to be lowest amongst all surveyed individuals and a significant relationship ($P < 0.001$) could be revealed with that of other studied Zones of Kolkata , but no significant variation was observed in relation to their BMI (body-mass-index) [Table-1] . The average systolic & diastolic blood pressures of the adult individuals of all Zones of Kolkata were observed to be higher in comparison to the same of Zone-4 , as revealed from Table-1 . Higher blood pressures were found among study subjects of Zone-3 , compared to that of other two zones of Kolkata and a significant relationship was revealed with the lowest average blood pressures of Zone-4 ($P < 0.001$).

Out of the total 593 adult sample population from Kolkata-city areas , 78 individuals (13.2%) were clinically found to be suffering from chronic respiratory signs & symptoms of cough & phlegm , and/or wheezing and/or breathlessness on exertion and/or at rest . Though the numbers of persons suffering from chronic respiratory illness were a bit higher in Zones 2 & 3 in comparison to Zone-1 , but no significant relationship was observed amongst these three groups . The incidence of signs & symptoms of chronic respiratory illness (as mentioned above) among control subjects of Zone-4 was found to be 9.5%, which is significantly lower than that of urban sample population.

Table 1

Some clinical parameters of study and control subjects. Values are mean ± S.D

Clinical parameters (Average values)	Zone-1 (n=190)	Zone-2 (n=195)	Zone-3 (n=208)	Zone-4 (n=252)	Statistical significance (By Student's "f'test)
Weight (kgs.)	63.88 ±4.47	60.99 ±4.88	58.54 ±4.10	55.51 ±4.44	Zone 1,2, 3 **vs** Zone 4 P < 0.001
Height (cms.)	170.07 ±8.09	167.53 ±9.39	164.18 ±9.02	160.11 ± 10.45	Zone 1,2, 3 **vs** Zone 4 P < 0.001
Body Mass Index (BMI)	22.10 ±1.59	21.62 ±1.68	21.76 ±1.67	21.68 ±1.71	No significant relationship
Systolic blood pressure (mm of Hg)	118.53 ± 10.92	118.26 ±15.58	122.16 ±15.82	115.44 ±13.37	Zone 3 **vs** Zone 4 P < 0.001
Diastolic blood pressure (mm of Hg)	76.64 ±8.28	77.20 ±9.92	79.37 ±8.42	75.90 ±9.65	Zone 3 vs Zone 4 P< 0.001

BIOCHEMICAL STATUS:

The biochemical parameters related to this research study are depicted in Table nos. 2,3&4 and also graphically represented in Figure nos. 9to 16.

Urinary Hydroxyproline , the biological marker for NO2 exposure was detected to be significantly higher among study-subjects of Kolkata than that of rural control-subjects (P<0.001) . Among three zones of Kolkata , the average Level of urinary Hydroxyproline was observed to be highest in Zone-2 and lowest in Zone-1 , but without any significant relationship (Table-2) . Kosminder et al. (1972) showed that long term exposure to NO2 induced an increase of hydroxyproline excretion in urine [5] . But due to practical limitations of collecting 24-hours urine samples , a partial sample may be estimated to detect the amount of excretion . Allison et al. (1966) reported that urinary creatinine was excreted at a nearly constant rate . Hence the ratio of urinary Hydroxyproline to Creatinine (HOP : C) might be used to estimate the daily amount of excreted hydroxyproline [7]. Also Whitehead (1965) and Kasuga et al.(1979) found that the ratio of partial sample coincided with the ratio of 24-hour sample . Furthermore, Younoszai et al. (1969) showed that using this ratio had the advantage of eliminating the effect of the subject's body-weight , since excretion rates of both hydroxyproline and creatinine depend on body-weight [5] . The average levels of HOP : C were found to be significantly higher ($P < 0.001$) among the study-subjects of Kolkata , in comparison to that of rural control-subjects (Table-3) and the highest urinary hydroxyproline to creatinine ratio of study-subjects from Zone-2 was probably due to their more exposure to environmental Nitrogen dioxide (NO2).

Table-2

Biochemical parameters on Urine-samples. Values are mean ± S.D

Biochemical Parameters (Average values)	**Zone-1 (n=190)**	**Zone-2 (n=195)**	**Zone-3 (n=208)**	**Zone-4 (n=252)**	**Statistical Significance (By Student's "t" test)**
Hydroxyproline (mg/l)	17.28 ± 1.53	17.80 ± 2.20	17.48 ± 2.19	12.30 ± 1.85	Zone 1,2,3 vs Zone 4 P < 0.001
Phenol (mg/l)	23.22 ± 2.28	23.41 ± 2.52	23.58 ± 2.54	18.04 ± 2.43	Zone 1,2,3 vs Zone 4 P < 0.001
Creatinine (gm/l)	1.10 ± 0.18	0.98 ± 0.13	0.99 ± 0.15	1.04 ± 0.17	Zone 1 vs Zone 2,3,4 P < 0.001 Zone 2,3 vs Zone 4 P < 0.001
pH	5.36 ± 0.59	5.40 ± 0.64	5.42 ± 0.49	5.03 ± 0.65	Zone 1,2,3 vs Zone 4 P < 0.001

