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HISTOPATHOLOGICAL CHANGES IN THE GILLS OF *NANDUS NANDUS* INDUCED BY ENDOSULFAN AND CARBARYL

Sarita Khare, Sudha Singh and Asha Mehrotra

Department of Zoology, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal – 462 016, India

ABSTRACT

Nandus nandus was exposed to sublethal concentration of endosulfan (0.04 ppm) and carbaryl (0.05 ppm) for one month. After the exposure significant histopathological alternations in different parts of gills were observed.

INTRODUCTION

Indiscriminate use of pesticides, has posed a serious threat to the mankind as it pollute the environment and eliminate useful insects causing ecological imbalance. The pesticide reach water resources through run-off during monsoon, wind action, reaching as combination of above causing toxicity to aquatic flora and fauna. The contamination of water by pesticides may be detrimental to aquatic food chain reaching to man. Acute concentration of pesticides in water cause mortality to fish, while subacute conditions render them unsuitable for human consumption.

Earlier toxicological studies on the insecticides on fishes were made by several workers (Hayes, 1964; Anees, 1975; Singh and Shrivastava, 1984; Gajghate *et al.*, 1988). All these studies showed that the effect of pesticides on mortality of fish is dose dependent.

Modern insecticides and pesticides belong to different classes of chemical compounds, but synthetic organic compounds (e.g. organo-chlorine and Organophosphorous) predominate over them. Present attempt has been undertaken to study the toxic effect of Endosulfan and Carbaryl on gills of *Nandua nandus*.

MATERIAL AND METHOD

The fishes were brought to laboratory and acclimatized in a tank for at least one week before using for experimentation. During acclimatization, they were fed with commercial fish food to avoid mortality due to starvation.

Three glass aquaria of 20 lits. capacity were used for experimentation. In each aquarium 10 fishes were exposed. The size of fishes were 9 to 10 cms. and weight was 70 to 75 gms in two aquaria endosulfan (0.04 ppm) and carbaryl (0.05 ppm) was added and exposed for specific duration of 8, 15, 30 days respectively. Controls was kept in one aquarium without toxicants. The water & the toxicants in control & experimental equaria were changed daily. 3 fishes from each experimental and control groups were removed & gills were dissected out and blocks were prepared.

RESULT AND DISCUSSION

In the present investigation, significant histopathological changes were observed after expo-

sure of two pesticides – Endosulfan and Carbaryl. After short term exposure gill arch remained unaffected in the case of carbaryl while there was a slight damage in the endosulfan treated fishes. After 30 days exposure, there was a severe damage in gill arch region. There was destruction in muscular and cartilagenous parts. Cartilagenous cells were shrunken and some of them disappeared. Blood vessels were also found in shrunken condition, while some of them broken. There was an increase in the destruction of blood capillaries and blood spaces (Fig. 1-6). These findings are similar to the findings of Shrivastava *et al.* (1984, 1987), Jauhar and Kulshreshtha (1985), Ramamurthy *et al.* (1987), Banerjee *et al.* (1987), Singh and Sahai (1990), Singh (1993) and Prasad *et al.* (2000).



Fig. 1. Transverse section of the gill of control *Nandus nandus* (x 40).

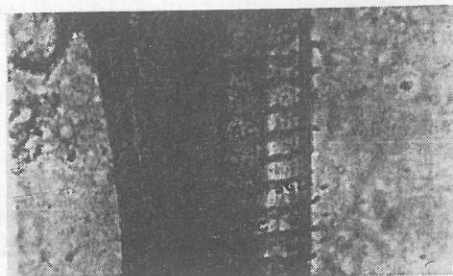


Fig. 2. Transverse section of the gill of control *Nandus nandus* (x 150).



Fig. 3. Transverse section of the gill of *Nandus nandus* after short term exposure at 0.04 ppm of endosulfan (x 40).



Fig. 4. Transverse section of the gill of *Nandus nandus* after long term exposure at 0.04 ppm of endosulfan (x 100).



Fig. 5. Transverse section of the gill of *Nandus nandus* after short term exposure at 0.05 ppm of carbaryl (x 150).



Fig. 6. Transverse section of the gill of *Nandus nandus* after long term exposure at 0.05 ppm of carbaryl (x 150).

Short term exposure resulted in slight damage in the epithelium of primary gill lamellae in endosulfan treated fishes, while in the case of carbaryl they remained unaffected. After 30 days exposure, there was a separation of epithelium from basement membrane and hyperplasia at several places in the case of both pesticides. It was seen that endosulfan produced more toxic effect than carbaryl. Similar observations are made by Singh and Sahai (1984), Shrivastava *et al.* (1984, 1987), Jauhar and Kulshrestha (1985), Ramamurthy *et al.* (1987), Bhargava and Bhide (1987), Banerjee *et al.* (1987), Singh and Sahai (1990), Singh (1993), and Prasad *et al.* (2000).

It has been found that prolonged exposure of pesticides even the concentration below the lethal level, damage the various parts of gills. Due to which respiratory process is disturbed, which may impair the ability of a fish to survive.

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IMPACT OF MARBLE SLURRY ON SUB-SURFACE WATER:- A CASE STUDY OF KISHANGARH, DISTRICT AJMER

A.K. Pandey, S. Dutta and K.C. Sharma

Department of Environmental Studies, Maharshi Dayanand Saraswati University, Ajmer, Rajasthan.

ABSTRACT

In the present work the impact of marble slurry on sub-surface water quality was studied. The drinking water and sanitation deserve immediate attention from the point of clean environment and public health. The physico-chemical parameters of drinking water and ground water were collected from 12 different areas in and around Kishangarh and analysed. The quality parameters were compared with standards laid by WHO and ISI for drinking water quality. It is significant to note that the level for all physico-chemical parameters of the ground water sources exceeded the permissible level prescribed by the BIS (1993) and WHO (1992) standards for drinking water. The raw effluent released from the industry is highly hazardous both from salinity and sodicity consideration.

INTRODUCTION

Water is an important substance required by all living organisms and for all anthropogenic activities (Khatri, 1985; Dagaonkar and Saksena, 1992). It is also an established fact that the very high mortality rate is found among the population using pond/reservoirs/rivers/earthen wells as the main source of drinking water (Ghosh, 1985; ICMAR, 1985). In India, only 12% of population get clean drinking water (Kudesia 1986). Due to industrialization, urbanization, mining and unmindful disposal of waste materials in recent years, the nature of water quality has been greatly deteriorated causing environmental hazards. Rapid growth of industries along with urbanization have not only decreased the water availability but also deteriorated the quality of both surface and sub-surface waters. The runoff from industries not only affects the water bodies of the area but exert an impact on the physiochemistry of groundwater. Earlier studies have clearly shown that underground water is contaminated due to hazardous substances and microorganisms (Somani et al. 1972, Oloniya and Saxena, 1977, Kishore et al. 1990, Gupta and Nathawat 1991, Chacharkar et al. 1992). Therefore, a continuous periodical monitoring of water quality is necessary, particularly in area of industrial settlements, so that appropriate steps may be taken for water resource management practices.

Kishangarh town a part of Ajmer district is famous for processing of marble, granite and related works. It is also known as one of the biggest marble business center of northern India. The slurry produced by the units is generally disposed of either in the designated Vishwakarma dumping site north of Madanganj-Kishangarh or in unauthorized area nearby the industrial units. This dumping can possibly contaminate the sub-surface water, as slurry is in a semisolid form and chances of its percolation and contamination of underground water is very high. Therefore, the present study was carried out to find out the impact of marble slurry on subsurface water quality of Kishangarh.

STUDY AREA

The study area Kishangarh is located about 27 km away from Ajmer city on the National

Highway 8, Delhi-Ahemadabad (Fig. 1). This town of Ajmer district covers an area of 67 sq. km. with a population of 0.14 million. It is situated between $74^{\circ}48'E$ to $74^{\circ}55'E$ longitude and $26^{\circ}33'N$ to $26^{\circ}37'N$ latitude and lies in Survey of India Toposheet No 45 J/14. To meet the growing demand of the water the government of Rajasthan has allowed the various marble cutting units to dig tubewells for industrial purpose.

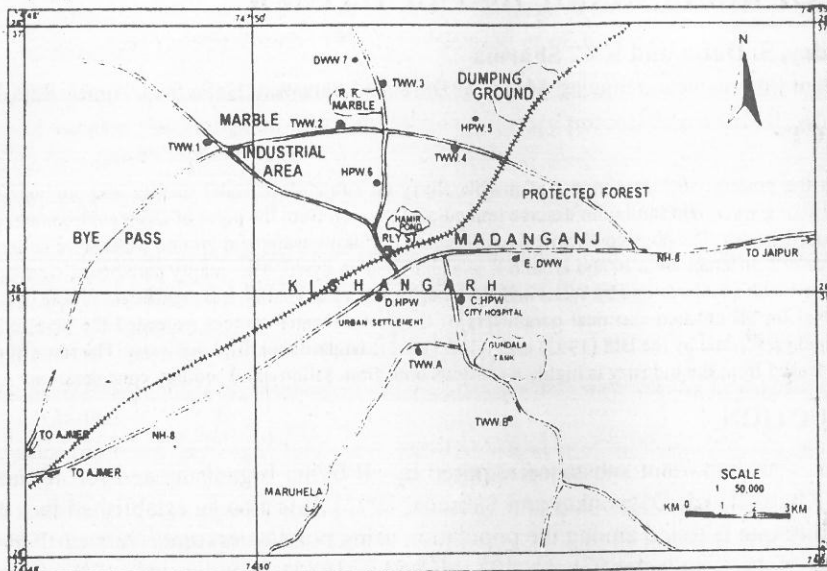


Fig. 1. Study area

MATERIALS AND METHODS

The quality test survey of the subsurface water was conducted in the month of April 2000 by collecting underground water sample from dugwell and tubewell in different localities of Kishangarh area. In all 12 sites were selected, out of these 7 sites were located in the marble industry area and the remaining in Kishangarh town. The samples were collected during the morning hours between 5 to 9 a.m. About one litre of sample were collected in polythene cans and taken to the laboratory for analysis. All samples were stored in the refrigerator at $4^{\circ}C$ till the analysis was over. The samples were analyzed for various physico-chemical parameters such as pH, Electrical conductivity, Total solids, Total dissolved solids, Total suspended solids, Turbidity, Free carbondioxide, Total hardness, Total alkalinity, Carbonates, Bicarbonates, Calcium, Magnesium, Chloride, Nitrate, Phosphate using standards method (APHA 1995).

RESULT AND DISCUSSION

The physico-chemical characteristic of various sample stations are tabulated in table 1 and 2. The presence of crushing, cutting and polishing units in this area produces a lot of slurry (waste material). The basic raw material used by these units is silica and water which after use forms slurry. It is disposed off on the open land without any treatment. As the slurry is in liquid form with rich in calcium/magnesium content, these therefore percolates in the groundwater and cause an increase in salinisation.

Table 1. Physico-chemical characteristics of different sub-surface water samples in city area of Kishangarh

Sample sites	pH	Cond.	TS	TDS	TSS	FCO ₂	Turb	TH	TA	Bicarb	Ca	Mg	Cl	NO ₃	PO ₄
WHO	7.5			1500				500			200	150	250	45	0.10
ISI	8.5			2000				600			200	150	250	50	0.10
A Tube well	7.99	1.278	800	600	200	ND	ND	600	440	410	16.03	97.45	1562	2.93	1.32
B Tube well	8.36	1.781	4400	2800	1600	ND	ND	1340	900	ND	92.18	134.0	205.9	2.10	0.91
C Hand pump	7.84	2.020	4000	1800	2200	ND	ND	760	730	ND	208.41	292.51	504.1	1.96	0.89
D Hand pump	7.92	8.780	38600	23800	14800	4.4	1.0	2610	830	ND	468.93	1754.2	1782.1	0.83	0.70
E Dug well	7.68	13.49	9000	3800	5200	ND	1.45	3610	390	280	765.52	414.20	319.5	3.13	1.13

Note: All the value are in mg/L except pH, conductivity (m mho cm⁻¹)
 ND : Not detectable

Table 2. Physico-chemical characteristics of different sub-surface water samples in marble industrial area of Kishangarh

Sample sites	pH	Cond.	TS	TDS	TSS	FCO ₂	Turb	TH	TA	Bicarb	Ca	Mg	Cl	NO ₃	PO ₄
WHO	7.5			1500				500			200	150	250	45	0.10
ISI	8.5			2000				600			200	150	250	50	0.10
1. Tube well	7.96	2.990	14400	2400	11600	ND	ND	620	390	ND	677.35	36.54	397.6	2.62	0.12
2. Tube well	7.84	4.420	2800	2400	400	ND	ND	874	720	690	188.37	487.29	524.4	2.01	1.76
3. Tube well	7.81	4.210	5800	2540	3276	13.2	ND	890	540	ND	192.38	499.47	1391.6	2.73	1.62
4. Tube well	8.12	3.670	26000	1400	24600	4.1	ND	1270	340	ND	420.84	268.01	433.1	1.99	0.89
5. Hand pump	7.11	3.040	43600	43400	200	4.4	ND	790	700	ND	140.28	536.02	582.2	1.81	1.10
6. Hand pump	7.35	3.470	23500	22600	900	ND	ND	430	1090	100	64.12	328.92	702.9	0.91	0.86
7. Dug well	8.95	1.956	52200	45800	6400	ND	1.12	6600	360	410	2520.0	316.74	681.6	3.96	2.10

Note: All the value are in mg/L except pH, conductivity (m mho cm⁻¹)
 ND : Not detectable

Hydrogen Ion Concentration: The pH was mild alkaline for all samples and falls within the permissible limit prescribed by WHO and ISI. The pH range of industrial area was between 7.11 to 8.95 whereas the range in city area was between 7.68 to 8.36, so there is not considerable change in the pH of industrial as well as of city area.

Electrical Conductivity: The bicarbonates contamination from marble slurry increases the electrical conductivity of the water. EC ranged between 1.278 to 13.490 m mho cm^{-1} in city area. The highest concentration was recorded in dugwell sample no-E in city area which was 13.490 m mho cm^{-1} and in industrial area it varied between 1.956 to 4.420 m mho cm^{-1} .

Total solids: The total solids concentration ranged between 800 mg/l to 38600 mg/l in city area whereas in industrial area it was recorded in between 2800 to 52200 mg/l. The highest concentration was found in dugwell sample no-7 in industrial area.

Total Dissolved solids: Total dissolved solids denoted various kinds of minerals present in water. TDS is composed mainly of carbonates, bicarbonates, Cl^- , SO_4^{2-} -S, PO_4^{2-} -P and NO_3^{-1} -N, Ca^{++} , Na^+ , K^+ , Fe^{++} (Trivedy and Goel, 1984). TDS of natural water ranges from less than 10 ppm for rain and snow, to more than 3,00,000 ppm as in the case of brines. Water for most domestic and industrial use should be below than 3,000 ppm (Davis and Dewiest, 1966). The BIS (1993) and ICMR (1975) prescribed the desirable limit of 500 mg/l and the permissible limit of 200 and 1500 mg/l respectively. But the TDS of dugwell and handpump samples varied between 600 mg/l to 23800 mg/l in city area and in industrial area it ranged between 1400 to 45800 mg/l. Thus the TDS of groundwater fluctuated between 600 and 45800 mg/l, exceeding all the permissible standards. It clearly indicates that the effluent has effected the groundwater.

Free Carbondioxide: Free carbondioxide in the water accumulates due to microbial activity and respiration of organisms. This imparts the acidity to the water because of the formation of carbonic acid. Free CO_2 concentration ranged in industrial as well as city area between 4.1 mg/l to 13.2 mg/l.

Total hardness: The principle cations that impart hardness are Ca^{++} and Mg^{++} , the anions responsible for hardness are mainly bicarbonates, carbonates, SO_4^{2-} , S, Cl^- , NO_3^{-1} , SiO_3^{-1} -S. The content of Ca^{++} and Mg^{++} in potable water ranged between 75 and 200 mg/l and 50-100 mg/l respectively (ICMR 1975). The desirable limit of 300 mg/l and the permissible limit 600 mg/l are prescribed by the BIS for drinking water. Total hardness of groundwater in industrial area was high in dugwell sample no 7. In industrial area fluctuated between 430 to 6600 mg/l. But in city area ranged between 600 to 3610 mg/l which is lower than the industrial area. When effluent from the marble industries passes through the soil, the calcium concentration in the soil increases gradually. This clearly explains the observation that in some tubewell and dugwell hardness is even higher than the permissible limit. Though no adverse effect on health is known, but some indicates its role in heart disease (Péter 1974).

Turbidity: Turbidity was found in only few samples. Majority of the samples showed ND (not detectable). The desirable limit of 5 NTU and the permissible limit 10 NTU are prescribed by the BIS for drinking water. Only one sample in industrial area showed some turbidity in sub-surface water. But in city area it fluctuated between 1.0 to 1.45 mg/l which is slightly higher than the industrial area. The reason behind this is that the city dugwell is not much used for general purpose.

Alkalinity: Alkalinity is a measure of the capacity of water to absorb hydrogen ion. Total alkalinity of the water increases with an increase in pH and Calcium. Sample in city area showed the alkalinity value fluctuate between 390 mg/l to 900 mg/l but in industrial area it ranged between 340 mg/l to 1090 mg/l which clearly indicates that the presence of calcium in sub-surface water has increased the alkalinity in industrial as well of city water samples. Generally groundwater contains more than 10 ppm but less than 800 ppm of bicarbonates. The desirable limit of 200 mg/l and permissible limit of 600 mg/l has been recommended by BIS (1991).

Calcium: It is an important ion in imparting the hardness to the water. The desirable limit of calcium prescribed by BIS and ISI was 75 mg/l and permissible limit was 200 mg/l. The calcium concentration varied in city area between 92.18 to 765.52 mg/l. In industrial area it fluctuated between 64.12 to 2520.0 mg/l which was recorded much above the permissible limit and it can cause ortho-diseases.

Magnesium: Magnesium also occurs in all kinds of natural water, but its concentration remains generally lower than the Calcium. The permissible limit of Magnesium 150 mg/l was prescribed by the ISI and BIS. Magnesium concentration varied from 36.54 to 1754.2 mg/l in industrial area as well as city area. The maximum value 1754.26 mg/l was recorded at handpump sample no - D which is situated in city area.

Nitrate & Phosphate: The permissible limit for nitrate is upto 45 mg/l (WHO 1992) and 50 mg/l (BIS 1993) and in the present instance its value varied between 0.83 mg/l to 3.13 mg/l in city area sample and in industrial area it fluctuated between 0.91 to 3.96 mg/l which was recorded in range prescribed by the ISI and WHO. Phosphate content concentration varied from 0.70 to 1.32 mg/l in city area but in industrial area it fluctuated between 0.89 to 2.10 mg/l which is much above the permissible range of 0.10 mg/l.

Bicarbonates: Presence of bicarbonates in drinking water increases hardness and Electrical conductivity. Bicarbonate ions had been found in all type of drinking water sources. In present analysis it varied in city area from 280 to 410 mg/l and in industrial area it fluctuated between 100 to 690 mg/l.

Chloride: Chloride concentration in natural groundwater varied from 0.1 ppm in Arctic snow to 15,000 in brines where as a concentration of 1000 ppm is more common in groundwater (Davis and Dewiest, 1966). The desirable limit is of 250 mg/l and the permissible limit is of 1000 mg/l (BIS, 1993). Except for sample A in city area, all the values varied between 205.9 mg/l to 1782.1 mg/l which was much higher than the prescribed value. The same condition followed in industrial area but the range was between 397.6 mg/l to 1391.6 mg/l these value were much higher than the city area. The reason being that the rocks are rich in calcium – magnesium which reacts with the chloride of the water used mainly during cutting and grinding process and the slurry so formed is rich in calcium chloride which percolates down and contaminates the sub-surface water. Therefore, all groundwater sample showed a very high concentration of chloride. The ground water gradient is from the industrial area towards the city area. Therefore, the sample of city area also showed the chloride concentration towards higher side.

The results suggest that the water of tubewell and handpump of industrial area shows higher concentration of Total solids, Total dissolved solids, Total hardness, Total alkalinity, Calcium, Mag-

nesium, Chloride, Nitrate and Phosphate concentration in comparison to city area. So it is not fit for drinking purpose particularly at and around the industrial sector Kishangarh. Therefore, proper disposal of Marble slurry after treatment as well as recycling of waste water along with periodical of subsurface water are some of relevant ingredients of management strategies for industrial areas of Kishangarh.

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Environment News

EPA Maps Air Pollutants

A clear picture of which toxins may be tainting your neighborhood air is now online. The U. S. Environmental Protection Agency (EPA) has posted new interactive maps estimating concentrations of the most toxic air pollutants across the United States. Another available on www.scorecard.org

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World's First Heavy Metals Ban

Denmark today announced that it is to become the first country in the world to impose a wide ranging ban on the heavy metal lead. The move is in defiance of opposition from a majority of European Union's 15 member states and a negative opinion from the European Commission's chief scientific Committee.