TabIe-3

Hydroxyproline-Creatinine Ratio (HOP:C) and Phenol-Creatinine Ratio (PH:C) of study & control subjects. Values are Mean ± SD

Ratio (Average values)	Zone-1 (n=190)	Zone-2 (n=195)	Zone-3 (n=208)	Zone-4 (n=252)	Statistical Significance (By Student's "t" test)
HOP : C ratio	16.62 ±0.91	18.42 ±1.03	17.98 ±1.14	12.92 ±0.94	Zone 1,2,3 vs Zone 4 = P< 0.001
PH : C ratio	21.89 ±1.49	25.92 ±1.93	24.98 ±1.77	18.12 ±1.05	Zone 1,2,3 vs Zone 4 = P< 0.001

TabIe-4

Biochemical parameters on Blood-samples. Values are Mean ± SD

Biochemical Parameters (Average values)	Zone-1 (n=190)	Zone-2 (n=195)	Zone-3 (n=208)	Zone-4 (n=252)	Statistical Significance (By Student's "t" test)
Bicarbonate (m mol/l)	26.99 ± 2.67	27.12 ± 3.10	28.09 ± 2.79	17.19 ± 2.01	Zone 1,2,3 vs Zone 4 = P < 0.001
Lead (ug/dl)	7.75 ± 0.92	8.08 ± 1.01	7.91 ± 0.83	3.98 ± 0.44	Zone 1,2,3 vs Zone 4 = P < 0.001 Zone 2 vs Zone 1 = P < 0.001

The serum Bicarbonate level , the biologic-marker of CO_2 exposure was estimated to be highest in the sample population of Zone-3 . Though the industrial areas of North Kolkata (Zone-3) were found worst affected by CO_2 exposure in comparison to other two studied-areas of Kolkata , but its levels among sample-population of all areas of the City were observed to be significantly high ($P < 0.001$) than that of rural-sample subjects of Zone-4 (Table-4) . CO_2 is present in normal atmosphere in concentration varying from 0.03% to 0.06% .Taking into account of the elevated CO_2 concentrations in the environment , it may be possible that the increase of inspired amounts of CO_2 might cause elevated levels of blood-bicarbonate and finally , increase in urinary excretion of bicarbonate . It is believed that urinary-bicarbonate levels of normal adults are usually negligible . But Hamashima et al. (1993) found significant excretion of urinary-bicarbonate and increase in urinary-pH among Tokyo-Citizens [8] ,due to elevated carbon-dioxide concentrations in ambient air. Though urinary-bicarbonate estimations were not performed in this particular research-study , but estimations of urinary-pH of all individuals revealed significantly increased values in all Zones of Kolkata , highest being in Zone-3 , in comparison to that of rural-subjects in Zone-4 ($P < 0.001$), as evident from Table-2 .

Benzene is rapidly metabolized and the metabolic products are excreted mainly as water soluble metabolites in urine in the form of phenol , catechol , quinol , hydroxyquinol and muconic acid . Among all these metabolites , urinary phenol is the best biologic marker of benzene exposure . The correlation between benzene exposure and increased urinary phenol levels has been reported previously [9]. Recent studies in Japan & China indicates that there is a close quantitative relation between levels of benzene vapour in air and urinary phenol level. By contrast , the same series of studies found that catechol & quinol levels in urine are not closely enough correlated to be as reliable markers of exposure [4]. The sample population of Kolkata city were found to be more exposed to environmental benzene exposure compared to their rural counterparts , as evident in their excreted urinary phenol levels and the estimated average urinary phenol levels of all three zones of Kolkata were observed to be significantly higher than that of rural areas ($P< .001$), as depicted inTable-2 . Due to practical limitations of being able to collect 24-hour urine samples it became necessary to estimate phenol-excretion from a partial sample . Allison et all. reported that urinary creatinine was excreted at a nearly constant rate . Therefore the ratio of urinary phenol to creatinine had been used to estimate the daily amount of phenol excretion in urine [7]. Significantly higher urinary phenol : creatinine (PH : C) were observed in the urine samples of Kolkata population ($P < 0.001$), compared to that of rural population (Table-3) and among all zones of Kolkata highest value of PH : C ratio was observed in Central Kolkata (Zone-2) , which suggested strongly the higher absorption of benzene (probably due to more environmental exposure of the same).

Lead (Pb) produces a cascade of effects on every tissues/ organs , it comes in contact. After absorption , Pb is carried in the blood bound with erythrocyte-membrane and mainly deposited in skeletal tissues . It is a cumulative toxin , as Pb has a long biological half-life and the total body burden increases with time . Though blood-Pb levels are very poor indices for assessment of its toxic effect on human tissues , but they are most commonly used measures for understanding its body burden and absorbed (internal) doses of environmental lead (Pb) exposure [10] The average blood lead levels of different urban sample population of Kolkata were detected to be significantly higher compared to that of rural adult individuals of Zone-4 , as revealed from Table-4 (PO.001) . The study-subjects of Zone-2 were probably more exposed to environmental lead than the studied individuals of other two zones of Kolkata city , and the rural sample population were found to be least exposed to the same, as evident from Table-4 . The average blood-lead level of urban commercial areas (Zone-2) was observed to be significantly higher ($P < 0.001$) than that of urban residential areas (Zone-1). Chronic low-level Lead exposure was reported to be associated with significant elevation in blood pressure . Some recent studies reported that small , but significant increase in B. P. (particularly in adults) might result from Pb exposure , denoting blood-lead levels to be as low as 5 ug/dl [10] . Like elevated blood-Pb levels, both systolic & diastolic blood pressures were also observed to be higher in the sample population of all zones of Kolkata , compared to that of sample population of Zone-4, but significant relationship ($P < 0.001$) was found only between the highest average blood pressure level of Zone-3 with the lowest average systolic & diastolic blood pressures of Zone-4 (Table-1) . However there is doubt regarding whether the elevated blood pressures of the urban sample population from different areas of Kolkata are really due to an effect of lead exposure or due to the cumulative effect of other confounding variables.

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FIG.3: TYPE OF HOUSES OF DIFFERENT ZONES