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U. N. Talks Clinch Toxic Chemical Treaty

Delegates from more than 120 countries clinched agreement on the text of a treaty to curb or ban some of the world's most dangerous pollutants. The talks, organized by UNEP, targeted persistent organic pollutants, or POPs, 12 of which have been singled out for urgent attention. POPs, which include chemicals such as DDT and PCBs (polychlorinated biphenyls), have been blamed for deaths, cancer, disease and birth defects among humans and animals. The UNEP "Dirty Dozen" are: dioxins, furans, PCBs, hexachlorobenzene, mirex, heptachlor, DDT, dieldrin, chlordane, toxaphene, aldrin and endrin. *Copyright 2000 Los Angeles Times http://www.greenpeace.org/~toxics/toxic_2.html*

cpinfo@unep.org Anila. Shah@unep.org

India Hot Dumping Ground for Toxic Wastes

More than 100,887 tonnes of hazardous wastes have entered India illegally, some in violation of a 1997 Supreme Court order banning the imports of hazardous wastes into India, according to import data compiled by Greenpeace from Government of India statistics for 1998-1999.

Economic Times, India

Arsenic Patients Higher Than Govt. Estimate

The number of arsenic patients in the country is more than the government's estimate of about 8000 which includes only those having external symptoms of the disease. David Kinniburgh of British Geological Survey presented a paper on "The groundwater arsenic problem in Bangladesh." He said, even in heavily contaminated areas, water from dug-wells are less contaminated with arsenic. The percentage of manganese exceeds the WHO limit in water of thirty five percent of tube-wells and shallow tube-wells, he said. Kazi Quamruzzaman of Dhaka Community Hospital in his paper on "Arsenic Mitigation: An Integrated Approach" revealed that three million people of the country are drinking water highly contaminated with arsenic. According to him, nearly 80 million people across the country are now exposed to the threat posed by arsenic contamination while the government figure is about 20 million.

Daily Star

WATER MONITORING AND SEAWATER INTRUSION IN KALAPET, PONDICHERRY

Anisa B. Khan

Salim Ali School of Ecology & Environmental Sciences, Pondicherry University, R.V. Nagar,
Pondicherry-605 014

ABSTRACT

Rapid industrialization has deleterious effects on the environment, more so on ground water. Depletion of groundwater near to the coastal regions results in the intrusion of nearby marine waters. The annual depletion rate in Pondicherry is 1-1.5 m. The analysis of groundwater collected from Kalapet region of Pondicherry indicates a chloride content as high as 1808 mg/L and total dissolved solids at 3276 mg/L. Resource recovery and management through water harvesting and recharge of surface water and storage irrigation tanks are urgently required.

INTRODUCTION

Groundwater is a valuable resource for both agricultural and industrial development. It is reported by State Ground Water Board (SGWB) in 1997 that Pondicherry region has a resource potential of 125 MCM (1998) of water, which is annually replenishable to the aquifer systems. Coupled with the population growth and increased tempo of industrialization, the demand for groundwater is on the increase. The abstraction levels in Pondicherry have touched a record of 110 MCM (1998), leaving a narrow gap between the resource available and exploited.

With increased availability of institutional finances for construction of wells, electrification of villages, and energization of the irrigation pumpsets, the development of groundwater has undergone considerable expansion. The anthropogenic exploitation of groundwater has resulted in the limited available potable water resources to be deteriorated by seawater intrusion (Todd 1991). The resources may experience disastrous and almost irreversible impact if they are not timely managed. Accumulation of salt as a consequence of water use in urban areas affects future water supplies (Peter Fox 2000). Hence, an immediate focus on coastal aquifers is needed.

Study area

Pondicherry is situated on the southern part of Coromandal coast. It is bounded on the east by Bay of Bengal and on the remaining sides by the lands of South Arcot districts of Tamil Nadu. The present study area extends along the Kalapet coast. The study period is from January 1999 to March 2000.

OBSERVATIONS

Agricultural and industrial area

The land-use pattern of Pondicherry region for the period 1992-1997 is presented in table 1. For the land under non-agricultural use and from the number of industries registered under Factories

Act (1990-1997) it is clear that within 7 years total number of industries in Pondicherry have increased by 1652. Table 2 shows the total number of registered industries in Pondicherry (1990-1997).

Table 1. Particulars of land use in Pondicherry region (1992-97)

S.No.	Description	1992-93	1993-94	1994-95	1995-96	1996-97
1.	Total area according to village papers	48,581	48,581	48,842	48,842	48,842
2.	Forests	-	-	-	-	-
3.	Land not available for cultivation	14,128	14,268	14,413	14,581	15,235
4.	Other uncultivated land excluding fallow lands	2,452	2,550	3,425	3,714	3,793
5.	Fallow lands	4,516	5,108	3,454	4,506	4,704
6.	Net area sown	27,485	26,655	26,550	26,041	25,110
7.	Area sown more than once	19,930	18,240	19,870	18,433	19,482
8.	Total cropped area	47,415	44,895	46,420	44,474	44,592

Source: Directorate of Economics & Statistics, Pondicherry
Area in hectares

Table 2. Number of small, large and medium scale industries registered

S.No.	Items	1990-91	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97
1.	Small scale industries	3,883	4,239	4,563	4,835	5,135	5,400	5,492
2.	Large scale and medium	70	74	79	83	95	102	113
3.	Factories (1+2)	3,953	4,313	4,642	4,918	5,231	5,502	5,605

Irrigation

Three decades back in Pondicherry, irrigation activity was mainly from surface water storage, which was available in 86 major tanks and 400 small ponds. There were only 3500 tube-wells. But over the years due to disuse of these tanks, the natural recharge is reduced. This has increased the load on groundwater causing an increase in number of tube-wells. Presently there are about 7000 tube-wells (Fig. 1). As the depletion of groundwater takes place, instead of getting recharged by rainfall, it gets filled by nearest marine water. This is the problem faced by most coastal regions, wherever the groundwater is extensively used for industrial, agricultural and human settlements. Hence, there is an urgent need to identify the areas affected and to implement methods to protect them.

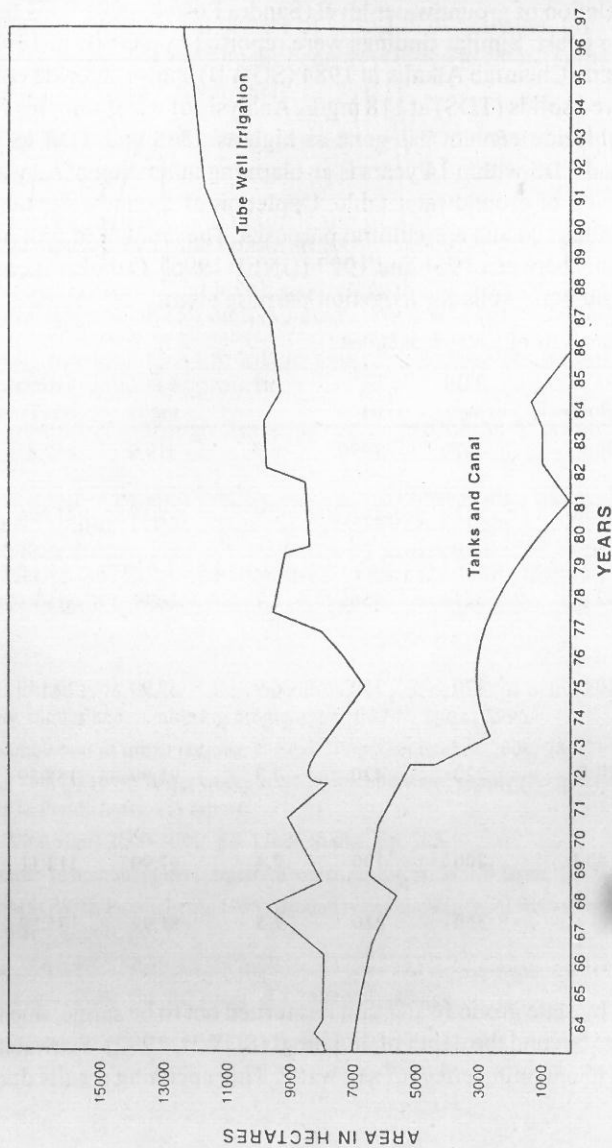
Present status of ground water development in Pondicherry

Since late 90's there was rapid groundwater depletion in Pondicherry (Table 3). There is large-scale development of groundwater towards central, western and north-western parts of Pondicherry, consequently resulting in the depletion of groundwater in Pondicherry. The annual depletion rate is 1-1.5 m due to over-sinking of tube wells and excessive pumping of water added to poor natural recharge.

Table 3. Groundwater level in Pondicherry 1991-97

Year/month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1991	-	-	-	-	6.18	6.52	6.65	6.84	6.95	6.86	5.72	5.1
1992	-	5.26	5.75	6.14	6.23	6.65	7.04	7.25	7.41	7.22	6.75	6.1
1993	6.15	6.33	6.78	7.26	7.56	7.52	7.84	7.9	7.95	7.88	6.51	9.95
1994	4.35	4.4	4.55	5.05	5.5	5.95	6.2	6.35	6.49	6.85	5.65	5.6
1995	5.72	5.65	6.25	7.0	6.9	7.25	7.71	7.68	7.54	7.44	7.23	7.58
1996	8.1	8.32	8.93	9.35	9.7	10.0	9.92	8.91	9.3	9.4	9.93	9.87
1997	8.74	9.84	-	11.5	12.3	-	12.4	11.6	-	11.9	10.8	11.3

Source: Seminar on water resources (25th March 1998); Units: Meters below ground level (mbgl)



Source : Groundwater Unit, Pondicherry

Fig. 1. Area irrigated by tanks, canals (surface water) and tube well (ground water)

In 1970 Public Works Department (PWD) in Pondicherry had 11 bore-wells for the supply of water to the whole of urban and semiurban parts of Pondicherry. By 1999 PWD has 120 bore-wells indicating a faster pace of groundwater development (PWD, Pondicherry, 1998). Due to depletion, salt water from sea has intruded and got mixed with groundwater in Kalapet and also places in Bahour commune making it unsuitable for consumption. Water samples from Kalapet region have been analysed to find the extent of seawater intrusion into the aquifers.

RESULTS AND DISCUSSION

Results of analysis of water samples collected from Kalapet (Table 4) show that seawater intrusion has contaminated the ground water aquifer, especially around areas 1, 3 and 4. In all these samples chloride-bicarbonate ratio has exceeded above 1. The over-withdrawal of water by industries results in the depletion of groundwater level (Sandra Postel 2000). This depletion causes intrusion of water from the coast. Similar findings were reported by SGWB in 1992. Samples collected from the borewell beside Chemfab Alkalies in 1984 (SGWB) shows chloride content as low as 14.18 mg/L and total dissolved solids (TDS) at 118 mg/L. Analysis of water samples from the same aquifer in 1999 shows that chloride content has gone as high as 1808 and TDS to 3276 mg/L. Such an increase in chloride and TDS within 14 years is an alarming indication of seawater intrusion that has resulted due to depletion of groundwater table. Depletion of groundwater table is due to over-extraction of water for industrial and agricultural purposes. The number of groundwater wells in India increased by 13 millions between 1951 and 1997 (UNEP 1996). Ozhukarai commune, under which Kalapet comes, has 100 bore-wells for irrigation purpose alone.

Table 4. Results of analysis of Kalapet samples

S.No.	Sample	Depth of the Borewell	TDS mg/L	EC μ s	pH	Cl mg/L	Estimated NaCl mg/L	HCO ₃ mg/L	Cl/HCO ₃ ratio
1.	Near Hotel Ashok	70 ft.	1080	1490	6.9	519.9	857.456	200	2.599
2.	Chemfab Alkalies (1984)	42 mt.	118	165.2	6.9	14.18	21.624	71.3	0.1988
3	Chemfab Alkalies (1999)	42 mt	3276	4586	8.1	1808	3163.47	170	10.635
4.	Ramco Cement Agencies	20 ft.	170	180	6.9	47.99	79.149	60	0.799
5.	Residence (Opposite to Ashok Hotel)	70 ft.	720	420	7.3	95.99	158.29	64.0	0.149
6.	Navodaya School	140 ft.	200	190	7.4	67.99	112.11	80	0.849
7.	Agro Chemicals	-	350	210	7.5	81.99	135.29	92	0.891

A bore-well dug by state groundwater unit has turned out to be saline, due to seawater intrusion and this interface exists beyond the depth of 465 mbgl (SGWB, 1992). Seawater intrusion into bore-wells has resulted due to upconing effect of salt water. This upconing results due to over-pumping of

water. The World Bank warns that in the coming decades, lack of freshwater is likely to be one of the major factors limiting economic development (Serageldin 1994).

Water samples collected and analysed from shallow bore-wells in Ramco Cement Agencies and from residential areas opposite to Hotel Ashok show good quality of water. Though salt water-freshwater interface occurs over here, upconing of seawater has not taken place in these bore-wells, which could be due to low rate of extraction. The other possible reason for good quality of water may be due to the percolation of rain water into this aquifer.

Water samples taken from Navodaya School and Agro-chemicals show low level of chloride and TDS value. Hence, it is inferred that seawater has not intruded to that distance.

CONCLUSION AND RECOMMENDATIONS

'Prevention is better than cure' but in case of intrusion of seawater, prevention is the only way since there is no cure. No technology has so far been developed to retard saline waters once intruded or to desalinate at the under-ground level itself.

In order to avoid over-exploitation of groundwater, at the first instance along the seacoast following propositions are suggested:

1. It is essential to enact a legislation to regulate use of water resources and to prevent contamination of precious groundwater in the coastal tract.
2. To prevent over-extraction at local level, restricting the number of wells, pump sets and maintaining a minimum spacing of 250 meters between two wells is necessary.
3. Since seawater has intruded to 6 km inland any further development of groundwater needs a systematic study and judicious approach.
4. Direct recharge of aquifers through water harvesting, recharge of surface water storage irrigation tanks is necessary.
5. The present reversal of hydraulic gradient along the coast is also due to indiscriminate sinking of tube wells in adjoining Tamil Nadu (SGWB 1992). Unless a joint effort is made by both Tamil Nadu and Pondicherry, the efforts taken by Government of Pondicherry alone will not bear any fruits. Hence GWB may be requested to take up the matter with Tamil Nadu for regulation and control of groundwater.

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Environment News

House Plants Combat Pollution

House plants can protect against pollution indoors, where pollutant concentrations are two to five times higher than outdoors, according to U. S. Environmental Protection Agency. Indoor toxins are becoming more of a problem because buildings are more efficient and well sealed and that is why potted plants can play a very useful role according to B. C. Wolverton, a returned NASA scientist. Green leafy plants or palms can remove toxins in the air and suppress bacteria, spores and molds.

Envirenews, Lucknow

Hydrogen-Powered Buses to Help Keep Cities Clean

These early years of the new millennium will see the introduction of cleaner, "greener" road transport to streets when the first of new-style hydrogen powered buses, built by Daimler Chrysler, are brought into public service.

Introduction of hydrogen fuel-cell buses to London is part of a Europe-wide, European Union-funded project to look at the viability of hydrogen as a fuel for motor transport vehicles. Renewable energy sources, such as hydrogen as a fuel, are expected to be economically competitive with conventional energy sources in the medium to long term.

These first buses will be supplied to those operators who committed themselves to a demonstration project in April 2000. They will run in Amsterdam, Barcelona, Hamburg, London, Luxembourg, Porto, Stockholm, Stuttgart and Reykjavik.

During a two-year trial the bus operators will jointly accumulate and evaluate their experiences and technical findings related to all aspects of bus operations and the new hydrogen infrastructure involved. All the operators will have filling stations for gaseous hydrogen built by BP and other fuel producers in their related territories.

Each of the low-floor buses will be 12 metres (39 feet) long and able to accommodate up to 70 passengers, depending on the individually specified vehicle layout. The buses will have an operating range of 200 to 250 kilometres (125-155 miles) and can achieve a maximum speed of 80 kmph (50mph).

The fuel-cell unit which has an output of more than 200 kilowatts, as well as compressed hydrogen storage tanks (which are pressurised to 350 bar), are housed within the roof of the Citaro, while the electric motor, transmission, prop shaft and mechanical rear axle are located at the rear of the vehicle. The vehicle's electric motor is powered by electrical energy produced when hydrogen reacts with oxygen in the fuel cells to form water. The only emission or "exhaust gas" produced is water vapour.

Spectrum

India Assisted In Protecting Ozone Layer

India will get an \$82 million grant to gradually phase out the production of ozone depleting substances such as CFCs in the country. The project, implemented through the World Bank, will provide financial compensation for CFC-producing enterprises to meet annual production ceilings agreed to by India and the executive committee of the Montreal Protocol Multilateral Fund. It said CFC production in India will be phased out in 2010.

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LANGLIER'S INDEX AND ITS RELATION TO PHYTOPLANKTON IN TWO LAKES OF MYSORE CITY

J. Mahadev and S.P. Hosmani*

Department of Studies in Environmental Science

*Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore-570006

ABSTRACT

Langlier, calcium carbonate saturation index is commonly used to evaluate the scale forming and the scale dissolving tendencies of water. Assessing these tendencies are useful in corrosion control programmes and in prevention of calcium carbonate scaling in pipes in equipments such as industrial heat exchangers and domestic water heaters. Phytoplankton growth in such waters have a tendency to reduce such processes. Members of the chlorococcales and blue greens seem to dominate such saturated waters.

INTRODUCTION

Freshwater bodies are being increasingly polluted in urban centers like Mysore city. The pollutants that enter such waters tend to increase or decrease the saturation with regards to many chemicals. One such saturation is the calcium carbonate saturation index also known as "Langlier's Index". Water over-saturated with respect to calcium carbonate tends to precipitate it while water under-saturated with calcium carbonate tends to dissolve it. Saturated water or water in equilibrium with calcium carbonate will have no net tendency of either scaling or corroding. This aspect of study has been poorly represented, in general, and especially with waters of Mysore city. The only work reported so far is by Hosmani & Vasanth Kumar (1996). The impact of phytoplankton growth on the saturation index has been described presently.

Two water bodies, one heavily polluted with sewage, and the other protected, have been studied. This water bodies often experience algal blooms, and therefore an attempt has been made to relate the blooms to the saturation index.

MATERIAL AND METHODS

Dalvoii lake and Karnaji lake are situated at a distance of about 5 km and are in the vicinity of Mysore city. Dalvoii lake receives heavy sewage from the city and is, therefore, considered polluted. Karnaji lake is not polluted and lies in a protected area. The water in it is used by the Mysore zoo. The method followed for determining the index is described by Trivedy & Goel (1983) and Hosmani & Vasantha Kumar (1996) and is the difference between the actual pH of the water (pH) and its pH at saturation with calcium carbonate (pHs). pH-pHs is the calcium carbonate saturation index. A chart of Langlier's index indicates the following.

Saturation index	Tendency of the water
+2 and higher	Heavy scale
+2	Light scale
+0.05	Light scale or corrosion
-1.0	Significant corrosion
-2.0	Heavy corrosion
-2.0 and below	Intolerable corrosion

The methods for the collection, preservation, identification and enumeration of phytoplankton are described in detail by Hosmani & Bharati (1980).

RESULTS AND DISCUSSION

The results of the saturation index in the two water bodies are indicated in table 1 and for the purpose of discussion they have been represented seasonally. The average planktonic population and the number of algal species in each season is also shown in the same table. The group-wise phytoplankton in each water body is represented in table 2.

Table 1. Seasonal variation of Langlier's Index and Phytoplankton

	Dalvoi Lake			Karanji Lake		
	Rainy	Winter	Summer	Rainy	Winter	Summer
Calcium carbonate Saturation Index	-0.14	+2.07	+2.86	+2.27	+1.29	+5.48
Tendency of the water	Light scale of corrosion	Heavy scale	Heavy scale	Heavy scale	Light scale	Heavy scale
Average planktonic population per L	24500	24675	14050	6517	7770	19405
No. of planktonic species	15	15	09	15	20	32

Table 2. Distribution of phytoplankton species in two lakes of Mysore city

	Euglenaceae	Bacillariophyceae	Chlorococcales	Myxophyceae	Desmids
Karanji lake	7	5	11	6	4
Dalvoi lake	7	5	3	4	0

On an overall consideration of the index in Dalvoi lake during monsoon, it was -0.14 (light scale to corrosive) while during winter it was $+2.07$ which is the tendency of heavy scale and continued to be same during summer months. Considering the index in Karanji lake it was with a heavy scale tendency during monsoon and summer, but slightly lowered down to a light scale during winter.

On considering the phytoplankton growth in these waters it is found that the light scale or corrosive deposition properties limit the growth of algae but with a wide diversity. Dalvoi lake is dominated by a mixed bloom of blue greens represented by *Phormidium fragile*, *Arthrospira feneri*, *Merismopedia tenuisima* and *Microcystis aeruginosa*. During summer months when the tendency of water rises to $+2.86$ i.e., heavy scale, the diversity increases with decreasing total population. The species that appeared in sufficient numbers were *Synedra ulna*, *Navicula sphaerophora*, *Navicula*

rhomboides, *Euglena elastica*, *Phacus longicauda*, *Phacus orbicularis*, *Trachelomonas volvocine* and *Lepocinclis ovum*, *Phacus curvicauda*, *Euglena gracilis* and *Gyrosigma accuminatum*. The greatest diversity was that of the members of Chlorococcales represented by *Scenedesmus orbicularis*, *Scenedesmus protuberens*, *Scenedesmus armatus*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Pediastrum duplex*, *Pediastrum tetras*, *Coelastrum cambricum*, *Tetraedron tribolatum* and occasional occurrence of *Oocytis gigas*.

It is of interest to note that in Karanji lake, during summer when the tendency of the water was heavy scale, almost similar organisms that were observed in Dalvoi lake during that season, were present in this lake also. This indicates that tendency of heavy scale also tends to diversify the organisms, while light scale tendency decreases the diversity of organisms, but increases a particular population. During the other seasons, Karanji lake had a uniform growth of similar phytoplankton. A few desmids were observed in Karanji lake that included *Cosmarium lundelli*, *Cosmarium divergens*, *Euastrum spinolosum* and *Closterium lunula* but were completely absent in Dalvoi lake indicating heavy pollution of water.

CONCLUSION

Summer season in Karanji lake has the highest tendency of scale forming (+5.48). Average plankton population is also significantly high during this season (19,405). Correspondingly the total number of planktonic species is also high. Dalvoi lake receives city sewage regularly and has a tendency of light scale deposition (-0.14) during rainy season. Sewage plus rainwater may have an importance in increasing the heavy scale nature of water, which is observed in winter and summer. Although plankton population is low, blooms are of common occurrence during summer season. Both the water bodies are heavily polluted and are saturated. Phytoplankton growth in abundance seems to regulate the tendency of water. Phytoplankton can be used in the treatment of wastewater and reducing either heavy scale deposition of corrosiveness of water and making it potable. Absence of desmids in Dalvoi lake is also an indication of heavy pollution of water. Calcium carbonate saturation index in both the water bodies has a tendency of heavy scale deposition.

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Conferences/Symposia

Eleventh National Symposium on Environment, June 5-7, 2002

Contact: Dr. L.L. Sharma
 Symp. Organising Committee (NSE-11)
 Head, Deptt. of Limnology and Fisheries
 Mohata Badi, University New Campus
 Udaipur-313 001 Rajasthan, India

Asian Congress of Mycology and Plant Pathology, October, 1-4, 2002

Contact: Dr. S.R. Niranjana
 Organising Secretary, ACMPP
 DOS in Applied Botany, Seed Pathology and Biotechnology
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 Mysore-570 006, Karnataka, India

International Conference on Environmental Threats to the Health of Children: Hazard and Vulnerability, March 3-7, 2002

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International Symposium on Biodiversity of Flowering Plants of South Asia November 1-5, 2000, Karachi, Pakistan

Contact: Prof. Dr. M. Qaiser
 Department of Botany
 University of Karachi, Karachi - 75270, Pakistan
 Email: flora@super.net.pk
 Phone: 92-21-496 3617; 924 3685

Worldwide Occurrence of Earthquakes

<i>Description</i>	<i>Magnitude</i>	<i>Annual Average</i>
Great	8 or higher	1
Major	7 - 7.9	18
Strong	6 - 6.9	120
Moderate	5 - 5.9	800
Light	4 - 4.9	6,200
Minor	3 - 3.9	49,000
Very Minor	1 - 3	3,285,000 (9,000 daily)

DISTRIBUTION OF CALCIUM AND MAGNESIUM IN GOPALPUR CREEK WATERS BAY OF BENGAL

Tapan R. Mahapatro, Sobhan K. Sahu and S.N. Padhy

Department of Marine Sciences, Berhampur University, Berhampur – 760 007, Orissa, India

ABSTRACT

The seasonal variations of calcium and magnesium concentrations in the surface waters of Gopalpur Creek were studied during the year 2000-2001. Calcium and magnesium concentration increased from the upper reaches of the creek (4th st.) towards the mouth (1st st.) and varied linearly with chlorinity value. Premonsoon period recorded higher values than other seasons, whereas monsoon period encountered lower value. The calculated value of Ca/Cl and Mg/Cl ratios ranged from 0.0194-0.0460 and 0.0432-0.0905 respectively. The ratios were found to be high during monsoon and low during premonsoon period.

INTRODUCTION

Gopalpur is situated on the east coast of India between 19°6'N lat. and 84°55'E long. A brackish water creek locally known as "Haripur Creek" is located at the north-eastern side of the Gopalpur township. A sandy barrier at its southern extremity impounds the creek and is fed with freshwater from an ephemeral stream called 'Nadia Nala'. The creek remains cut off from the sea during premonsoon months. During the period, the water exchange between the sea and creek takes place through seepage. The general condition of the creek satisfies the requirements of a pocket lagoon according to Phleger (1981).

Although all of the alkaline earth's elements present in seawater, calcium and magnesium are major elements with strontium constituting a minor component. Analyses of ocean waters for these ions are not numerous. These elements have yielded metal ion-chlorinity ratios, which remain fairly constant. Conservative or semi-conservative properties of seawater and their relation with chlorinity may be useful in delineating the characteristic of different water bodies (Sengupta et al. 1978). Therefore, it is of interest to study the concentration of major elements and their ratios to chlorinity in creek water. Hence, the present study signifies on the conservative and semi-conservative behaviour of the creek water in relation to the chlorinity. The chlorinity value was subjected to seasonal fluctuation due to the tidal flushing at its mouth and drainage by the ephemeral streams around the creek. No such reports on the distribution of calcium and magnesium with their behaviour are available to the date.

MATERIAL AND METHODS

Surface water samples were collected at selected stations during premonsoon (May—June), monsoon (July-Oct) and postmonsoon (Nov-Feb) period 2000-2001 in the creek water of Gopalpur (Fig. 1) by using a precleaned polyethylene bucket. The samples were stored in prerinsed polyethylene bottles with leak-proof stoppers and preserved in a refrigerator until analysis, which were to be completed within a month of collection. Chlorinity determinations were made using argentometric

titration procedure (Grasshoff 1976). Calcium and magnesium were determined applying the method of Culkun and Cox (1966) after titrating the sample to the photometric end point with EGTA and Zincon Zn-EGTA indicator. Complexometric titration's with EDTA and Eriochrome black-T indicator yielded combined concentrations of calcium, magnesium and strontium from which the calcium concentration was subtracted to obtain the magnesium concentration. The magnesium concentration was further corrected for strontium and for a systematic overestimate of about 1% in the titration end point (Sengupta et al. 1978).

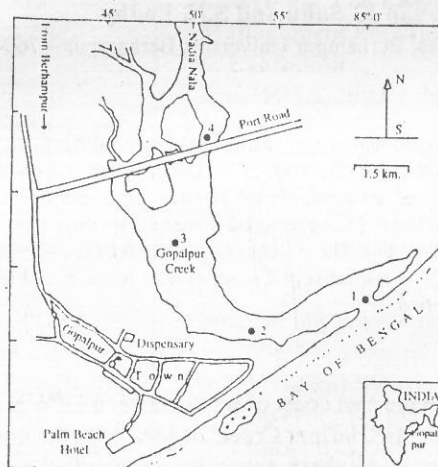


Fig. 1. Gopalpur creek showing sampling stations

RESULTS AND DISCUSSION

Chlorinity

The chlorinity ranged from 6.908-17.553‰ during the observation. The average value was 11.718‰. During the study period maximum chlorinity value was observed at st. 1 during premonsoon, and whereas minimum at st. 4 during monsoon. Overall, the chlorinity decreases from st. 1 to 4 in all the three seasons. The maximum value at st. 1 is due to the dominance of coastal water and minimum value at st. 4 resulted from the dilution of creek water by the drainage of freshwater from 'Nadia Nala' (Fig. 2a).

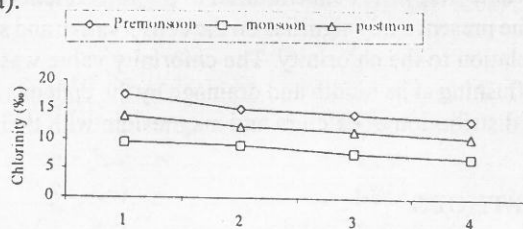


Fig. 2a. Seasonal variation of chlorinity at selected stations

The premonsoon is a dry season and the addition of freshwater from the 'Nadia Nala' to the creek was scanty. Hence higher values were recorded during this season. As there is maximum freshwater inflow through 'Nadia Nala' in monsoon, comparatively lower values were observed during the season.

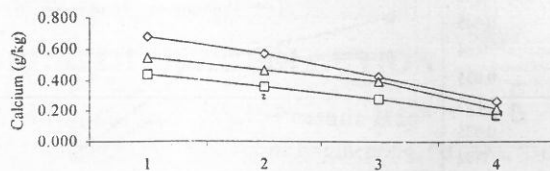


Fig. 2b. Seasonal variation of calcium at selected stations

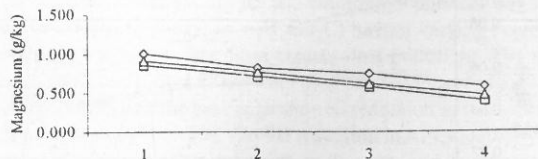


Fig. 2c. Seasonal variation of magnesium at selected stations

Calcium

The calcium value ranged from 0.172-0.676 g/kg. The average calcium value was found to be 0.399 g/kg. During the study period maximum calcium value was observed at st. 1 during premonsoon, whereas minimum value was observed during monsoon. During all the seasons the value was decreased from st. 1 to 4 (Fig. 2b). Calcium values of all the stations were observed more in premonsoon and less in monsoon. The lower value of calcium in monsoon may be due to dilution effect and higher concentrations in the subsequent seasons appeared to be largely due to the recovery of calcium for gradual attainment of coastal water by seepage. Naik (1978) and Noronha et al. (1981) reported similar findings. The values of calcium are always lower in postmonsoon season than the premonsoon season owing to an active growth of the ayad larvae of muscles in postmonsoon season and uptake of calcium during this period (Rao et al. 1975). Calcium linearly varied with chlorinity. The Ca/Cl values appeared highest during monsoon at st. 1 (0.0460), whereas lowest during premonsoon at st. 4 (0.0194). The average Ca/Cl was observed was 0.0340. Seasonal variation also indicated the involvement of chlorinity in changing the ratio. The higher values were noticed during monsoon period due to lower chlorinity and *vice versa* in premonsoon season (Fig. 3a). The higher values of Ca/Cl in monsoon may also be due to addition of freshwater to the creek during this season (Noronha et al. 1981).

Magnesium

Magnesium, the second most abundant cation in seawater, bears an average concentration of 0.699 g/kg. The values ranged from 0.393-1.003 g/kg. Maximum magnesium value was observed at st. 1 during premonsoon, whereas minimum at st. 4 during monsoon period. Comparatively higher values were observed at all the stations in premonsoon than postmonsoon and monsoon periods (Fig. 2c). While evaporative process concentrates the salts during premonsoon there is addition of

freshwater in postmonsoon and monsoon, which dilutes the seawater and decreases magnesium content (Palanichamy & Balasubramanian 1989, Nasolkar et al. 1997).

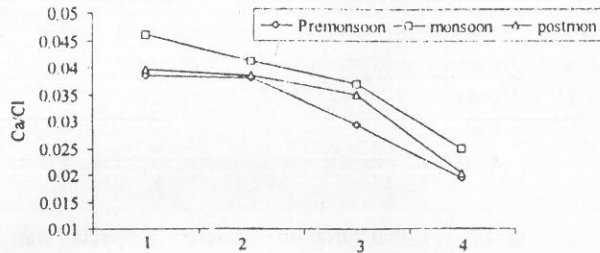


Fig. 3a. Seasonal variation of Ca/Cl at selected stations

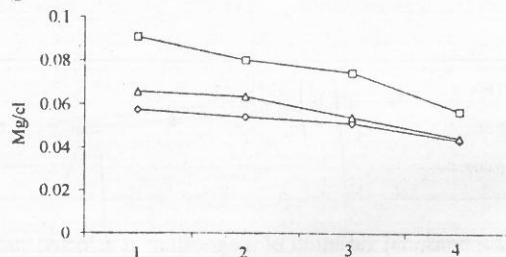


Fig. 3b. Seasonal variation of Mg/Cl at selected stations

The Mg/Cl ratio varied from 0.0432-0.0905. Maximum value was observed at st. 1 during monsoon and minimum at st. 4 during premonsoon (Fig. 3b). The average value was 0.061. The higher value of Mg/Cl during monsoon may be due to drastic drop in chlorinity associated with heavy monsoonal floods.

ACKNOWLEDGEMENT

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BIO MONITORING OF DUST POLLUTION

N. Srinivas, B. Chandrapaul* and P.V.V. Prasada Rao*

Department of Environmental Studies, College of Engineering, GITAM, Visakhapatnam – 530045

*Department of Environmental Sciences, Andhra University, Visakhapatnam-530 003

ABSTRACT

The response of seedlings of *Tridax procumbens* and *Parthenium hysterophorus* to particulate air pollutants on the morphological changes and chlorophyll pigment was studied. Seedlings were collected from three different sites (site A, B and C) having varying degrees of air pollution (Site-A, which serve as control area is free from cement dust pollution). The maximum reduction in leaf area, lengths of shoot and root, API and total biomass was observed in *Tridax* seedlings compared to *Parthenium* seedlings. Both the seedlings showed reduction in total chlorophyll and carotenoids in site – C. The present study indicates that the reduction in API, chlorophyll content and total biomass is an indication of presence of air pollutants in the area, and *Tridax procumbens* seems to be more sensitive to air pollution, hence may be suggested as an indicator species of air pollution.

INTRODUCTION

In the last few years, the effects of air pollution on plant growth and the use of higher plants as bioindicators in industrial and urban areas have attracted the attention of researchers (Varshney & Garg 1978, Chaphekar 1982, Rao 1972, Panday 1983, Ali 1993, Kousar et al. 1998). The presence or absence, distribution, morphology and chemical characteristics of plants can be conveniently used for bio-monitoring of air pollution. Plants can act as indicators of air pollution and play a vital role to arrive at a conclusion regarding the air quality of an area. Since many plant species are sensitive to air pollution, it is possible to monitor the levels of air pollution through proper quantification and standardization of plant responses of sensitive *species* (Trivedy & Goel 1995). Chlorophyll has been traditionally used as a sensitive parameter for air pollution studies. Agarwal (1991) has reported a direct relationship between SO₂ and SPM concentration in air and decrease in chlorophyll content. Nandi et al (1980) have examined protein content of germinating seeds of *Phaseolus aureus* exposed to different pollutants and obtained reliable results. Banerjee et al (1983) also suggested it as a useful tool for air pollution monitoring. Varshney (1985) compared the sulphur content in leaves and bark of 11 common tree species growing around Indraprastha power station in New Delhi with sulphur content in tree bark at unpolluted sites and reported that sulphur content in tree bark varied widely with air pollution levels.

The present study reports the response of two weed seedlings, *Tridax procumbens*, and *Parthenium hysterophorus* of Asteraceae family to dust pollution from a cement plant on the morphological changes and chlorophyll pigment responses at Visakhapatnam.

MATERIALS AND METHODS

Naturally grown seedlings of *Tridax procumbens* and *Parthenium hysterophorus* were collected from three sites (Site A, Site B, and Site C) selected from three different impact zones of a

cement plant. Site A which is sheltered by hill and is free from dust pollution was situated far from the cement plant, hence it was considered as control area. Sites B and C was situated at an arial distance 275 m and 150 m respectively in the windward direction and are within the impact zone. Seedlings of the two species were collected up to the expansion of the third leaf. The total leaf area and shoot and root length measurement were made by graph paper method, and biomass was estimated by oven dry weight (80°C, 48 hours) method. Total chlorophylls were estimated following Arnon (1949) and carotenoids by Liaanen-Jension and Jenson (1971) method using Systronics UV-Vis Spectrophotometer. Air pollution Index (API) values of leaf, shoot and root were calculated by the method of Chaphekar et al. (1980). Twenty-five seedlings of each species were collected from each site and their mean values were computed.

RESULTS AND DISCUSSION

The effects of dustfall on leaf area and on the lengths of shoots and root of the two seedlings are presented in table 1. The main pollutants emitted through the chimney of the cement plant are in the form of suspended particulate matter which contains lime, silica and alumina in higher quantities along with small quantities of iron oxides, magnesium oxides and oxides of sulfur (Jain & Jain 1986).

Table 1. Growth factors and biomass of the two seedlings at different sites

Plant species	Site	Root length (cm)	Shoot length (cm)	Leaf area (cm) ²	Biomass (mg/plant)			Total biomass (mg/plant)
					Root	Shoot	Leaf	
<i>Tridax procumbens</i>	A	6.68	1.1	30.9	2.40	0.61	4.86	7.87
	B	2.78	1.0	16.75	0.82	0.61	4.20	5.63
		(-51.05)	(-9.09)	(-45.81)	(-65.83)		(-13.58)	(-28.46)
	C	2.36	0.64	14.95	1.23	0.43	3.21	4.87
		(-58.45)	(-41.85)	(-51.61)	(-48.75)	(-29.51)	(-33.95)	(-38.11)
<i>Parthenium hysterophorus</i>	A	2.72	0.5	14.29	0.60	0.62	4.22	5.44
	B	2.32	0.92	10.72	0.60	0.62	3.21	4.43
		(-14.70)	(+84.00)	(-24.97)			(-23.93)	(-18.57)
	C	2.26	1.02	7.15	0.82	1.04	3.00	4.86
		(-16.91)	(+104.00)	(-49.92)	(+36.66)	(+67.74)	(-28.9)	(-10.66)

Values in parenthesis indicate % of inhibition/stimulation

Both *Tridax* and *Parthenium* seedlings showed reduction in leaf area and root length at Sites-B and C compared to Site A. The shoot length showed reduction in *Tridax* seedlings whereas in *Parthenium* seedlings continuous increase in shoot length was observed in Site B and Site C compared to Site A. *Tridax* seedlings showed much reduction in leaf area, and shoot and root lengths compared to *Parthenium* seedlings. The development of root and leaf biomass for both the seedlings was reduced in both the polluted sites. The maximum reduction of total biomass (38%) was observed in Site-C in *Tridax* seedlings. The data presented in table 2. indicate that *Tridax* seedlings seem to be more sensitive to air particulate than *Parthenium* seedlings. Reduction in root length, shoot length and leaf area was observed in *Tridax* seedlings whereas only root length and leaf area was reduced in *Parthenium* seedlings. The reduction in *Parthenium* is much lesser compared to *Tridax* seedlings.

Air Pollution Index (API) of *Tridax* and *Parthenium* seedlings after exposure to cement dustfall at different sites is presented in figs. 1 and 2. Of the two species *Tridax* seedlings are found to be more sensitive to cement dustfall than *Parthenium* seedlings. The complete reduction of root, shoot and leaf API values was observed in *Tridax* seedlings whereas only root and leaf API values were reduced in *Parthenium* seedlings. Neither reduction nor inhibition of shoot API was observed in *Parthenium* seedlings.