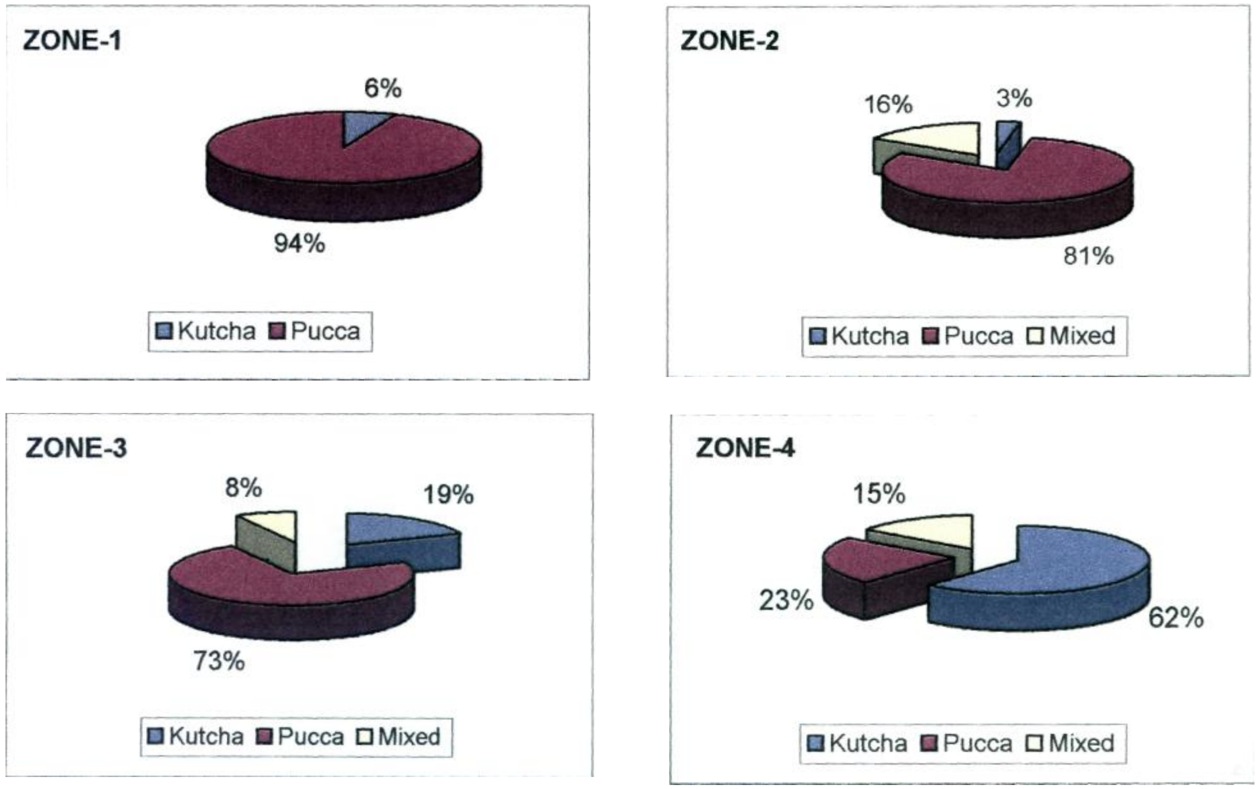

FIG.4: VENTILATION FACILITIES OF DIFFERENT ZONES

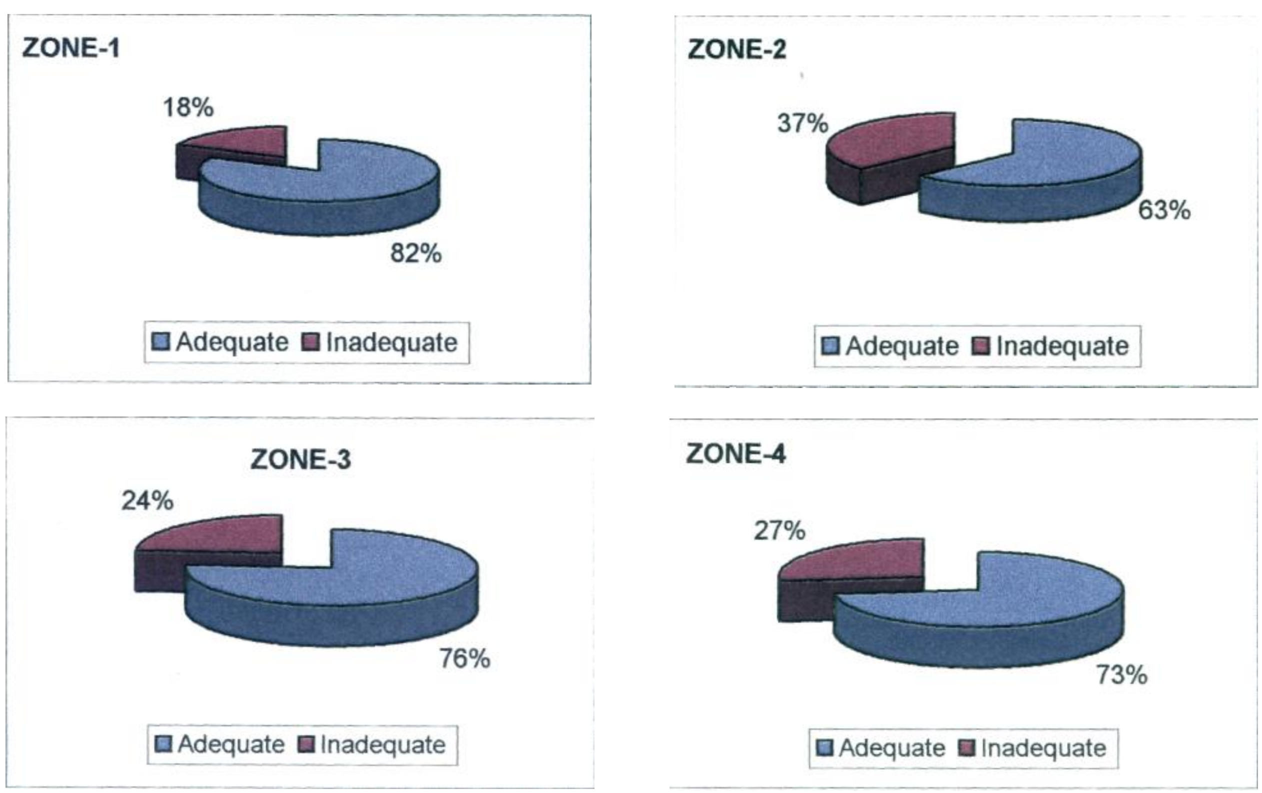

FIG.5: DRAINAGE FACILITIES OF DIFFERENT ZONES