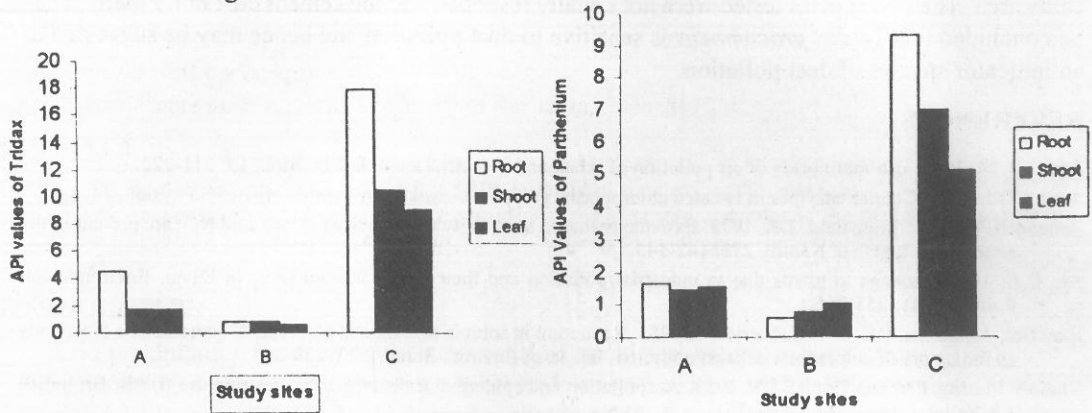


Fig. 1. Air pollution index (API) of *Tridax* seedling after exposure to cement dust fall at different sites

Fig. 2. Air pollution index (API) of *Parthenium* seedling after exposure to cement dust fall at different sites

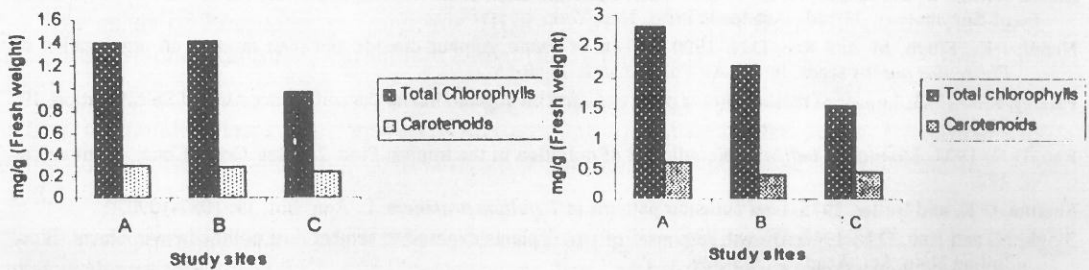


Fig. 3. Total chlorophylls and carotenoid content in *Tridax* seedling after exposure to cement dust fall at different sites

Fig. 4. Total chlorophylls and carotenoid content in *Parthenium* seedling after exposure to cement dust fall at different sites

The total chlorophyll and carotenoid content in *Tridax* and *Parthenium* seedlings are presented in figs. 3 and 4. Both the species exhibited a reduction in total chlorophyll and carotenoid at site C and the rate of reduction in *Parthenium* is quite high as compared to *Tridax*.

CONCLUSION

Many studies have shown that air pollution effect plant morphology in a variety of ways. Singh and Rao (1978, 1980) reported the role of cement dust on chlorophyll content, biomass and productivity in different plant systems. The present study indicates that the reduction in chlorophyll content and total biomass is an indication of the presence of air pollution particularly air particulates in the study area. The two species tested were not equally responsive to the cement dust fall. Finally it can be concluded that *Tridax procumbens* is sensitive to dust pollution and hence may be suggested as an indicator species of dust pollution.

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EFFECT OF SIMULATED ACID RAIN ON *PHASEOLUS VULGARIS* VAR. HUR-15

T.I.Khan and Shikha Devpura

Indira Gandhi Centre for H.E.E.P.S., University of Rajasthan, Jaipur.302004, India

ABSTRACT

The seedlings of *Phaseolus vulgaris* var. HUR-15 tolerated simulated acid rain exposure down to pH 3.1. Seeds succumbed at pH level 2.1. At pH 3.1, the percentage reduction in length and dry weight of root and shoot varied between 75-90%, whereas in chlorophyll content reduction was 81.5%.

INTRODUCTION

Acid precipitation has been associated with industrial and automotive emissions of sulphur oxides and nitrogen oxides. The gaseous sulphur and nitrogen oxides react with atmospheric water and result in sulphuric and nitric acid formation that is ACID RAIN. Acid rain interferes with soil texture and soil chemistry, injures the leaves of the tree and other plants. Reports of acid rain on biological system are given by Evans et al. (1982), Sheriden (1985), Nath and Nath (1990), Khan et al. (1996), Raj (1998). However, there is a dearth of investigations on the effect of acid rain on crop legumes, and hence the present study was made to determine the effects of simulated acid rain on *Phaseolus vulgaris* var. HUR-15, an important leguminous crop.

METHODOLOGY

The seeds of *Phaseolus vulgaris* var. HUR-15 were obtained from Durgapura Research Experiment Station, Rajasthan Agriculture University Jaipur, India.

The experiment was conducted in the Petriplates placed in germinator at 25°C for a period of 10 days. Artificial acid rain was prepared by mixing sulphuric acid and nitric acid in 3:1 ratio and by adding double distilled water to prepare a range of solutions with pH values of 6.8 (control), 5.1, 4.1, 3.1, 2.1 and 1.1.

In each petriplate five seeds were placed on filter paper moistened from below by sterilized cotton in the germinator at 25°C + 2°C temperature. Root and shoot lengths were measured on the tenth day, after termination of the germination experiment. Thereafter roots and shoots were separated and dried in a hot air oven at 80°C to estimate dry weight.

Chlorophyll was estimated by the standard method suggested by Arnon (1949). The absorbance values at 645nm and 663nm were used in the following formulae for chlorophyll estimation.

$$\text{Chlorophyll a} = 12.7A_{663} - 2.69A_{645} \text{ (mg/l)}$$

$$\text{Chlorophyll b} = 22.9A_{645} - 4.68A_{663} \text{ (mg/l)}$$

$$\text{Total Chlorophyll (a+b)} = 8.02A_{663} + 20.20A_{645} \text{ (mg/l)}$$

Where A_{663} is absorbance at 663nm and A_{645} is absorbance at 645nm.

The amount of chlorophyll was calculated in mg/g dry weight of the leaves.

RESULTS

Phaseolus vulgaris var. HUR-15 seedlings tolerated lowering of pH upto 3.1. Below this pH level the seedlings faced mortality. Plant could not attain its normal growth. Leaf size also reduced and developed necrosis. Plant growth decreased with the decrease in pH level. A reduction of 75-90% was observed in root and shoot length at pH 3.1 (Table-1).

Root and shoot dry weight also decreased with the decrease in pH. Root dry weight decreased more steeply than shoot dry weight (Table-1).

Table 1. Effect of pH on seedling growth of *Phaseolus vulgaris* var. HUR-15

pH level	Length (in cm)		Dry weight (in mg)	
	Root	Shoot	Root	Shoot
6.8(control)	12.7±0.5	20±0.8	63±3.6	218.3±5.5
5.1	8.7±0.4 [31.07]	10.2±0.4 [49.68]	45.3±3.4 [28.07]	87.1±4.8 [60.10]
4.1	2.4±0.2 [80.99]	7.0±0.2 [65.37]	15.3±3.2 [75.79]	62.6±1.8 [71.26]
3.1	1.4±1.8 [89.12]	4.8±0.1 [76.34]	8.4±1.0 [86.66]	45.9±2.1 [78.97]
2.1	M	M	M	M
1.1	M	M	M	M

*Data in parenthesis denotes percentage reduction

*M denotes Mortality

Chlorophyll a, b and (a+b) decreased with the decrease in pH levels. A reduction of 81.5% in total chlorophyll was recorded between control level and pH 3.1. (Table 2)

Table 2. Effect of pH levels on chlorophyll content (mg/gm dry weight) of *Phaseolus vulgaris* var. HUR-15

pH level	Chlorophyll content (in mg/gm of dry weight of leaf)		
	Chlorophyll-a	Chlorophyll-b	Total chlorophyll
6.8(control)	0.78±0.04	0.34±0.03	1.12±0.07
5.1	0.51±0.17 [34.66]	0.19±0.11 [43.73]	0.7±0.28 [37.10]
4.1	0.21±0.01 [72.79]	0.18±0.09 [46.94]	0.39±0.04 [64.70]
3.1	0.1±0.06 [86.65]	0.1±0.01 [70.26]	0.21±0.06 [81.45]
2.1	M	M	M
1.1	M	M	M

* Data in parenthesis denotes percentage reduction

* M denotes Mortality

DISCUSSION

Visible adverse effects on plant growth were observed by Harcourt and Farrar (1980). In the present study seedlings of crop legume *Phaseolus vulgaris* var. HUR-15 tolerated pH down to 3.1 (Fig. 1).

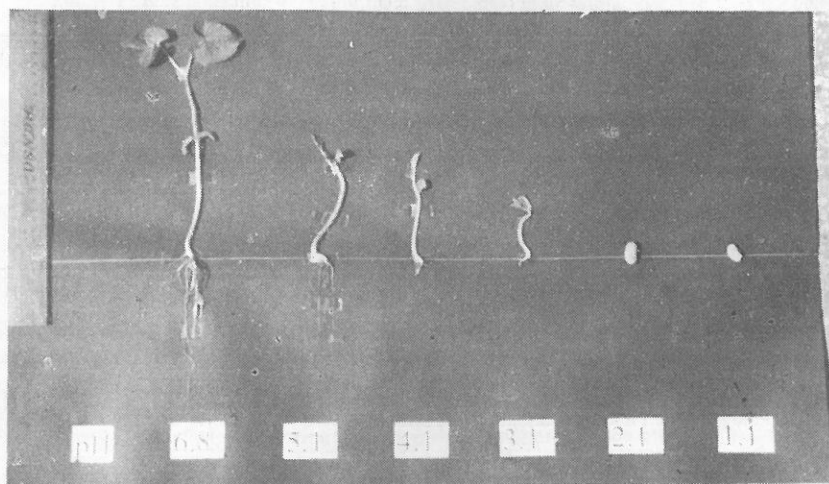


Fig. 1. *Phaseolus* seedlings exposed at different pH

Below pH 3.0 plants in tropical and subtropical ecosystems were observed to be severely affected (Choudhary et al.1988). Plants fail to reach normal height with visible necrosis (Shriner, 1976; Jacobson.1980; Irving, 1983; Khan et al., 1996; Raj, 1998). Present study showed that length and weight of seedlings decrease with the decrease in pH levels. The growth was also adversely affected as the reduction in the dry weights was recorded. Wood and Bormann (1974), Farenbaugh (1976) and Babich (1981) found that plants could not attain a normal height and that there was sufficient reduction in the heights of the treated plants below pH 3. These plants had necrosis and wrinkled leaves. Choudhary et al.(1988) and Kakralaya et al.(1988) also found that simulated acid rain adversely affected the growth of leguminous seedlings, and pH 3.5 induced spotting and necrosis on the leaves. With lower pH chlorophyll content, plant dry weight and root systems showed considerable reduction. Simulated acid rain has reduced chlorophyll, as reported by Sheridan and Rosenetreter (1973) and Khan et al (1996). In the present study a reduction of 89.12% in the root length and 76.34% in shoot length was observed at pH 3.1. Root dry weight decreased to 86.66% and shoot dry weight to 78.77% at pH 3.1. Total chlorophyll also decreased with decrease in pH level.

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STUDIES ON THE EFFECT OF SALINITY ON ENGINEERING PROPERTIES OF SOIL

M. Murali and M. Sreenivas*

Department of Environmental Studies, College of Engineering, GITAM,
Visakhapatnam-530045, A.P. India
(Author for all correspondence)

* Department of Civil Engg., S.V.H. College of Engineering, Machilipatnam-520002, A.P. India

ABSTRACT

Industries generally discharge their effluents in a nearby stream or onto ground surface. These effluents seep through the pores of soil and vary the pore fluid characteristics, which in turn change the soil properties. This paper aims at study of such effect on a typical expansive soil with the salinity of pore fluid. The variations of soil properties in terms of volume change behaviour as well as strength characteristics are studied with varying salt concentrations. The results are compared and discussed.

INTRODUCTION

Soil is the base material on which all structures rest. It is also the place where most refuse and wastes are dumped. Initially it is presumed that the soil is an inert material. But it is not so. Soils, particularly clays, possess unbalanced charges on their surface and hence are very active chemically (Holtz et al. 1954). Other parameters remaining same, the quality of pore fluid has sometimes tremendous influence on the properties of soils (I.I.Sc., 1991).

Industries often discharge their effluents in streams or on land. These effluents seep through the pores of soil and change the pore fluid characteristics, which in turn change the soil properties (Sreenivas et al. 1996). Any overlook of this aspect may seriously influence the performance of the structure. In fact the performance is predicted from the laboratory test results which will be conducted with distilled water as pore fluid and hence the difference between prediction and performance exists (Murali et al. 1996).

In this paper an attempt is made to study that how engineering properties of soil will vary with salinity of pore fluid. A typical expansive soil is taken and analysed. The variation of soil properties in terms of volume change behaviour and strength characteristics are studied with varying salt concentrations. From the observations made, it can be emphasised that the prediction of soil behaviour be made exclusively from tests conducted on samples with the existing pore fluid only.

METHODOLOGY

A natural soil with good expansive properties on which the structures are observed to suffer damages is taken for testing. The following tests are conducted on the soil with distilled water, in accordance with standard procedures (BIS, 1989).

1. Atterberg Limits: As per IS: 2720.

2. Different Free swell: 20 g of soil is taken, each in 24 hours, the difference in volume of soil in both jars is noted.

$$\text{Differential Free Swell} = \frac{\text{Difference in volumes}}{\text{Volume in Kerosene}} \times 100$$

3. Swell Potential and Swell Pressure: It is conducted in Oedometer ring restrained against movement in downward direction and sides. Dry sample is compacted in layers in the ring, up to the top level. It is fixed in position and submerged under water. The soil swells in the presence of water. The increase in height (ΔH) of the specimen is recorded by a dial gauge. Swell potential is determined as

$$\text{Swell Potential} = \frac{\Delta H}{H}$$

where H = Height of specimen

The pressure required to bring the swollen specimen to its original volume is defined as swell pressure (expressed in kg/cm^2).

4. Standard Proctor Test
5. Direct Shear Test: Soil is remoulded to its maximum dry density at respective optimum moisture content. The test is conducted in an unconsolidated, undrained condition.

Synthetic samples of 5, 12, 20, 25 and 30 ppt (parts per thousand) of common salt were prepared in distilled water (APHA 1985) and the same tests were conducted on soil with synthetic samples instead of distilled water to study how salinity of pore fluid modified the properties of soil.

RESULTS AND DISCUSSION

The test results of the soil with distilled water and with synthetic samples of different ppt are presented in table 1.

Table 1.

S.No.	Property	Distilled water	5 ppt	12 ppt	20 ppt	25 ppt	35 ppt
1.	Liquid limit, %	77	65	57	56	51	50
2.	Plastic limit, %	17	32	30	29	25	25
3.	Plasticity index, %	54	33	27	27	26	25
4.	Differential free swell (%DFS)	145	90	85	60	50	45.5
5.	Swell potential, %	17.6	6.65	5.5	5.4	5.4	-
6.	Swelling pressure kg/cm^2	0.437	0.381	0.344	0.268	0.20	-
7.	Cohesion, kg/cm^2	0.19	0.23	0.32	0.23	0.19	0.17
8.	Angle of internal friction (Degrees)	19	22	26	26	24	21
9.	Optimum moisture content, %	26	26	25.5	26.5	27	26

1. The swelling pressure is gradually decreasing; whereas the % free swell decreased drastically up to 12 ppt and then remained constant (by Oedometer test). The same thing is also confirmed by DFS tests.
2. There is no noted effect on the OMC and MDD with the variation of salt content.
3. The DFS is also observed to decrease with increase in salt content.
4. The observed decrease in swelling nature of soil with increased salt concentration is more at early stages, i.e. at lower salt concentration. The percentage reduction at later stages is small.
5. It is observed that cohesion and angle of internal friction are increasing with increase in salt content up to certain extent and later decrease. The cause may be taken as the stabilisation of soil, where the cations participate in the base exchange making plastic nature more non-plastic. But after a range is exceeded, the values of cohesion and angle of internal friction are decreased. This may be attributed to the free salt available, thus, reducing strength.
6. There is a marked change in the value of Atterberg limits. The change tends towards a more stabilised soil in terms of swelling nature. The decrease in liquid limit with increase of plastic limit indicates the same.
7. Except for OMC, the other tests indicate that around 12-15 ppt of salt concentration (salt concentration of the brackish water), is a remarkable point, for this soil. After this point the properties either remained unchanged or decreased.
8. It is observed that the change in swelling properties like percentage swell, and swelling pressure are decreasing and adding to advantage. But cohesion and angle of internal friction are increasing and then decreasing.

CONCLUSION

It is recommended to use only the pore fluid existing at the place of work to assess the performance of the soil.

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**International Conference of SAARC Countries on Biotechnology in Agriculture,
Industry and Environment, December 28-30, 2001,
Karad, Maharashtra (India)**

Yashwantrao Chavan College of Science, Karad jointly with Microbiologists Society, Karad has organized an International Conference of SAARC countries on Biotechnology in Agriculture, Industry and Environment. The Conference was sponsored by UGC, DST, CSIR, CPCB and Shivaji University, Kolhapur. The Organizing Secretary of the conference was Dr. A. M. Deshmukh with Dr. P. K. Goel and Dr. G. R. Pathade who worked as Joint Organizing Secretaries.

Since biotechnology is emerging as one of the most important sciences, which can mould the future of mankind; the conference was aimed to bring together the researchers on a common platform to discuss the strategies and latest developments in this field. The broader areas of the deliberations in the conference were agriculture, industry and environment, which included the following general themes.

1. Aquaculture and marine biotechnology
2. Bioinoculants biopesticides
3. Mushroom technology
4. Tissue culture, plant transformation
5. Bioenergy
6. Microbial production of industrial compounds
7. Molecular biology
8. Solid state fermentation
9. Bioprocess monitoring, modelling and control
10. Biosensors, Biocatalyst, Biopolymers, Bioseparation, Biobleaching, Biotransformation, Bioremediation, Biobleaching
11. Waste treatment
12. Vaccine production

A total of 283 abstracts were received for presentation in oral and poster sessions. The Conference was attended by over 450 delegates from India and SAARC Countries. Keynote addresses were delivered by Dr. Nimal Adikran (Srilanka) and Dr. Ashok Pandey, RRL Trivandrum (India). The deliberations of the Conference included 20 invited lectures, presentation of about 60 oral papers and 160 posters. The conference provided free internet and telephone facilities to all the delegates.

The Conference also unanimously passed three resolutions as below:

1. Banning of human cloning except for the purpose of research in medical science.
2. Formulations of guidelines and standards for biofertilizers and biopesticides by a competitive authority.
3. Propagation of biotechnology related subjects through opening of undergraduate and postgraduate courses in Universities and colleges.

The messages of well-wishes for successful holding of the conference were received from Hon. Shri K.R. Narayanan, President of Republic of India, Dr. R.A. Mashelkar, Director General of CSIR and Prof K. Kannaiyan, Vice-Chancellor of Tamil Nadu Agricultural University, Coimbatore.

EFFECT OF ULTRAVIOLET-A AND ULTRAVIOLET-B ON THE BIO-CHEMICAL CHANGES OF INTEGUMENT AND FECUNDITY OF THE TOBACCO CATERPILLAR *SPODOPTERA LITURA* (FABRICIUS)

K. Madurakavi

Post Graduate Department of Zoology, ANJA College, Sivakasi-626 124 (Tamil Nadu)
Present Address: B1/9, SECL, Gevra Project (P.O.) Korba (Dt), Chattisghard - 495 452

ABSTRACT

An attempt has been made to study the quantitative variations in the integumentary protein, chitin, lipid and fecundity of the UV-A and UV-B irradiated larvae of tobacco caterpillar *Spodoptera litura* (Fab.). When compared to the control, the UV-A and UV-B irradiated third, fourth and fifth instar larvae showed a significant reduction in the amount of lipid and chitin, but the amount of protein increased significantly and the fecundity reduced in irradiated larvae.

INTRODUCTION

Control of pests has been aimed to be brought about by newer concepts, without disruption of the ecosystem and these concepts constitute mainly the integrated control and the various methods that brings about reproductive suppression rather than mortality of an existing population. One of the reasons for the success of the insects in general is the possession of an integument (Chapman, 1982). The tobacco caterpillar *S. litura* (F.) is a polyphagous pest having 122 host plants belonging to 44 families (Moussa et al. 1960, Chari and Patel 1983). UV-rays cause excitation of the molecules but not ionization. This excitation sometimes causes chemical changes (Yeomans 1976).

MATERIALS AND METHODS

The freshly emerged larvae and egg masses were collected from castor plants in and around Sivakasi (9°27'N, 77°49'E) Tamil Nadu, South India, at altitude of MSL + 160 meters (Mohandoss et al. 1987). The freshly emerged first instar larvae were separately irradiated with UV-A lamp 20 WTL/12 and UV-B lamp 15 WTL/12 made in Holland, at a distance of 40 cm for 30 minutes and maintained separately, such as UV-A, UV-B irradiated and control larvae in well aerated plastic containers. The larvae were fed with fresh leaves of castor.

After irradiation, the larval integument was prepared (Kurup et al. 1963) from the third, fourth and fifth instars. The amount of chitin and lipid was estimated gravimetrically using monopan balance, following (Ishaaya and Casida 1974) and (Ananthakrishnan 1986) respectively. The amount of protein was estimated by Lowry's method (Lowry et al. 1951).