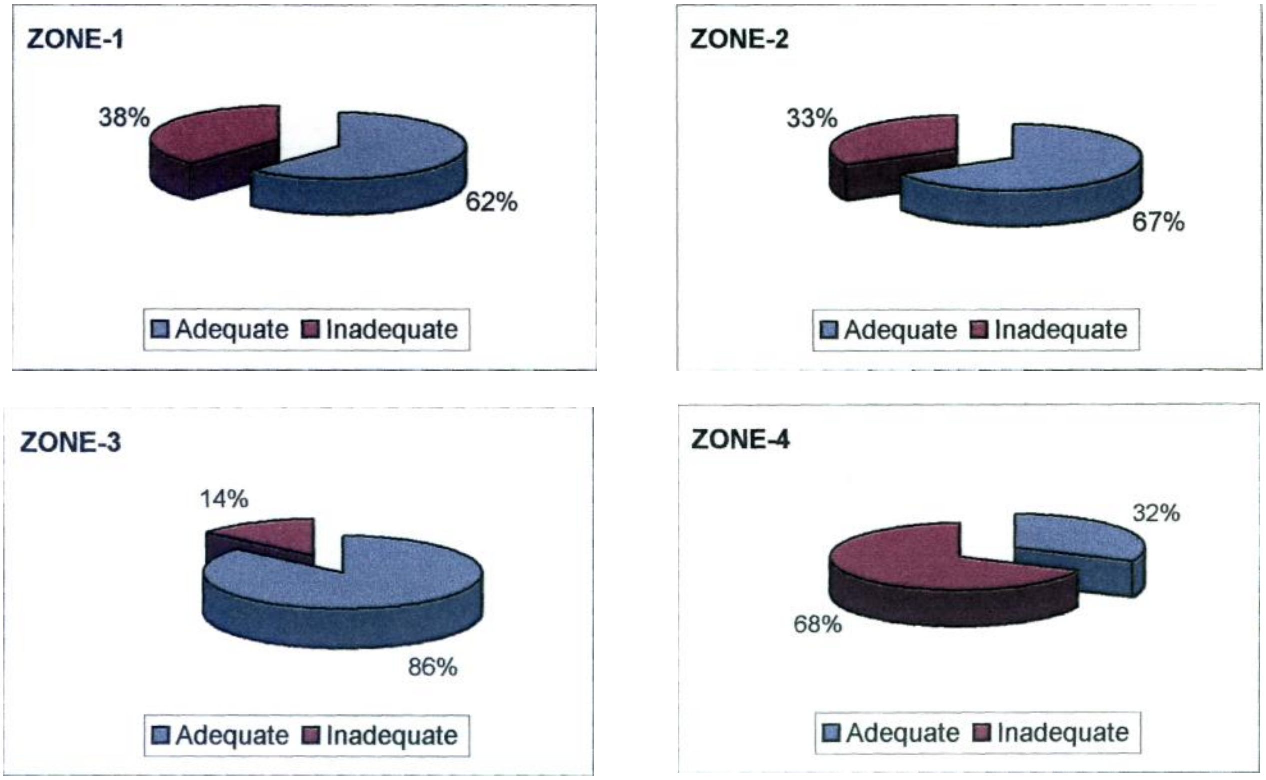

FIG.6: SMOKING STATUS OF DIFFERENT ZONES

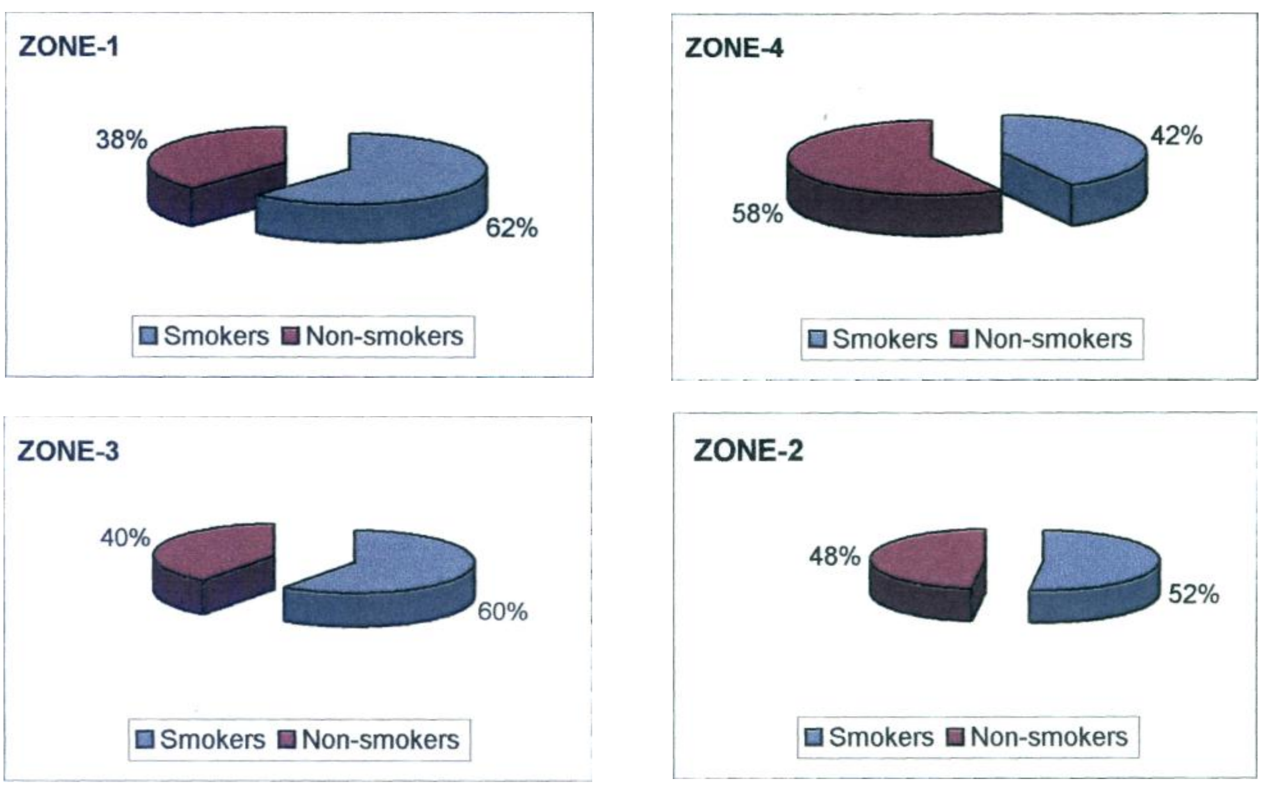

FIG.7: STATUS OF ADDICTION TO ALCOHOL OF DIFFERENT ZONES