After emergence of adults, both male and female were placed in inverted funnel to facilitate mating and eggs laying. They were provided with 5% sucrose solution soaked with cotton as food. The number of eggs laid were counted using fine brush to know fecundity.

RESULTS AND DISCUSSION

Results furnished in table 1, reveal that the chitin, protein and lipid contents fluctuate differently in the control and the irradiated forms. The significant reduction in the amount of chitin may probably be due to the harmful and penetrating effect of UV-B irradiation on the integument, and thus affecting the chitin synthesis. It has been observed that the reduction in the amount of chitin due to UV-B irradiation is found to be statistically significant. Highly penetrating rays have been reported to disturb the neuro-endocrine system, thus affecting damage in the macromolecular synthesis (Couch and Mills 1968). UV-B radiation has been found to be more damaging than UV-A radiation (Gies, 1976; Peak *et al.* 1985).

Table 1. Variations in the amount of integumentary Chitin, Protein and Lipid (mg/g dry weight) in the control, UV-A and UV-B irradiated larvae of tobacco caterpillar, *Spodoptera litura* (Fab.)

Amount (mg/g dry wt)	Treatment in larvae integument	III instar	IV instar	V instar
Chitin	1. Control	126.3±14.52201	194.6±5.4365	296.0±12.6754
	2. UV-A irradiation	120.3±7.8457 (t=1.0815)	186.0±5.7155 (t=2.1279)	281.6±56.0733 (t=0.3632)
	3. UV-B irradiation	102.5±6.5996* (t=5.0999)	177.2±3.3993* (t=7.2388)	250.3±9.5335* (t=6.7791)
Protein	1. Control	165.3±0.5312	113.2±8.6538	102.7±13.8884
	2. UV-A irradiation	187.5±0.4922* (t=63.7931)	184.8±0.8219* (t=123.1934)	178.3±1.3072* (t=81.7916)
	3. UV-B irradiation	181.6±0.4109* (t=56.1101)	148.3±8.6538* (t=5.7360)	172.5±10.1077* (t=9.7659)
Lipid	1. Control	35.5±0.4922	33.9±0.6532	32.6±0.4922
	2. UV-A irradiation	30.2±0.5887* (t=12.7312)	30.0±0.9416* (t=5.8576)	25.6±0.4496* (t=22.0195)
	3. UV-B irradiation	29.5±1.0656* (t=7.9628)	25.3±0.5735* (t=21.2084)	22.0±5.8878 (t=2.5461)

(* = Significant)

The present study reveals that there is a significant increase in the protein content in UV-A and UV-B irradiated larvae than the controls. It is crystal clear that UV-B irradiation causes damages to DNA and reduces translation rate *in vivo* as well as *in vitro*, and induces the synthesis of protein, not found during the normal course of embryogenesis (Berry 1982). But there is an apparent increase in the amount of protein as UV-radiation causes DNA damage which forms many peptides. The CO-NH group forms a purple complex with copper ions in an alkaline medium. Therefore, it is assumed that the colour developed during the present study may be due to the presence of large number of peptides instead of a complete protein.

The decrease in the amount of lipid in present study indicates the interruption of UV-irradiation on the lipid synthesis. The decrease in the lipid from the third instar to the fifth instar larvae may be due to aging (Suraj *et al.* 1970).

The number of eggs laid by the control moths is higher than the irradiated forms (Table 2). The egg production in the adult may be greatly influenced by the nutrition of the larva. Under feeding of the larvae of *Drosophila* or *Tineola* reduces the number of eggs laid (Wigglesworth 1972).

Table 2. Variation in the fecundity of tobacco caterpillar *Spodoptera litura* (Fab.) in the control, UV-A and UV-B irradiated forms.

Larval form	Number of eggs laid
Control	369±9.4163
UV-A irradiated	341±12.1928 (t=3.2476)
UV-B irradiated	279±4.1096 (t=30.9715)

Protein is required not only for adult maintenance but also to supply energy and nutrients for provisioning the egg and egg product in insects (Slansky 1982). The less number of egg production in the UV-B irradiated forms has been found when compared to UV-A irradiated forms. The reduction may be due to the predominant lesion in these shorter wave length range of UV-B light (Calkins *et al.* 1987).

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MUTAGENICITY OF HERBICIDE PARAQUAT DICHLORIDE IN A N₂-FIXING CYANOBACTERIUM

D.K. Shrivastava

Department of Botany, Govt. Pt. J.L.N. Arts & Science College, Bemetara-441335, Dist. Durg, Chattisgarh, India

ABSTRACT

The herbicide paraquat dichloride (1,1'-dimethyl-4,4'-bipyridylum dichloride) has been found toxic and lytic to the filamentous, heterocystous and nitrogen-fixing cyanobacterium *Nostoc muscorum* in N₂ or 5 mM NO₃⁻ medium at a dose of 75-100 µg/ml for 15 minutes. The toxicity has been found relatively more on solid (agar) medium, since a herbicide dose of 8.30 µg/ml for 30 minutes has permitted nearly 50% survival of the organism. A forward mutation for resistance to 1.0 µg/ml HgCl₂, an auxotrophic mutation for glutamine-requirement and a reverse mutation for conversion of nif⁻ nit⁻ auxotroph to nif⁺ nit⁺ prototrophy could be induced using this dose of the herbicide with frequencies parallel to those obtained through induction with the well known alkylating agent MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). The bipyridyl ions capable of liberating free radicals, alone or in combination with the alkyl groups present in the herbicide may be responsible for the observed strong wide-range mutagenicity of the chemical in this organism.

INTRODUCTION

Indeed photosynthesis and nitrogen fixation are the two most important biological processes on the earth. All the green plants including Cyanobacteria, have the capacity of carbon fixation (photosynthetically), while the ability to fix nitrogen is restricted to only certain micro-organisms, despite the vast presence of nearly 79% N₂ in the atmosphere (Kumar 1991). Regarding the usable form of nitrogen, as a nutritional component, the living organisms directly depend on soil-nitrogen of agricultural lands, where N-supply is made either by naturally occurring N₂-fixers or through the application of synthetic N-fertilizers in agricultural practices.

Under the modern agronomical practices a wide use of agronomicals (i.e. pesticides/insecticides/herbicides) has been evaluated to be a potential danger to naturally occurring biofertilizer, i.e. N₂-fixing Cyanobacteria (Hawxby et al. 1977, Venkataraman & Rajyalakshami 1972). As a toxic or mutagenic chemical, some of the herbicides, often strongly inhibit the normal physiological processes of these organisms and ultimately disrupt their growth and development (Wright 1978). This is because Cyanobacteria are photosynthetic in nature, and synthetic agrochemicals, particularly the herbicides, are generally designed to be inhibitors of carbon fixation in the target species (Dodge 1975).

While toxicity and mutagenicity of some herbicides are known (Singh 1973, Hawxby et al. 1977, Karr and Singh 1978, Vaishampayan et al. 1978, Singh & Vaishampayan 1978, Singh et al. 1979, Prasad et al. 1986), systematic efforts to thoroughly test agrochemicals for their forward, auxotrophic and reverse mutation induction capacity have not yet been made. The present work relates to the strong mutagenic activity of a bipyridylum herbicide paraquat dichloride (1,1'-dim-

ethyl 4,4'-bipyridylum dichloride) in the $\text{het}^{\text{--}}\text{nif}^{\text{+}}\text{nit}^{\text{+}}$ cyanobacterium *Nostoc muscorum* and its $\text{het}^{\text{+}}\text{nif}^{\text{--}}\text{nit}^{\text{--}}$ mutant strain.

MATERIAL AND METHODS

The heterocystous, N_2 -fixing and NO_3^- metabolizing ($\text{het}^{\text{+}}\text{nif}^{\text{+}}\text{nit}^{\text{+}}$) Cyanobacterium *Nostoc muscorum*, previously isolated from the paddy fields of Darbhanga, north Bihar and its $\text{het}^{\text{+}}\text{nif}^{\text{--}}\text{nit}^{\text{--}}$ mutant, earlier isolated by Vaishampayan and Prasad (1982) have been used as test materials in the present investigation. The parent organism forms heterocysts and fixes nitrogen in N_2 medium (un-supplemented with a combined nitrogen source) and not in medium supplemented with a combined nitrogen, e.g. 5 mM NO_3^- .

Paraquat dichloride is a water soluble salt. Earlier it was shown to cause abrupt phytotoxic action by rapid cellular disruption (Brian et al. 1958, Mess 1968) and reported as mutagenic/antimutagenic in the spring onion (Alemprew 1967).

Unialgal/axenic clonal cultures of *N. muscorum* were grown routinely in Chu-10 medium, as modified by Gerloff et al (1950) with combined nitrogen (5mM potassium nitrate) at a continuous light intensity of $2,800 \pm 200$ lux and a temperature of $28 \pm 2^\circ\text{C}$ in aseptic condition (Vaishampayan 1981).

Parent *N. muscorum* was cultured in 5 mM NO_3^- medium. Physiological effects of the graded concentrations (25, 50, 75 & 100 $\mu\text{g}/\text{ml}$) of paraquat dichloride (used for 15 minutes) were examined on this organism in both N_2 and NO_3^- media, un-supplemented or supplemented with 3mM glucose after Prasad et al (1986). Growth was measured every alternate day by optical density determination at 663 nm. Heterocyst frequency of the N_2 -growing samples was assessed daily following the procedure adopted by Vashampayan (1983).

For survival studies equal volumes of the untreated and various herbicide treated (for 30 minutes) samples were diluted 100 times and a known volume of each was spread on N_2 -agar (1.0%) plates. The inoculated plates were incubated for a fortnight in growth chamber to permit the appearance of colonies growth of the viable cells on the solid medium. As such, the per cent survival was scored by comparing the number of colonial coming upon each treated set in relation to that obtained from amongst the untreated set. The 50% survival permitting dose of the herbicide was detected. Through the same procedure the identical survival permitting dose of the known mutagen N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) was determined for having a comparative assessment of the mutagenic activity of the chemical.

Mutagenicity screening was done for the following to previously established markers i.e. (i) forward mutation for 1.0 $\mu\text{g}/\text{ml}$ HgCl_2 resistance (Hg^{r}) Prasad et al (1990); (ii) auxotrophic mutation for glutamine dependence ($\text{gln}^{\text{--}}$) (Verma et al. 1990); (iii) reverse mutation of the $\text{het}^{\text{+}}\text{nif}^{\text{--}}\text{nit}^{\text{--}}$ auxotrophic mutant to $\text{het}^{\text{+}}\text{nif}^{\text{+}}\text{nit}^{\text{+}}$ prototrophy (Vashampayan and Prasad 1982). For conducting the experiments Cyanobacterial plating technique of Stewart and Singh (1975) was used, altering the dose of MNNG to 12.35 $\mu\text{g}/\text{ml}$ for 30 minutes. Spontaneous, paraquat dichloride-induced and MNNG-induced frequencies of mutation for these three markers were determined after Stewart and Singh (1975). Each isolate was characterized for the acquisition of (i) Hg^{r} , (ii) $\text{gln}^{\text{--}}$, and (iii) $\text{het}^{\text{+}}\text{nif}^{\text{+}}\text{nit}^{\text{+}}$ characteristics, respectively, using the methods adopted earlier by the respective groups of workers.

MNNG and glutamine were obtained from Sigma chemicals Co., St. louis, MO, USA. All other analytical chemicals and medium constituents were of C.P. grade, B.D.H., India.

RESULTS AND DISCUSSION

The herbicide appeared to result in growth inhibition and toxicity to the parent organism by

causing fragmentation and/or lysis of the cyanobacterial filaments / cells (Table 1). The toxicity was severe with the increasing concentration of the herbicide, and neither inorganic nitrogen (NO_3^-) nor exogenous carbon (glucose) source could protect the organism from the toxicity (Table 1). This suggested that the herbicide was neither an inhibitor for inorganic nitrogen assimilation nor CO_2 fixation, as exploited in a photoheterotroph (Pelroy et al. 1972). The herbicide proved toxic on agar medium as well. The survival values of the parent organism under treatment with the graded concentrations of the chemical are given in table 2. An 8.30 $\mu\text{g/ml}$ concentration of paraquat dichloride and 12.35 $\mu\text{g/ml}$ of MNNG applied for 30 minutes permitted 50% survival of the organism. These doses were chosen for mutagenicity test. Data on spontaneous, paraquat dichloride - and MNNG -induced frequencies of forward mutation to the Hg^+ marker, auxotrophic mutation for gln^- marker and reverse mutation from $\text{het}^+\text{nif}^-\text{nit}^-$ auxotrophy to $\text{het}^+\text{nif}^+\text{nit}^+$ prototrophy in the cyanobacterium *N. muscorum* have been recorded in table 3.

Table 1. Data* on growth (increase in O.D. after 10 days of inoculation)** of *Nostoc muscorum* in N_2 and 5 mM NO_3^- media with and without 3 mM glucose, treated*** with graded concentrations of paraquat dichloride.

Herbicide Concentration ($\mu\text{g/ml}$)	Growth (O.D. at 663 nm)			
	N_2		NO_3^-	
	- Glucose	+ Glucose	- Glucose	+ Glucose
0	0.592±0.016	0.685±0.013	0.626±0.016	0.696±0.009
25	0.487±0.014	0.511±0.015	0.471±0.018	0.478±0.018
50	0.313±0.012	0.336±0.018	0.332±0.015	0.373±0.012
75	0.105±0.014	0.114±0.021	0.092±0.011	0.097±0.014
100	0.007±0.005	0.019±0.004	0.021±0.008	0.027±0.011

* The values are means of independent determinations±S.E.

** Initial O.D. was 0.15 for all the samples

*** Treatment time was 15 minutes

Table 2. Data* on percent survival of parent *Nostoc muscorum* treated** with graded concentrations of N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) and paraquat dichloride on N_2 (agar) medium

Concentrations in mg/ml	% Survival on treatment with	
	MNNG	Paraquat dichloride
0	100.00	100.00
1	100.00±0.03	100.00±0.06
2	92.88±2.53	87.83±3.77
3	86.03±4.62	78.92±3.09
4	79.34±8.35	71.06±7.63
5	74.77±10.44	65.02±9.58
10	50.61±15.61	44.21±12.77
25	31.87±11.83	20.00±8.59
50	16.26±9.52	8.23±2.33
100	6.03±3.65	1.62±0.62
150	2.64±2.04	0.18±0.05
200	1.03±0.83	0.00±0.00
250	0.00-	0.00

* The values are means of five independent determinations±S.E.

** Treatment time was 30 minutes

Table 3. Data[#] on spontaneous, MNNG-induced and paraquat dichloride-induced mutation frequencies of 1.0 µg/ml HgCl₂ - resistance (Hg^r), glutamine-auxotrophy (gln⁻) and reversion of nif⁻nit⁻ auxotrophy to nif⁺nit⁺ prototrophy

Markers	Mutation frequency		
	Spontaneous	MNNG-induced ^{##}	Paraquat dichloride-induced ^{##}
Hg ^r * (Forward)	5.05±0.67x10 ⁻⁷ (18.22/20)	2.20±0.38x10 ⁻⁵ (15.16/18)	2.24±0.73x10 ⁻⁵ (15.22/20)
Gln ^{**} (Auxotrophy)	3.17±0.85x10 ⁻⁸ (16.22/17)	1.62±0.43x10 ⁻⁶ (8.12/10)	1.17±0.36x10 ⁻⁶ (13.17/14)
nif ⁺ nif ⁺ *** Reverse	2.12±1.04x10 ⁻⁶ (11.17/13)	3.97±1.23x10 ⁻⁴ (16.13/18)	3.55±0.44x10 ⁻⁴ (14.15/15)

[#] The values are means of five independent determinations±S.E. (Numbers of stable colonies out of the colonies tested are given parenthesis).

^{##} Treated with 50% survival permitting dose (8.30 igml⁻¹ of paraquat dichloride and 12.35 µg/ml of MNNG for 30 min.)

* Characterized to be 1.0 µgml⁻¹ Hg-resistant

** Characterized to be a true glutamine auxotroph

*** Characterized to be significant N₂ fixer and NO₃⁻ reducer

The herbicide paraquat dichloride-induced mercury-resistant mutant was tested for its 1.0 µg/ml HgCl₂ resistance characteristic and designated as Hg^r strain of *n. muscorum*. Similarly gln⁻ mutant and het⁺nif⁺nit⁺ revertant were tested to be glutamin – dependent and N₂-fixing / NO₃⁻ reducing, respectively.

This suggests that the herbicide paraquat dichloride is as good as a strong mutagen as MNNG. The mutagenicity of paraquat dichloride may be either due to the presence of (I) bipyridylium ion, known to liberate free radicals in oxygenic organism which may cause rapid phytotoxicity (Dodge 1975) and mutagenicity (Vaishampayan 1984), or (ii) alkyl group known to cause mutation in the replicating DNA chains of the prokaryotes (Drake & Baltz 1976).

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DIVERSITY, DENSITY AND DISTRIBUTION PATTERN OF MOSQUITOES IN DEVAKOTTAI, TAMIL NADU, S. INDIA

R. Senthilkumar, R. Selvaraj Pandian and M. Ismail*

Postgraduate & Research Department of Zoology, The American College, Madurai-625 002, India

* Department of Microbiology, Madurai Medical College, Madurai-625 020

ABSTRACT

Systematic survey has been made to collect both the larvae and adult mosquitoes available in Devakottai, Tamil Nadu, India by using standard methods from October, 1999 to March, 2000. Analysis of the data shows that eighteen species of mosquitoes belonging to five different genera namely *Aedes*, *Anopheles*, *Armigeres*, *Cules* and *Mansonia* are available in Devakottai. Inter-generic variation with reference to species diversity and density has been observed. Regarding the distribution pattern, some species have exhibited a wide distribution pattern and many species are restricted within a few sites.

INTRODUCTION

Mosquitoes are of tremendous importance to man because they transmit various human diseases including malaria, dengue, yellow fever, Japanese encephalitis, filariasis, etc. The role of mosquitoes is becoming increasingly important in recent years because of changes in ecology owing to human intervention. There are several aspects, in the ecology, life cycle and behaviour of mosquitoes as well as faunistics, which still requires in depth investigation. Since the discovery of involvement of mosquitoes in the transmission of many diseases, considerable data have been collected in India on the distribution, systematics and bionomics of mosquitoes (Rahman et al. 1973, Reuben 1978, Rajput & Singh 1988, Pandian 1990).

Since the vector and non-vector are proliferating in large numbers, it is essential to use all appropriate technological and management techniques to bring about an effective degree of control in a cost effective manner. The management of mosquitoes requires adequate knowledge about the species diversity and distribution pattern to evolve suitable strategy and to implement the same for the meaningful control of the population and in turn to reduce the menace and the incidence of the diseases. Therefore, the present study is carried out on the bionomics of mosquitoes in Devakottai, an urban area, with reference to their species diversity and diversity in the spatial distribution pattern.

MATERIALS AND METHODS

The study area and period

The study area, Devakottai, is situated 74.98 m above the sea level (10°58'N, 78°49' E). The total area of Devakottai town is 67.79 hectares. It receives an average of 106 cm rainfall annually. In summer, the maximum temperature was found to be 38°C and in winter the minimum temperature was 20°C. The study area is blessed with North East and South West monsoons rains and in the rainy

season, paddy cultivation has been carried out around the Devakottai town. Further, the agriculture fields are irrigated by the river Viruchuzi. The town has adequate water storage facilities i.e. the availability of 14 tanks and 17 ponds. The study was conducted from October, 1999 to March, 2000. Ten sites were selected by random sampling method to collect the biting mosquitoes. Larval collections were made systematically every month and the adult biting collections were done for ten days covering all the ten selected sites during the study period.

Identification of breeding habitats & collection of immature stages

All the available larval habitats were searched for the presence of immature stages and the larval and pupal stages were collected with the help of a tea strainer. The teated pipettes which were ordinary glass pipettes with a wide opening, were used to collect the larva from the container habitats. Dipper method was also used to collect larvae from the larger water bodies (Reuben 1978). From the positive habitats, mosquito immatures were collected in separate wide mouthed plastic jars, brought to the laboratory and reared till emergence. The larvae were fed with yeast tablets and kept in tap water. The adults obtained from the rearings were kept separately in plastic vials. These mosquitoes were identified upto species level with the help of the Senior Entomologist of Department of Microbiology, Madurai Medical College, Madurai by using the standard key.

Collection of adult female mosquitoes

The method adopted by Pandian & Chandrashekar (1980) was used to record the biting activity cycle of mosquitoes. The mosquitoes biting human were collected by using transparent vials (4.5x2.5 cm) with a lid. The vials were directed towards the mosquitoes and placed suddenly over them, while they were biting. Then the mouth of the vial was closed with the lid carefully without allowing the mosquitoes to get out. They were then killed by using ether, and kept in separate vials which were labelled with time and area of collection. This procedure was continued over a period of 24 hours. The collected adult female mosquitoes were then identified by using the standard key.

RESULTS AND DISCUSSION

Surveys conducted in connection with species diversity of mosquitoes in Devakottai from October, 1999 to March, 2000 show the occurrence of 18 species of mosquitoes belonging to five different genera. *Aedes* consists of four species namely *Ae. aegypti*, *Ae. albopictus*, *Ae. edwardsi* and *Ae. vittatus*. *Anopheles*, *Armigeres* and *Mansonia* consist of two species each namely *An. subpictus*, *An. pallidus*, *Ar. kushingensis*, *Ar. subalbatus*, *Mn. annulifera* and *Mn. uniformis*. *Culex* exhibits eight species namely *Cx. bitaeniorhynchus*, *Cx. influla*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. fuscus* and *Cx. raptor* (Table 1).

Table 1. Number of mosquitoes collected from the selected sites at Devakottai from October, 1999 to March, 2000.

Mosquito species	Jeeva nagar	Bus stand	Weekly market	Nithya Kalyani Puram	Chidaan baran athapu-puram	Aruna-giri Pattanam	Subramania Puram	Ram nagar	Othakadai	Government Hospital	Total
<i>Ae. aegypti</i>	0	3	8	0	5	2	1	0	1	2	22
<i>Ae. albopictus</i>	5	0	33	0	0	0	1	0	0	1	40
<i>Ae. edwardsi</i>	0	0	6	0	0	0	0	0	0	0	6

Contd...

<i>Ae. vittatus</i>	0	0	1	10	3	0	0	0	0	0	14
<i>An. pallidus</i>	0	2	0	0	0	0	0	0	0	0	2
<i>An. subpictus</i>	33	0	38	13	0	0	0	0	21	4	109
<i>Ar. kuchingensis</i>	0	0	7	1	108	0	52	35	1	4	208
<i>Ar. subalbatus</i>	0	36	4	0	0	0	0	0	0	0	40
<i>Cx. bitaeniorhynchus</i>	1	0	0	0	0	0	0	0	0	0	1
<i>Cx. infula</i>	5	2	2	0	1	0	0	0	0	0	10
<i>Cx. pseudovishnui</i>	4	3	3	0	0	0	0	0	0	0	10
<i>Cx. quinquefasciatus</i>	10	80	52	47	131	71	335	246	200	333	1505
<i>Cx. tritaeniorhynchus</i>	13	3	5	7	3	0	0	0	13	0	44
<i>Cx. vishnui</i>	2	9	2	4	2	0	0	0	0	0	19
<i>Cx. (Lutzia) fuscans</i>	0	1	0	1	0	0	0	0	0	0	2
<i>Cx. (Lutzia) raptor</i>	0	1	0	3	1	0	0	0	0	0	5
<i>Mn. annulifera</i>	1	16	24	0	5	16	1	0	2	0	65
<i>Mn. uniformis</i>	0	0	0	0	0	2	0	0	0	0	2
Total	74	156	185	86	259	91	390	281	238	344	2104

Intergeneric variation with reference to species diversity has been recorded. *Culex* and *Aedes* show a high degree of diversity in their species composition. In addition to the diversity, density variation has also been observed. Most predominant species are *Cx. quinquefasciatus*, *Ar. kuchingensis*, *An. subpictus*, *Ae. albopictus*, *Ar. subalbatus*, *Cx. tritaeniorhynchus*, *Mn. annulifera* and *Ae. aegypti*. However, *Ae. vittatus*, *Cx. infula*, *Cx. pseudovishnui* and *Cx. vishnui* exhibit a moderate density. The remaining species exhibit a very low density (Table 2).

Table 2. Number of mosquitoes collected during the 24h biting and larval collections and their number of occurrence in various sites during the study period 1999-2000.

S.No.	Name of the species	Sites of occurrence	Number of	
			Larva	Adult
1.	<i>Aedes (Stegomyia) aegypti</i> (Linnaeus)	7	2	20
2.	<i>Aedes (Stegomyia) albopictus</i> (Skuse)	4	32	8
3.	<i>Aedes (Stegomyia) edwardsi</i> (Barraud)	1	0	6
4.	<i>Aedes (Stegomyia) vittatus</i> (Bigot)	3	5	9
5.	<i>Anopheles (Cellia) pallidus</i> Theobald	1	0	2
6.	<i>Anopheles (Cellia) subpictus</i> Grassi	5	109	0
7.	<i>Armigeres (Armigeres) kuchingensis</i> Edwards	7	2	206
8.	<i>Armigeres (Armigeres) subalbatus</i> (Coquillett)	2	0	40
9.	<i>Culex (Culex) bitaeniorhynchus</i> Giles	1	0	1
10.	<i>Culex (Culex) infula</i> Theobald	4	0	10
11.	<i>Culex (Culex) pseudovishnui</i> Colless	3	0	10
12.	<i>Culex (Culex) quinquefasciatus</i> Say	10	213	1292
13.	<i>Culex (Culex) tritaeniorhynchus</i> Giles	6	0	22
14.	<i>Culex (Culex) vishnui</i> Theobald	5	0	19
15.	<i>Culex (Lutzia) fuscans</i> Wiedemann	2	0	2
16.	<i>Culex (Lutzia) raptor</i> Edwards	3	0	5
17.	<i>Mansonia (Mansonioides) annulifera</i> (Theobald)	7	0	65
18.	<i>Mansonia (Mansonioides) uniformis</i> (Theobald)	1	0	2

The diversity and the density of the mosquito fauna collected in Devakottai vary from one place to another, indicating the occurrence of a spatial distribution pattern (Table 1). Among the *Aedes* spp., *Ae. aegypti* exhibited a wide range of distribution pattern and the other three species exhibited a restricted distribution pattern. Among the *Anopheles* spp., *An. subpictus* showed a wide distribution pattern and *An. pallidus* is restricted to one place. Among the two *Armigeres* spp., *Ar. kuchingensis* showed a wide distribution pattern whereas *Ar. subalbatus* shows a restricted distribution pattern. The distribution pattern of *Culex* species showed a wide range of distribution pattern. The occurrence of *Cx. quinquefasciatus* has been recorded almost in all the surveyed sites, indicating a wide range of distribution pattern (Table 2). The sites of occurrence decrease in the following order: *Cx. tritaeniorhynchus* > *Cx. vishnui* > *Cx. infula* > *Cx. pseudovishnui* > *Cx. raptor* > *Cx. fuscans* > *Cx. bitaeniorhynchus*. *Mn. annulifera* is present in all the collected sites and *Mn. uniformis* is restricted to one site.

Bioecology of mosquitoes is useful when there is a need to know the density of the species and its potential in transmitting any particular disease. Further, density variation is recorded in many places. This is dependent upon the availability of hosts and breeding habitats. The high density of *Armigeres* spp., *Cx. quinquefasciatus* and *Mn. annulifera* was recorded in this study and *Cx. fuscans* and *Cx. raptor* prefer to bite more on cattle and bite lesser on human beings. *Cx. quinquefasciatus* was the most dominant species found in all the breeding sites. The abundance of *Mansonia* spp. is associated with the vegetation. The larvae of *Mn. uniformis* were found attached to the roots of aquatic plants, where they take oxygen from roots and come to the surface rarely (Gakhar and Vandana 1996). Nelson et al. (1976) found that density of *Ae. aegypti* in indoor resting collection generally fluctuated with rainfall (Rao 1967). The prevalence of constant density throughout the year of *Cx. quinquefasciatus* observed in their study resembles with the work reported in Morogoro (Mrope et al. 1973).

In Hulu Perak area, *An. maculatus* is usually present in large numbers in June during the dry season (Rahman et al. 1992). *Cx. quinquefasciatus*, *Ae. aegypti* and *Mn. annulifera* occur almost in all areas. This is mainly due to the availability of their breeding ground i.e. stagnant sewage water bodies and improperly managed septic tanks. Similar biodiversity and behaviour patterns of mosquitoes have been reported elsewhere (Reuben, 1978). The variation in this study is correlated to the above findings.

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MEASUREMENT OF SELECTED MAJOR CONSTITUENTS OF STACK EMITTED DUSTS AROUND AN INTEGRATED STEEL PLANT

Rajnikant Sharma and Shamsh Pervez

Department of Chemistry Govt. Arts and Science College, Durg-491 001, Chhattisgarh State, India

ABSTRACT

A respirable dust sampler was positioned at selected 32 sites, within the radius of 10 km around an integrated steel plant at Bhilai. Samples of nonrespirable suspended particulate matter (NRSPM) (Size $> 10\mu$) and respirable suspended particulate matter (RSPM) ($< 10\mu$) were collected by operating the sampler for 24 hours, using recommended conditions of operation. All the samples were subjected to chemical analysis for major constituents like Fe, Al, Ca, Mg, Na and K. Sites of southwest and north-east direction from the plant have shown higher susceptibilities for RSPM and NRSPM depositions as compared to sites of other directions. Higher settling tendency of RSPM was observed at the distance of 7 km, while NRSPM have shown the tendency within the distance of 4 km in most of the cases. Meteorological irregularities were resulted in the exceptional pattern of settling tendencies of RSPM and NRSPM at few sites. Higher RSPM and NRSPM levels at all the sites, except few sites of south-east and northwest directions, compared to permissible standards (CPCB Publication, New Delhi) have been observed. It has been found from analysis data that except iron, all the elements have shown higher concentration in RSPM compared to NRSPM. Correlation coefficient and enrichment factors were also calculated.

INTRODUCTION

Particulates less than 10μ (PM 10) in diameter tends to pose the greatest health concern because they can be inhaled into and accumulated in the respiratory system. Early episodes of extreme pollution by PM 10 particles, the most famous of which occurred in Donora, London and Pennsylvania in the 1930s, 40s and 50s and killed thousands of people, highlighted the importance of evaluating this form of air pollution (EPA 2000). Raw material handling and processes involved in the integrated steel plant in Bhilai are the major sources of dust emission in Chhattisgarh region (Nayar 1996, Seth 1983a). India is the 10th largest manufacturer of steel in the world. A total mass of 1.401 tonnes of solid raw material is handled during the production of one tonne of steel (Seth 1983a). Formation of 30 tonnes of dust per annum as a result of production of one million tonnes of sinter in steel plant has been reported (Bhattacharya et al. 1995). Presence of Fe (30%), C (15%), SiO_2 (10%) and small quantities of oxides of Al, Mn and Ca in emitted particulate matter from blast furnace of steel plant have also been reported (Bhattacharya et al. 1995). The rates of dust fallout around this steel plant were measured by earlier workers (Seth et al. 1983a and 1983b). Many researchers have carried out the monitoring and impact studies of suspended particulate matter (Chandrasekaran et al. 1997, Joshi 1998, Naik et al. 1998, Panwar et al. 1997, Quaraishi et al. 1997 and Rastogi et al. 1997).

Both RSPM and NRSPM are associated with numerous health effects. NRSPM particulates can aggravate respiratory conditions such as asthma. Particulate matter less than 10μ , can be inhaled and deposited in the pulmonary region in respiratory system (Sinha et al. 1983). Exposure to insoluble dust can produce local effects in respiratory system. The most widely studied and acknowledged consequence of long term mineral based dust inhalation is pneumoconiosis, including premature death (EPA 2000).

On the basis of above observation, it has been considered useful to measure the concentration of respirable suspended particulate matter (PM 10) in ambient air of an integrated steel plant and determine the concentration levels of major constituents in the collected samples. Data, so obtained, could be useful in establishing the higher and lower susceptible zones of RSPM around the steel plant and determining the enrichment aspects of selected major constituents in respirable size range.

MATERIAL AND METHODS

Sample collection: Thirty two sampling sites at distance of about 2,4,7 and 10km from the steel plant at Bhilai were identified for sample collection (Fig.1). A respirable dust sampler (Envirotech, Model APM 460) was operated at each sampling site for a duration of 24 hours at a flow rate of 0.85-1.10m³/min. (Envirotech 2000). After the completion of the sampling, weight of RSPM and NRSPM, which were collected on glass microfilter sheets and attached cyclonic cups, respectively, were recorded. The concentration levels ($\mu\text{g}/\text{m}^3$) of the respective fractions were then calculated, and values have been shown in Table 1. The meteorological data (temperature, wind direction, wind velocity and average rainfall) were also recorded during the sampling period.

Analysis of collected samples: All the collected samples of RSPM and NRSPM were subjected to chemical analysis for Fe, Al, Ca Mg, Na and K using standard methods of chemical analysis (Jeferry et al. 1989). Triplicate analysis have been done for each sample. The results obtained have been shown in Table 2.

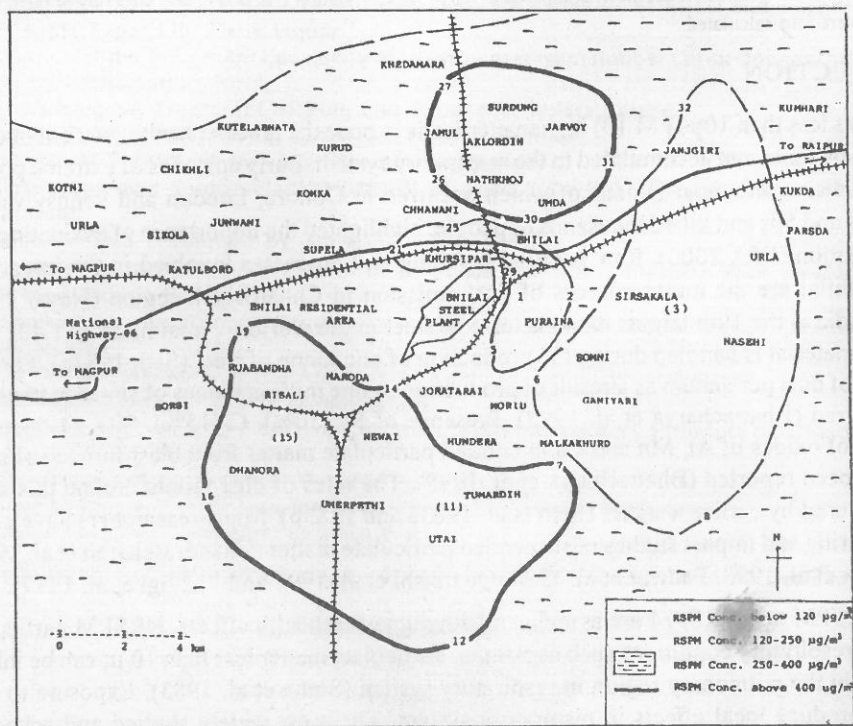


Fig. 1: Concentration zones of respirable suspended particulate matter around an integrated steel plant.

Table 1: Concentration levels ($\mu\text{g}/\text{m}^3$) of respirable and non-respirable suspended particulate matter around the steel plant.

Sampling site no.	Direction of site with respect to the plant	Distance of site from the plant (km)	Concentration of R S P M ($< 10\mu$)	Concentration of N R S P M ($> 10\mu$)
1.	East	2	42.48	35.13
2.	East	4	103.78	184.64
3.	East	7	176.47	141.34
4.	East	10	133.17	85.78
5.	South east	2	83.33	67.81
6.	South east	4	271.24	461.60
7.	South east	7	414.22	325.16
8.	South east	10	209.15	233.66
9.	South	2	169.93	404.41
10.	South	4	353.76	584.97
11.	South	7	642.16	482.03
12.	South	10	413.39	343.95
13.	South west	2	209.15	502.45
14.	South west	4	542.48	670.75
15.	South west	7	842.32	566.18
16.	South west	10	514.71	381.54
17.	West	2	167.48	426.47
18.	West	4	359.48	629.90
19.	West	7	643.79	519.61
20.	West	10	394.61	352.94
21.	North west	2	112.75	243.46
22.	North west	4	276.14	420.75
23.	North west	7	364.38	343.14
24.	North west	10	187.91	277.78
25.	North	2	74.35	179.74
26.	North	4	180.56	343.14
27.	North	7	345.59	341.50
28.	North	10	153.59	252.45
29.	North east	2	99.67	277.78
30.	North east	4	348.86	551.47
31.	North east	7	537.58	561.27
32.	North east	10	337.42	406.86

Evaluation of enrichment factors: Correlation coefficient values were worked out between the elements of RSPM, and the values have been shown in Table 3. Using the data with regard to the concentration and correlation coefficient of different elements in respirable suspended particulate matter, the enrichment factors of the elements in RSPM in relation to those in NRSPM were worked out and the values are shown in Table 4.

RESULTS AND DISCUSSION

Sites located at southwest and northeast directions from the plant have shown higher susceptibilities for RSPM and NRSPM depositions, compared to sites of other directions (Fig. 1). These high values were in agreement with wind direction, which was southwesterly and northeasterly during the period of measurements. Except for few cases, higher concentrations of RSPM and NRSPM were found in all the sampling sites compared to the permissible limits (RSPM = $60 \mu\text{g}/\text{m}^3$; NRSPM = $120 \mu\text{g}/\text{m}^3$) (CPCB 1995).

Table 2: Analysis data ($\mu\text{g}/\text{m}^3$) of major constituents of RSPM and NRSPM.

Sampling Site No.	Fe		Ca		Mg		Al		Na		K	
	RSPM	NRSPM	RSPM	NRSPM	RSPM	NRSPM	RSPM	NRSPM	RSPM	NRSPM	RSPM	NRSPM
1.	2.76	4.35	2.12	1.00	3.32	2.26	2.33	0.99	0.02	0.01	0.02	0.01
2.	9.32	28.44	7.43	6.83	5.30	4.92	4.24	3.21	0.10	0.03	0.08	0.07
3.	15.62	21.11	13.13	5.00	10.60	4.00	6.49	2.87	0.90	0.12	0.20	0.06
4.	14.49	11.49	10.14	4.87	7.78	2.44	3.00	2.04	0.01	0.002	0.01	0.01
5.	8.33	8.80	4.00	3.70	4.32	3.36	3.49	1.23	0.03	0.01	0.02	0.01
6.	27.52	71.26	15.31	14.31	14.49	13.50	10.23	8.26	0.09	0.04	0.08	0.12
7.	34.72	49.41	30.42	9.00	26.33	8.36	16.78	7.42	0.21	0.08	0.09	0.07
8.	19.30	38.33	16.62	5.05	11.78	1.26	7.44	5.92	0.004	0.001	0.004	0.004
9.	18.00	61.78	12.72	8.84	8.33	12.25	5.12	8.72	0.01	0.02	0.10	0.03
10.	37.22	89.37	19.33	15.37	16.24	14.55	13.46	13.01	0.09	0.08	0.81	0.82
11.	56.35	73.42	43.00	13.44	34.32	10.69	27.79	11.21	1.22	0.27	1.34	0.30
12.	47.67	50.43	30.26	10.55	14.21	8.73	12.01	8.72	0.92	0.08	0.09	0.004
13.	20.20	80.31	14.22	10.62	9.41	13.49	8.35	8.83	0.86	0.62	0.03	0.07
14.	39.00	103.44	23.13	18.78	20.26	15.02	16.68	14.22	1.21	1.02	1.89	1.32
15.	76.71	91.29	48.01	15.32	43.33	8.93	37.90	10.92	2.21	0.88	2.00	0.76
16.	51.32	62.99	39.35	7.37	20.21	6.33	19.23	7.30	0.92	0.80	0.05	0.003
17.	16.43	75.77	11.44	8.33	7.27	4.98	6.57	5.28	0.90	0.93	0.02	0.02
18.	35.31	98.32	21.34	16.92	18.19	14.39	13.89	12.81	1.81	0.76	0.88	0.73
19.	60.71	83.49	37.12	12.00	31.22	12.93	28.12	9.44	2.00	0.18	1.33	0.21
20.	41.21	56.32	26.43	7.00	15.97	8.42	14.46	7.26	0.80	0.60	0.33	0.10
21.	10.98	39.43	7.27	9.35	5.34	7.36	4.79	2.22	0.03	0.02	0.02	0.03
22.	30.88	64.49	17.55	12.89	11.71	10.37	9.01	8.65	0.09	0.12	0.18	0.50
23.	34.21	52.02	29.13	10.46	15.29	8.43	14.33	7.53	0.93	0.13	0.21	0.13
24.	18.41	43.31	14.23	8.74	10.12	5.93	6.19	5.54	0.01	0.09	0.02	0.003
25.	4.45	24.41	5.77	12.49	7.33	7.22	3.20	2.33	0.03	0.02	0.01	0.01
26.	12.33	39.26	17.67	14.22	16.95	16.01	5.46	12.44	0.05	0.05	0.10	0.34
27.	21.68	32.28	36.33	29.27	28.33	18.20	10.00	10.95	0.76	0.34	2.21	0.19
28.	15.89	27.37	10.22	16.56	6.92	8.40	5.56	9.59	0.12	0.09	0.09	0.01
29.	6.12	38.22	12.43	6.22	8.33	12.22	2.78	7.45	0.01	0.01	0.01	0.12
30.	28.45	46.88	28.61	40.45	25.78	18.56	11.87	28.55	0.08	0.09	0.41	1.22
31.	34.64	40.92	39.79	38.29	38.4	10.42	27.32	39.29	0.25	0.07	1.21	0.33
32.	16.22	11.22	51.55	26.35	14.15	9.22	16.12	42.44	0.03	0.01	0.01	0.01

Table 3: Correlation coefficient values ($\times 100$) between element in respirable particulates of steel plant.