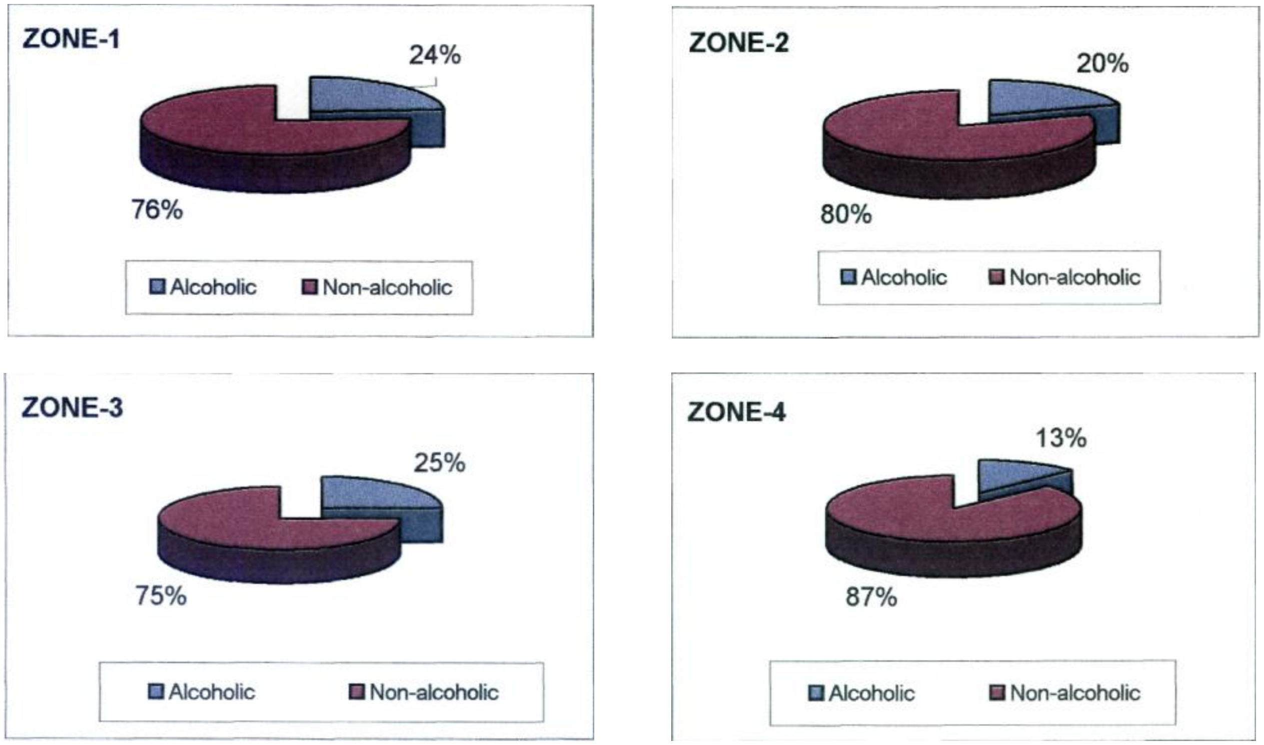

FIG.8: STATUS OF ADDICTION TO KHAINI OF DIFFERENT ZONES

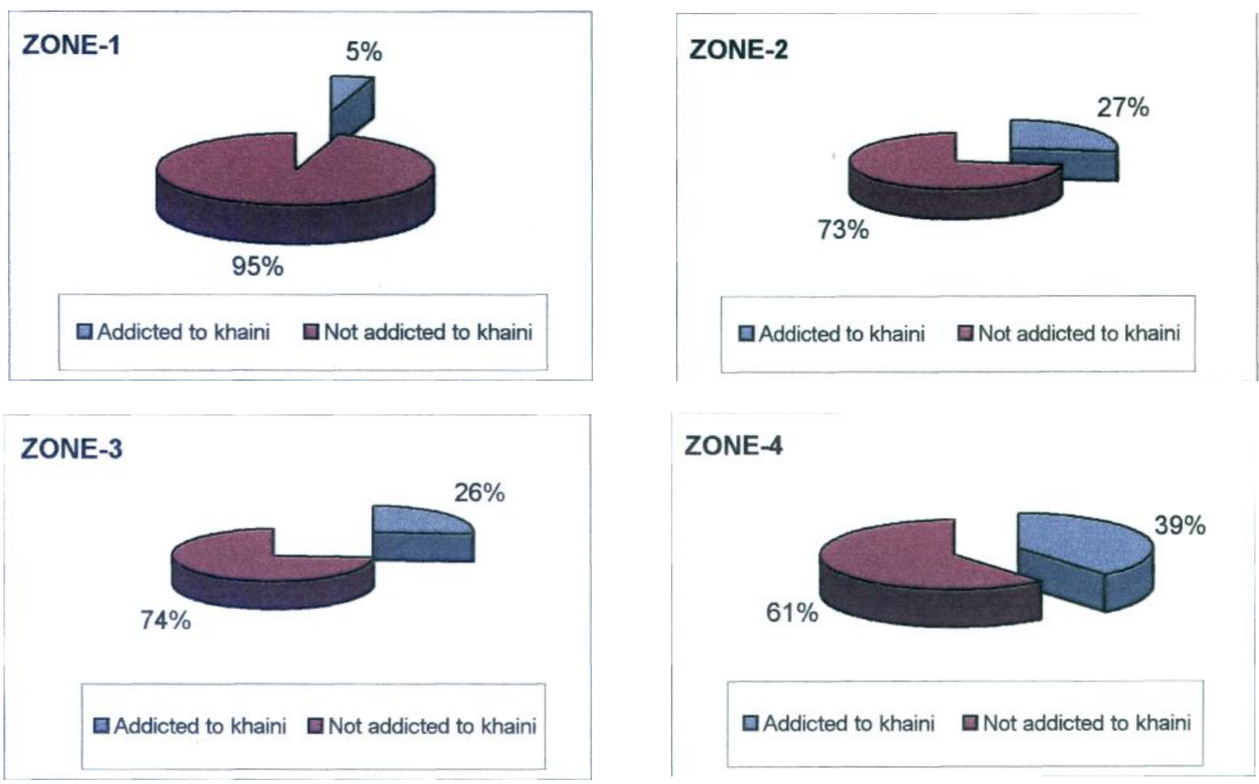

FIG.9 : URINARY HYDROXYPROLINE LEVELS (mg / l)