Element	Fe	Ca	Mg	Al	Na	K
Fe	-	75.4	79.52	90.41	76.29	60.72
Ca	-	-	83.74	85.82	52.12	56.87
Mg	-	-	-	91.03	58.23	77.52
Al	-	-	-	-	69.94	68.38
Na	-	-	-	-	-	63.79
K	-	-	-	-	-	-

Table 4: Enrichment factor values.

Sampling Site No.	Ca	Mg	Al	Na	K
1.	3.33	2.25	3.65	3.50	6.43
2.	3.32	3.35	4.09	10.00	3.85
3.	3.55	3.58	3.00	10.17	4.64
4.	1.65	2.57	1.17	3.00	0.45
5.	1.14	1.37	3.00	5.00	1.67
6.	2.77	2.79	3.08	5.00	1.65
7.	4.81	4.47	3.20	3.75	1.80
8.	6.52	20.33	2.60	6.67	2.10
9.	4.94	2.32	2.00	2.30	11.00
10.	3.02	2.75	2.40	2.22	2.39
11.	4.17	4.07	3.27	5.95	5.85
12.	3.04	1.71	1.47	9.50	24.05
13.	5.33	2.76	3.73	5.37	1.65
14.	3.26	3.71	3.07	3.10	3.77
15.	3.73	5.60	4.08	2.90	3.09
16.	6.55	3.90	3.08	1.38	17.80
17.	6.38	7.333	5.71	4.50	4.00
18.	3.51	3.47	3.00	6.37	3.33
19.	4.24	3.47	4.18	16.50	8.80
20.	5.17	2.60	2.69	1.72	4.44
21.	2.79	2.58	7.33	4.00	2.50
22.	2.85	2.38	2.23	1.50	0.76
23.	4.23	2.81	3.00	9.00	2.54
24.	3.83	3.93	2.62	0.50	15.94
25.	2.53	5.50	7.20	8.75	5.56
26.	3.96	3.37	1.38	4.00	0.91
27.	1.85	2.34	1.35	3.50	17.29
28.	1.06	1.42	1.00	2.67	14.25
29.	12.39	4.28	2.37	10.00	0.31
30.	1.17	2.28	0.69	1.50	0.54
31.	1.23	4.44	0.82	3.50	4.32
32.	1.35	1.06	0.26	2.00	1.64

Higher concentrations of NRSPM compared to RSPM were obtained with few exceptions. As far as settling tendencies of RSPM and NRSPM are concern, the highest tendency observed at the distance of 7 km for RSPM and at 4 km for NRSPM in most of the cases. The exceptional pattern of settling tendencies of RSPM and NRSPM in few sites was due to the meteorological irregularities during the day of sampling.

The occurrence of elements studied in RSPM and NRSPM samples were found in the order $Fe > Ca > Mg > Al > Na > K$. In cases of sampling sites in the east direction from the plant, the order is almost the same with few exceptions. Except iron, all the elements have shown higher concentration in RSPM compared to NRSPM. This is explainable on the basis of enrichment of elements in the respirable size range of the particulates. From the analysis data of the elements in RSPM, it was found that iron has the highest value of correlation coefficient with all other elements. A higher correlation coefficient value between metal pair is an indicative of the fact that the elements must have originated from a common source and similar chemical behaviour.

The data obtained (Table 4) have shown that out of all the elements analysed, potassium has the highest value of enrichment factor (Average value 5.60) while lowest value was found in the case of aluminium (Average value 2.89). A good caution is, therefore, needed in preventing the RSPM from getting into the atmosphere through anthropogenic sources.

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FLOTATION-CLARIFICATION OF WATER BY NATURAL COAGULANTS

Haribhakta V. Keshav and Malay Chaudhuri

Department of Civil Engineering, Indian Institute of Technology, Kanpur-208 016, Uttar Pradesh, India

ABSTRACT

An experimental study has shown pressure flotation using natural coagulants to be a promising low-cost option for water clarification. A household unit for flotation-clarification was designed and fabricated.

INTRODUCTION

The potential of natural coagulants for clarifying turbid waters by flocculation-sedimentation is well-known. The seed of *Moringa oleifera* is a popular natural coagulant in many African villages, whereas the seed of *Strychnos potatorum* is used in water clarification in the rural areas of the Tamil Nadu and Maharashtra states of India. A preliminary investigation on removal of turbidity by pressure (dissolved-air) flotation using the seed of *M. oleifera* undertaken by Folkard et al. (1986), generated interest on flotation-clarification of water by natural coagulants. The present study evaluated the efficacy of the two popular natural coagulants in removing turbidity, bacteria and viruses from water by pressure flotation.

EXPERIMENTS

Water from the Lower Ganga canal was used as raw water for the study. During the summer months, the water had the following characteristics: turbidity 10-20 NTU; pH 8.4-9.1; alkalinity 90-130 mg CaCO₃/L; dissolved solids 350-380 mg/L; and heterotrophic plate count 320-360 CFU/mL. A bentonite-kaolin mix in equal proportions was used to augment the turbidity of the raw water to the desired level (ca.50 and 100 NTU). Settled domestic sewage was added (0.1 mL to 10 litres of water) to introduce fecal coliforms (240-300 MPN/100 mL); this produced a heterotrophic plate count of 400-500 CFU/mL. Raw water for the tests to assess virus removal was the canal water spiked with poliovirus 1 (Sabin).

Dried *M. oleifera* seeds were collected from the Indian Institute of Technology campus; seed wings and coats were removed, thoroughly pounded and squeezed in a mortar, and a 2% suspension was made with distilled water. Dried *S. potatorum* seeds, obtained from the Maharashtra Engineering Research Institute, were powdered by pounding and a 0.2% suspension was made with distilled water.

Pour plate method, using R2A agar (35 ± 0.5°C and 5 days), were employed for heterotrophic plate count and the counts are reported as colony-forming units (CFU)/mL. The multiple-tube technique, using A-1 broth (44.5 ± 0.2°C and 24 ± 2 hours), was employed for enumeration of fecal coliforms and the values are reported as most probable number (MPN)/100 mL. For virus enumeration, plaque assay on MA-104 cells was used and virus concentrations are reported as plaque-forming units (PFU)/mL.

A bench-scale set-up (Fig. 1), comprising a 3.5-litre stainless steel pressurizing vessel (10 cm ID; 45cm height) with attached pressure gauge and air-release valve, and perspex flotation cell (7.5cm ID; 65cm height), was used for flotation-clarification tests. The set-up also included an air-regulating

valve and a needle valve to control the size of air bubbles. Compressed air was used to saturate 2 litres of water in the pressurizing vessel at a predetermined pressure (4-5 atm). A set procedure for a flotation-clarification test consisted of rapid mixing (fast hand-stirring for one minute with a stick at about 100 rpm) followed by slow mixing (slow hand-stirring for 5-10 minutes at about 30 rpm) of a coagulant (*M. oleifera* or *S. potatorum* seed suspension) into one litre of raw water in the flotation cell, and thereafter introducing a desired volume (250, 500, 750, or 1000 mL, i.e., 25, 50, 75, or 100% of the raw water) of air-saturated water at 200 mL/minute into the flotation cell. Following a 20-minute flotation time, a 100 mL subnatant sample was withdrawn for analysis.

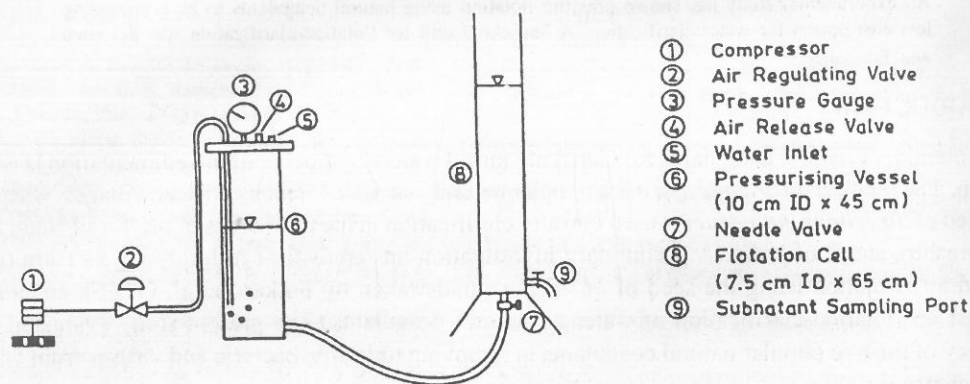


Fig. 1: Bench-scale flotation-clarification set-up.

Initially, the study was devoted to finding the optimum dose of *M. oleifera* or *S. potatorum* seed and the effect of varying the ratio of air-saturated water to raw water on turbidity removal by flotation-clarification. Tests were conducted using distilled water in the pressurizing vessel, and two raw water turbidity levels, viz., low raw water turbidity (ca. 50 NTU) and high raw water turbidity (ca. 100 NTU). An air compressor was used to saturate the water with air at 5 atm pressure which was found to be the optimum by Flokard et al. (1986). Tests at 4 atm pressure were also conducted. The pressurizing vessel was shaken during release of the air-saturated water. Subnatant samples were analysed for turbidity removal. Performance of *M. oleifera* seed is shown in Fig. 2 and that of *S. potatorum* seed in Fig. 3. The optimum seed dose range (based on subnatant turbidity) was much lower for *S. potatorum* (1.2 mg/L) and higher for *M. oleifera* (120 mg/L) for both the turbidities. A seed dose of 150 mg/L was observed to be the optimum by Folkard et al. (1986) for *M. oleifera* and this resulted in a subnatant turbidity of 3.5 NTU when the raw water turbidity was 100 NTU and the ratio of air-saturated water to raw water was 50%. In the present study, with an increase in the ratio of air-saturated water to raw water there was a decrease in the subnatant turbidity for both the seeds. For *M. oleifera* seed and low raw water turbidity (48-53 NTU), subnatant turbidity values were 7.0, 5.0, 4.0 and 3.0 NTU, whereas for high raw water turbidity (98-103 NTU), subnatant turbidity values were 8.5, 7.0, 5.5 and 4.5 NTU, respectively for air-saturated water to raw water ratios of 25, 50, 75 and 100%. In the case of *S. potatorum* seed, subnatant turbidity values were 6.0, 5.0, 4.0 and 3.5, and 6.5, 5.5, 5.0 and 4.5 NTU for low and high raw water turbidity levels, respectively. For both the seeds, the subnatant turbidity was slightly higher for high raw water turbidity under similar operating conditions. This presumably was due to the requirement of more air bubbles for flotation-clarification of high turbidity raw water. Tests using lower

pressure (4 atm) for air saturation resulted in higher subnatant turbidity for both the seeds – 9.0 and 10.0 NTU for *M. oleifera* (120 mg/L) and *S. potatorum* (1.2 mg/L), respectively for low raw water turbidity (48-53 NTU) and air-saturated water to raw water ratio of 100%.

Tests were also carried out using clarified canal water in the pressurizing vessel (turbidity 2-3 NTU) instead of distilled water, canal water of turbidity 48-52 NTU in the flotation cell and the optimum seed dose, and the ratio of air-saturated water to raw water of 50 and 60%. For both the seeds, an air-saturated water to raw water ratio of 60% at 5 atm pressure resulted in a subnatant turbidity of 5 NTU, the drinking water guideline value recommended by the World Health Organization (WHO 1993). Presence of dissolved solids in the pressurizing water resulted in slightly higher percent (60%) requirement of air-saturated water to raw water ratio as compared to distilled water (50%) for identical subnatant turbidity (5 NTU). In all batch flotation-clarification tests, the temperature of the water was in the range of 34-35°C at which the solubility of air in water is very low (70-75 mL/L at 5 atm) and this factor was also responsible for higher requirement of air-saturated water.

For bacteria removal tests, clarified canal water (turbidity 2-3 NTU) spiked with domestic sewage (0.1 mL to 10 litres) was used in the pressurizing vessel, and sewage and bentonite-kaolin spiked canal water (turbidity 48-52 NTU; heterotrophic bacteria 400-500 CFU/mL; fecal coliforms 240-300 MPN/100 mL) in the flotation cell. While both the seeds produced a subnatant turbidity of 5 NTU, *M. oleifera* seed was slightly more effective in removing both heterotrophic bacteria and fecal coliforms (36.4-

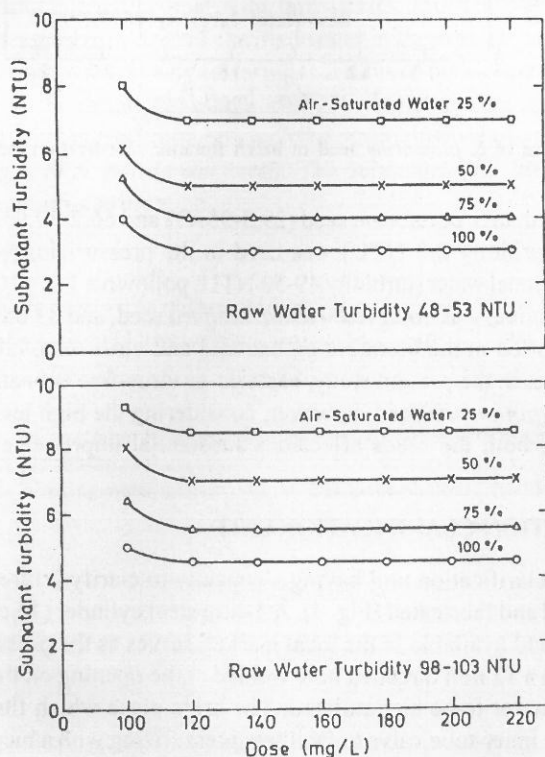


Fig. 2: Performance of *M. oleifera* seed in batch flotation-clarification test (5 atm; 34-35°C).

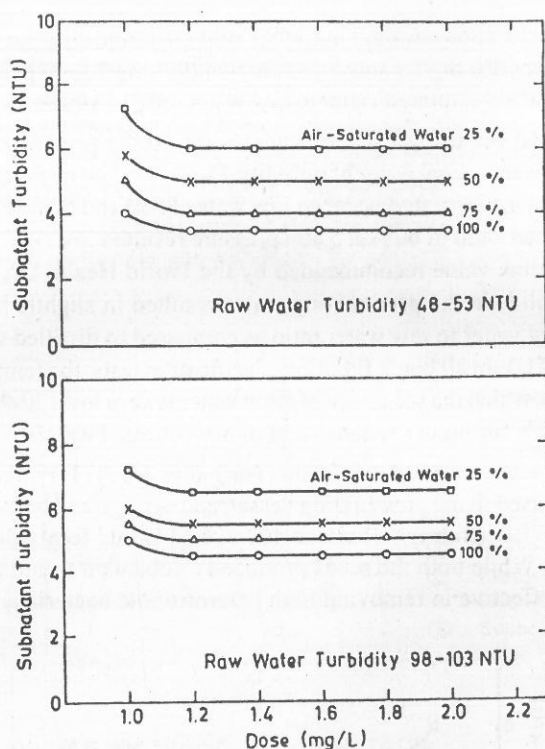


Fig. 3: Performance of *S. potatorum* seed in batch flotation-clarification test (5 atm; 34-35°C).

42.8% and 85.0-91.2%) than *S. potatorum* seed (29.5-35.4% and 66.2-77.9%). For virus removal tests, clarified canal water (turbidity 2-3 NTU) was used in the pressurizing vessel, and poliovirus and bentonite-kaolin spiked canal water (turbidity 49-50 NTU; poliovirus $1-2 \times 10^3$ PFU/mL) in the flotation cell. A 78.15% virus removal was observed with *M. oleifera* seed, and 83.65% with *S. potatorum* seed. No study has been reported in the literature on bacteria and virus removal by flotation-clarification using natural coagulants. In the present study, bacteria- or virus-free subnatant was not achieved with either *M. oleifera* or *S. potatorum* seed; however, considering the high levels of fecal coliforms and virus in the raw water, both the seeds effected a substantial improvement in the microbiological quality of the water.

HOUSEHOLD FLOTATION-CLARIFICATION UNIT

A household flotation-clarification unit having a capacity to clarify 2 litres of water in one cycle of operation was designed and fabricated (Fig. 4). A 5-litre steel cylinder (16 cm ID; 25 cm height), used for storing refrigerant and available in the local market, serves as the pressurizing vessel. It has a 20 mm hexagonal nut with a 12 mm threaded hole welded at the opening on the top. The hole is used for filling the vessel with water to be air-saturated. The brass piece which fits into the threaded hole is provided with a bicycle inner-tube valve to facilitate pressurizing with a bicycle pump. The cylinder is also provided with an opening at the bottom fitted with a needle valve for withdrawal of the air-saturated water. The flotation cell (10cm sq. \times 36cm) is made of galvanised iron sheet. It is provided

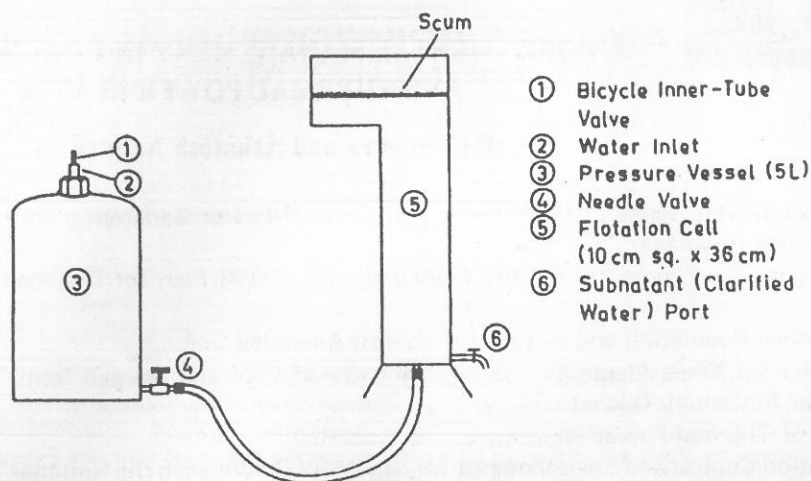


Fig. 4: A household flotation-clarification unit.

with a port at the bottom for withdrawal of the subnatant and a trough at the top for collection of the scum. The total material and fabrication cost of the unit was Rs. 750. The procedure for clarifying water by the unit would be to pressurize 2 litres of clarified water in the pressurizing vessel at 5 atm pressure (100 strokes of a bicycle pump) and slowly releasing 1.2 litres of the air-saturated water to the flotation cell containing 2 litres of the raw water coagulated (fast hand-stirring for one minute with a stick followed by gentle hand-stirring for 5 minutes) with the optimum dose of a coagulant (120 mg/L of *M. oleifera* seed or 1.2 mg/L of *S. potatorum* seed). The subnatant (3.2 litres) is withdrawn after 20 minutes, allowing for flotation-clarification.

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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF FIELD GROWN *VICIA FABA* L. PLANTS TO SUPPLEMENTAL ULTRAVIOLET - B RADIATION

S. B. Agrawal

Department of Biological Sciences, Allahabad Agricultural Institute (Deemed University), Allahabad-211 007, India

ABSTRACT

In a field experiment *Vicia faba* plants were grown under ambient and supplemental ultraviolet-B (sUV-B) radiation at 20% ozone depletion. Photosynthetic rate, concentrations of photosynthetic pigments, flavonoids, nitrogenase, catalase and peroxidase activities and contents of ascorbic acid were determined to evaluate the changes induced by artificially provided supplemental levels of UV-B irradiation. Decline in photosynthesis was associated with reductions in chlorophyll and carotenoid pigments at all ages of plants. Concentrations of UV-B absorbing pigments and flavonoids increased with age. The amount of antioxidant and ascorbic acid decreased after exposure of UV-B. Catalase activity decreased, while peroxidase activity increased at sUV-B. Significant reduction in nitrogen fixing ability, i.e., nitrogenase activity was observed in the fresh nodules of plants.