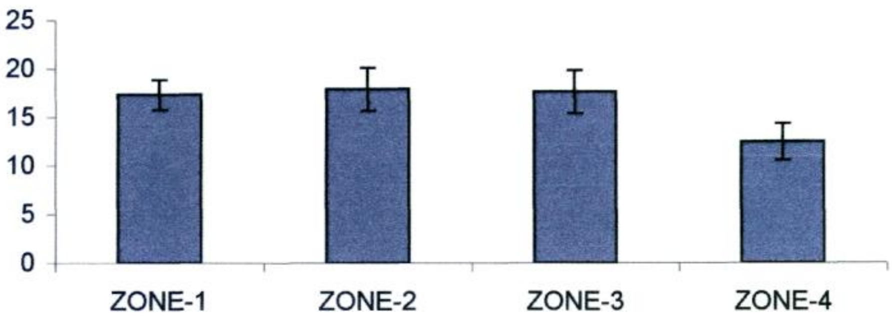

FIG.10 : URINARY PHENOL LEVELS (mg / l)

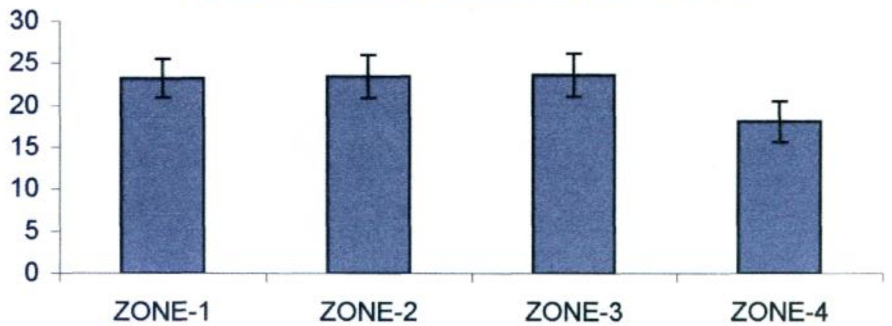

FIG.11 : URINARY CREATININE LEVELS (gm / l)

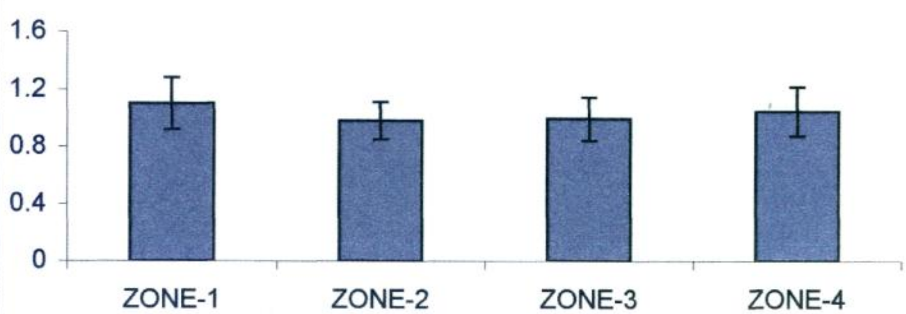

FIG.12 : URINARY pH LEVELS

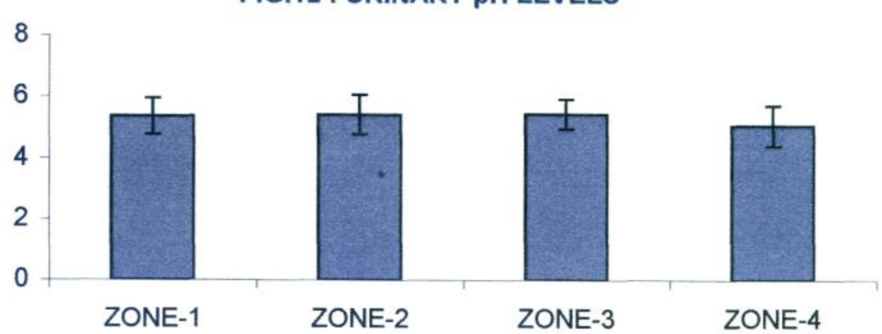

FIG.13 : URINARY HYDROXYPROLINE-CREATININE RATIO (HOP : C)

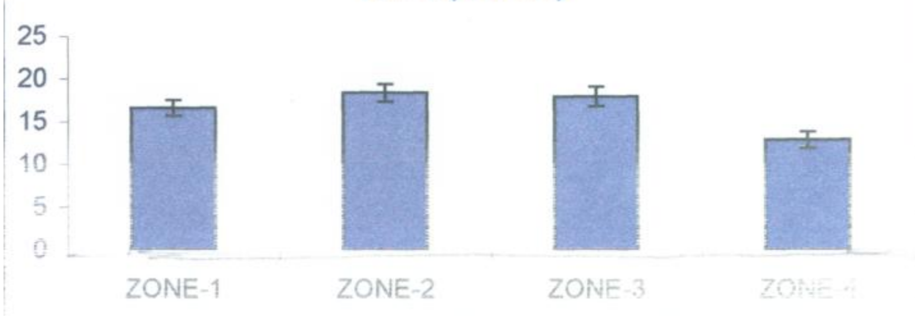

FIG.14 : URINARY PHENOL-CREATININE RATIO (PH : C)

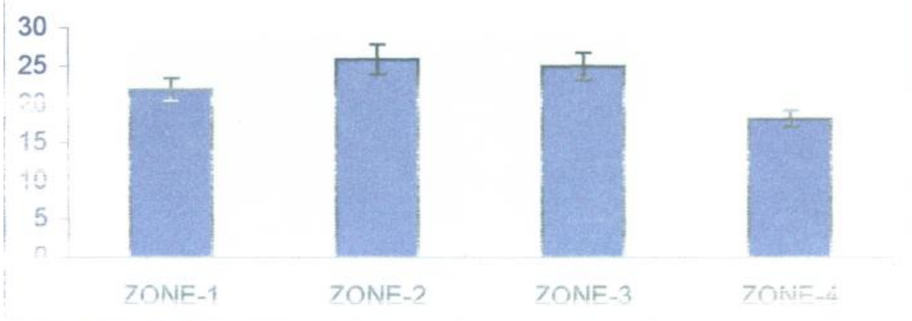

FIG.15 : BLOOD BICARBONATE LEVELS (m mol / l)

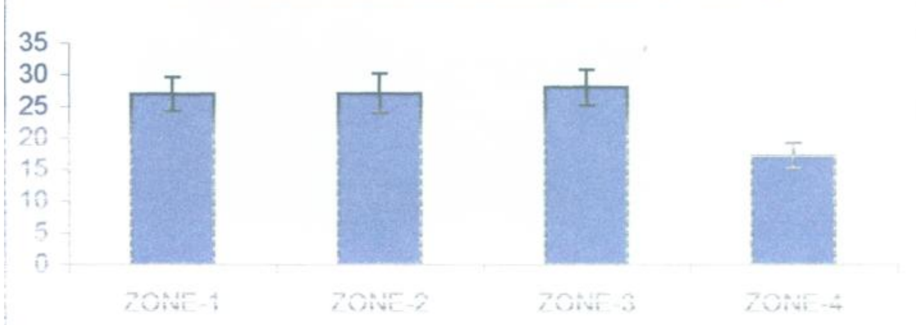

FIG.16 : BLOOD LEAD LEVELS (ug / dl)