INTRODUCTION

Crops growing in the mid latitudes are increasingly at risk of exposure to elevated ultraviolet-B (UV-B, 290-315 nm) radiation resulting as a consequence of stratospheric ozone depletion which may result from the continued release of chlorofluorocarbons, methane and other trace gases (Agrawal et al. 1991, Caldwell 1993, Middleton et al. 1996; Ambasht & Agrawal 1994, 1997, 1998). Elevated level of UV-B is considered a significant potential environmental stress in natural ecosystems and agriculture as it caused reductions in plant growth by alterations in leaf physiology and function (Tevini et al. 1991). In the last twenty years, most of the reports on effects of UV-B on plants are from those grown in growth chambers and greenhouse where the irradiance is totally different from normal sunlight and, therefore, the sensitivity of plants may vary (Teramura et al. 1991, Dai et al. 1994, Ambasht & Agrawal 1998). However, only a few realistic field studies have been conducted that confirm or contradict results obtained in growth chambers or greenhouses.

UV-B radiation is energetically capable of disrupting proteins, nucleic acids and other important plant pigments (Tevini & Teramura 1989). Several other studies indicated that supplemental UV-B (sUV-B) can deleteriously affect physiological processes and overall growth in a number of plant species (Sullivan & Teramura 1990, Tevini 1999), resulting in reduction of total dry weight. Searles et al. (1995) suggested that a minor ozone depletion in the tropics can influence the physiological functions and growth of tropical species. Krupa & Kickert (1989) have compiled the results of many experiments on UV-B effects on plants and suggested that C₃ plants are more sensitive than those with C₄ photosynthetic pathway. Among C₃ plants leguminous plants are categorized as sensitive in most of the experiments performed in growth chambers/greenhouses (Agrawal et al. 1991, Sharma et al. 1991, He et al. 1993). Recently, Ambasht & Agrawal (1997, 1998) reported unfavourable effects of sUV-B on a number of physiological and biochemical processes in *Sorghum vulgare* and *Oryza sativa* plants, leading to reduction in dry matter production.

The present investigation was aimed to assess the physiological and biochemical responses of *Vicia faba* L. (local variety) plants to sUV-B under natural levels of photosynthetically active radiation (PAR).

MATERIALS AND METHODS

The experiment was performed in winter (November-January), in the experimental plots at botanical garden of Banaras Hindu University, Varanasi (25°18'N, 83°1'E, about 76 m above sea level) situated in the eastern Gangetic plains of India. *Vicia faba* seeds were hand sown in rows, 0.2 m apart in six plots of 1.5 × 1.5 m each. Six plots were randomly divided into two treatments, i.e., with UV-B and without UV-B (control). Supplemental UV-B was artificially provided by Q panel UV-B 313 fluorescent lamps (Q-Panel, Cleveland, U.S.A.). Lamps were fitted 20 cm apart on an adjustable iron frame and suspended above and perpendicular to the planted rows. The lamps were fitted with dimmer switches to control the irradiance. Each lamp was covered with 0.13 mm thick cellulose diacetate film (Cardillac Plastics, Baltimore, U.S.A.) which absorbed radiation below 290 nm. For control (without UV-B) lamps were covered with 0.13 mm thick polyester films which absorbed radiations below 320 nm. Plants were exposed to radiation after one week of their emergence for 4 h per day (10.30 AM to 2.30 PM) in the middle of photoperiod till the maturity of pods. The UV-B irradiance on the top of plant canopy was measured by Ultraviolet Intensity Meter (UVP, San Gabriel, U.S.A.). The plants beneath cellulose diacetate film received sUV-B (+ 7.1 kJ m⁻²) that mimicked 20% reduction in stratospheric ozone at Varanasi (25°N) during clear sky conditions.

Three plants were selected randomly from each replicate plots after 35 and 65 days of UV-B exposure. Photosynthesis, transpiration rate and stomatal conductance were measured with LI-COR 6200 portable photosynthetic system (LI-COR, Lincoln, Nebraska, U.S.A.) under ambient conditions.

Total chlorophyll and carotenoids were extracted from leaf discs with 80% acetone and determined according to Maclachlan & Zalik (1963) and Duxbury & Yentsch (1956), respectively. Estimation of ascorbic acid and flavonoid levels in leaves were done by using the method of Keller & Schwager (1977) and Flint et al. (1985), respectively. Nitrogenase activity in fresh nodules was estimated by acetylene reduction method with the help of gas chromatography by adopting the method of Stewart et al. (1967) with some modifications.

RESULTS AND DISCUSSION

Chlorophyll *a*, *b* and total tended to be lower in sUV-B exposed plants than control plants and the percentage reduction was only significant at early stages, i.e., at 35 days plant age (Table 1). Carotenoid content showed reductions at all plant ages after exposure of UV-B, but it is significant at 50 and 65 days. Flavonoid level generally increased in control and UV-B exposed plants with increasing plant age (Table 2) but UV-B induced increases were significant at 50 and 65 days of plant age and not at 35 days. The reduction in chlorophyll concentration after UV-B exposure may be ascribed to inhibition of its biosynthesis or due to increased degradation of this pigment and its precursors due to UV-B treatment. Disruption of chloroplasts has been reported due to UV-B (Allen et al. 1978). Iwanzik & Tevini (1982) have reported that among photosynthetic pigments both carotenoids neoxanthin and β -carotene reduced by 80% at highest UV-B irradiance. The most important effect of UV-B in the present study was the increase of UV-B absorbing pigment, flavonoids. Flavonoid accumulation is regarded as a defence mechanism in higher plants to provide protection against UV-B (Tevini et al. 1991). Increase in flavonoid concentration is in support of results obtained by Agarwal

Table 1: Age-wise changes in chlorophyll and carotenoid contents of control and UV-B exposed *Vicia faba* plants (Mean \pm S. E.).

Plant age (days)	Parameter	Control plants (mg g ⁻¹ dry leaf)	UV-B exposed plants (mg g ⁻¹ dry leaf)
35	Chl <i>a</i>	7.04 \pm 0.32	6.01 \pm 0.35
	Chl <i>b</i>	6.87 \pm 0.11	6.82 \pm 0.42
	Total Chlorophyll	13.91 \pm 0.28	12.83 \pm 0.04
	Carotenoid	4.32 \pm 0.08	4.12 \pm 0.06
50	Chl <i>a</i>	7.25 \pm 0.04	7.05 \pm 0.21
	Chl <i>b</i>	7.36 \pm 0.32	7.34 \pm 0.18
	Total Chlorophyll	14.61 \pm 0.50	14.39 \pm 0.56
	Carotenoid	4.64 \pm 0.10	3.14 \pm 0.25
65	Chl <i>a</i>	7.56 \pm 0.26	6.81 \pm 0.05
	Chl <i>b</i>	8.02 \pm 0.36	7.87 \pm 0.44
	Total Chlorophyll	15.58 \pm 0.42	14.68 \pm 0.42
	Carotenoid	4.48 \pm 0.06	2.87 \pm 0.13

Table 2: Effects of UV-B radiation on changes in foliar concentrations of flavonoids in *Vicia faba* plants (Mean \pm S. E.)

Plant Age (Days)	Flavonoids (A ₃₀₀ mg ⁻¹ fresh leaf)	
35	Control	0.19 \pm 0.002
	UV-B exposed	0.23 \pm 0.003
50	Control	0.53 \pm 0.003
	UV-B exposed	0.81 \pm 0.005
65	Control	0.76 \pm 0.003
	UV-B exposed	0.98 \pm 0.004

et al. (1991), Ambasht and Agrawal (1997, 1998). Thus, *Vicia faba* plants may activate a defense against UV-B stress by increasing nonphotosynthetic pigments, flavonoids.

The rate of photosynthesis was maximum at 35 days in both the control and UV exposed plants (Table 3). The rate of photosynthesis was reduced significantly after UV-B exposure at 35 and 65 days. Transpiration rate was maximum at 65 days in control and UV-B treated plants, i.e., 0.0538 and 0.0315 mol m⁻²s⁻¹, respectively. Stomatal conductance increased with plant age, but it reduced significantly after exposure with UV-B at all plant ages. Negash (1987) and Deckmyn et al. (1994) and Ambasht & Agrawal (1998) have suggested that reduction in rate of photosynthesis may be due to stomatal closure (decrease in stomatal conductance) under supplemental levels of UV-B. The present results are also in support of this mechanism. Brandle et al. (1977) have demonstrated that photosystem-II activity is impaired in *Pisum sativum* plants exposed to UV-B. Lingakumar & Kulandaivelu (1993) have suggested that strong UV-B radiation, besides acting directly at photosystem level, also induces a change in stoichiometry of PS-I to PS-II. Disruption of chloroplast membrane by sUV-B has been shown to occur in pea (Brandle et al. 1977). UV-B induced decrease in photosynthesis could also be due to inactivation of primary carboxylase reactions (He et al. 1993, Lingakumar & Kulandaivelu 1993).

The level of ascorbic acid declined significantly at the age of 65 because of sUV-B treatment (Table 4), the reduction was higher at 65 days. The catalase activity also declined significantly at both the plant ages, whereas peroxidase activity increased significantly in sUV-B plants as compared to

control plants (Table 4). Maximum catalase activity was recorded at 35 days of plant age while maximum peroxidase activity was noticed in plants of 65 days. Low content of ascorbic acid in sUV-B exposed plants suggested generation of oxy-radicals in *Vicia* plants. Ascorbic acid acts as an *in vivo* biological antioxidant and functions as a co-substrate of plant peroxidases (Halliwell 1982). Both catalase and peroxidase are important components of antioxidant system which protect plants by scavenging free radicals and H_2O_2 (Bowler et al. 1992). Ambasht & Agrawal (1998) also found similar results with *Sorghum vulgare* plants i.e., decrease of catalase activity *vis a vis* increase in peroxidase activity. It was suggested from the molecular structure of both the enzymes and from the *in vitro* relationship that the tetrameric molecules of catalase might disintegrate *in vivo* into monomeric units with peroxidase activity (Nandi et al. 1984). Panagopoulos et al. (1989) also reported changes in peroxidase and catalase activities in UV-B exposed plants while Booker et al. (1992) did not observe significant changes in either enzymes.

Nitrogenase activity in the root nodules of control plants remained always higher in comparison to UV-B exposed plants. Nitrogenase activity declined by 30.7 and 61.3 respectively at the plant age of 35 and 65 after sUV-B exposure (Table 4). Earlier studies showed suppression of root growth and nodulation following UV-B exposure (Teramura & Murali 1986). Singh (1993) also noted reduction in nitrogenase activity as a result of sUV-B exposure in some leguminous plants. The reduction in nitrogenase activity may be an indirect effect of UV-B induced alteration in plant metabolism which

Table 3: Age-wise changes in photosynthetic rate, stomatal conductance and transpiration rate of control and UV-B exposed *Vicia faba* plants (Mean \pm S.E.).

Parameter	35 days		65 days	
	Control	UV-B exposed	Control	UV-B exposed
Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	13.65 \pm 0.12	11.32 \pm 0.24	10.65 \pm 0.25	7.85 \pm 0.53
Stomatal conductance (cm s^{-1})	4.96 \pm 0.08	2.53 \pm 0.04	5.62 \pm 0.16	2.76 \pm 0.08
Transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$)	0.0232 \pm 0.0008	0.0196 \pm 0.0006	0.538 \pm 0.005	0.0315 \pm 0.002

Table 4: Age-wise changes in ascorbic acid content, catalase, peroxidase and nitrogenase activity in control and UV-B exposed *Vicia faba* plants (Mean \pm S.E.).

Parameter	35 days		65 days	
	Control	UV-B exposed	Control	UV-B exposed
Ascorbic acid (mg g^{-1} fresh leaf)	1.26 \pm 0.06	1.14 \pm 0.03	1.73 \pm 0.05	1.48 \pm 0.05
Catalase (mmoles H_2O_2 decomposed $\text{min}^{-1} \text{g}^{-1}$ fresh leaf)	3.80 \pm 0.05	2.6 \pm 0.1	3.4 \pm 0.01	1.6 \pm 0.04
Peroxidase activity ($\mu\text{moles purpurogalallin formed min}^{-1} \text{g}^{-1}$ fresh leaf)	64.8 \pm 1.5	68.6 \pm 2.2	66.5 \pm 2.0	78.9 \pm 2.0
Nitrogenase activity (nmol of C_2H_4 mg^{-1} dry wt. h^{-1})	46.4 \pm 0.88	32.4 \pm 1.2	68.8 \pm 1.42	26.6 \pm 1.06

reduces the amount of photosynthate translocated to the roots essential for deriving energy needed for symbiotic nitrogen fixation.

The results of present study showed that supplemental UV-B has led to significant reductions in photosynthesis, antioxidant defense system and nitrogen fixation capacity *vis a vis* accumulation of flavonoids as a defense mechanism.

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HEAVY-METAL ACCUMULATION IN INDUSTRIAL SOLID WASTE AMENDED SOILS

Sapna Pande and V. S. Shrivastava

Centre for P. G. Research in Chemistry, G. T. P. College, Nandurbar-425 412, Maharashtra, India

ABSTRACT

The accumulation of As, Cd, Cu, Hg, Ni, Pb and Zn in industrial solid waste amended soil has been studied. The soil samples were taken at the depth of 15 cm from the surface. The status of the metals in seven different samples has been evaluated. The concentration of these metals was detected by Inductively Coupled Plasma Atomic Emission Spectroscopy. The concentration of some metals was found to be higher. The detected concentration of metals has also been correlated with the organic matter (OM) and chemical oxygen demand (COD) of the samples.

INTRODUCTION

Metal specification has become an important area of inquiry because of its importance in understanding of the fate and effect of metals in the environment (Dissanayake 1982). Heavy metals also complex with organic compounds adsorbed or occluded in carbonate or oxide minerals or in the structure of primary and secondary minerals (Hickey & Kittrick 1984, Chang et al. 1984). Some workers (Lund et al. 1976) have concluded that the organic compounds constitute only 2 to 3 percent of the sediment, and known to play an important role in various geochemical processes such as solubility, mobility, concentration and accumulation of the metals.

The mobility of certain metals such as Mn, Zn, Cu and Fe was investigated as affected by soil organic matter. It was found that the increasing concentration of soil organic matter caused a decrease in their mobility. Some researchers (Gerritase et al. 1982) also found the relation between the adsorption of heavy metals, organic matter and pH of the soil. When adsorption was related to soil organic matter content, for many trace elements, these research workers found the strength of adsorption was dependent only on pH, showing an increase with rising pH. Das and Patnaik (2001) have reported the use of industrial waste for reduction of COD from paper mill effluent. COD is always related with metal organic fractions in the solid waste. In view of the above it has been considered worthwhile to study the accumulation of heavy metals in industrial solid waste amended soils.

MATERIAL AND METHODS

The solid waste amended soil samples were collected from different locations of Sachin and Pandesara GIDC areas of Surat city at the depth of 15 cm from the surface.

The metal concentration was detected by ICP-AES method at RSIC, IIT, Mumbai. The OM and COD were detected by standard methods (Lund et al. 1976, APHA 1989).

RESULTS AND DISCUSSION

The results obtained during the present investigations are given in Tables 1 and 2. The amount of OM in these solid waste amended soil samples was found very high. It ranges from 4633.69 to 100782.75 µg/g. The COD ranged from 25.6 to 377.6 µg/g.

The amount of As and Cr in all solid waste amended soil samples were detected less than 0.5 and 0.6 $\mu\text{g/g}$ respectively.

Cd was present in detectable range of 0.026 - 0.048 $\mu\text{g/g}$. Cd is a well known carcinogen and at high levels it causes kidney problems and anaemia. Correlation obtained between Cd and COD was negative (-0.779) and between Cd and OM was very weak (-0.1656).

The Cu was present in all the samples and ranged from 0.34 - 1.84 $\mu\text{g/g}$. Many workers (Stevenson 1982, Shrivastava et al. 1989a, Shrivastava et al. 1994) studied the Cu-OM complexation. They concluded that there is greater affinity between Cu and MO, which results in low mobility of Cu in soil. The correlation of Cu with COD is 0.379 and with OM is -0.00267, both insignificant.

Mercury has not been detected in most of the samples except in site no. 6. Hg was detected 1.34 $\mu\text{g/g}$. Hg is converted by natural biological processes to the poisonous neurotoxin methyl mercury. Methyl mercury and dimethyl mercury are the examples of Hg-OM complexation.

Nickel was found in only three sites. It ranged from 0.42 - 0.70 $\mu\text{g/g}$. Ni has negative correlation with OM and COD. The correlation of Ni with OM is -0.2391 and with COD is -0.1769. Organic complexation of Ni apparently was not as strong as that of Cu (Dudley et al. 1986).

Lead has been found in all the solid waste amended soil samples. It ranged from 0.045-1.80 $\mu\text{g/g}$. Lead is strongly bonded to humic material. Organic forms of Pb are introduced in significant quanti-

Table 1: Concentration of metals (SAS), OM and COD in $\mu\text{g/g}$.

Sr. No.	Site of sample collection	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	OM	COD
1.	Nr. Bank of Baroda, Pandesara	ND	0.038	ND	1.74	ND	ND	0.78	10	100782.7	377.6
2.	Nr. Parag Lab, Pandesara	ND	0.026	ND	1.76	ND	ND	1.08	1.88	27802.1	310.4
3.	Sachin Amended soil, GIDC	ND	0.036	ND	1.54	ND	0.70	1.51	3.0	33594.2	236.8
4.	Sachin Amended soil, GIDC	ND	0.045	ND	0.34	ND	0.60	1.72	3.0	4633.69	25.6
5.	Sachin Amended soil, Sunder chemical	ND	0.038	ND	0.36	ND	0.42	0.045	2.24	88080.05	118.4
6.	Sachin Amended soil, Nr. Priyadarshani chemicals	ND	0.045	ND	1.77	1.34	ND	0.46	2.24	33594.25	32
7.	Reference soil sample Nr. Sachin	ND	0.045	ND	1.84	ND	ND	1.8	2.98	33594.25	38

Table 2: Correlation coefficients among metals, COD and OM.

	COD	Cd	Cu	Ni	Pb	Zn	OM
COD	1						
Cd	-0.779	1					
Cu	0.379	-0.1516	1				
Ni	-0.1769	-0.0242	-0.02030	1			
Pb	-0.2056	0.0864	0.02030	-0.22751	1		
Zn	-0.5865	0.0045	0.02335	-0.2511	-0.1335	1	
OM	0.4989	-0.1656	-0.00269	-0.2391	-0.6314	0.6413	1

ties into the environment principally through gasoline fuels. Craig et al. (1980) concluded that any biological methylation demonstrated only increase in the existing environmental loading for lead compounds. The correlation of Pb with COD was -0.2056 , and with OM -0.6314 .

Zinc was found in all the soil samples and existed in the range of $1.88 - 10 \mu\text{g/g}$. Generally Zn has been found to be only weakly complexed by the organic matter in sewage (Baham et al. 1979). Dudley (1983) reported that Zn was complexed under most favourable conditions, i.e., high charge density of organic matter, low pH and low solubility of complexing metals. The correlation of Zn with COD was -0.5868 and with OM 0.6413 .

The accumulation of heavy metals in the soil profile is often the result of repeated sewage sludge disposal (Chang et al. 1984). The concentration of some metals were found higher in these samples. This clearly indicates that the metals accumulate in the top soils by an external atmospheric input. Chang and co-workers (1984) concluded that metal concentration in soil was proportional to sludge application.

In most of the samples a negative correlation was obtained between metals and organic constituents. In these samples though there is higher concentration of some metals, they show negative correlation. However, it does not mean that all the elements in higher concentration should show positive correlation, because their association depends not only on the anthropogenic process but also on natural processes such as weathering and leaching (Ratha 1995).

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A NOTE ON HERBAL MEDICINAL PLANT *ALOE*

M. P. N. Singh, J. Ahmed, S. Ahmed, S. Columba and M. P. Sinha

Centre for Biotechnology, Marwari College, Ranchi - 834 001, Jharkhand (India)

ABSTRACT

An attempt has been made to study the medicinal importance of *Aloe*, which cures the intestinal disorder and problems of spleen and liver. It helps in curing diabetes and heart disease and also cures many gynecological problems of women. Its use as medicine makes the ovary healthy and cures the menstrual problem of women. It is also effective in curing asthma and dry cough.

INTRODUCTION

The native place of *Aloe* is Northern Africa, Kanari Island and Spain. In India it is found from Himalyan range to Kanyakumari. It is generally known as Kumari in Sanskrit, Gwarpatha in Hindi, Kuwarpatha in Gujarati, Korphar in Marathi, Kuwargandal in Punjabi, Ghiritkumari in Bengali, Guwarpatho in Rajasthani, Chiriuli in Tamil, Kumari in Malayalam, Sabbarat in Arabi, Darkhat-E-Sibr in Farsi, Mussabar in Unani and Indian *Aloe* in English languages.

The initial description about the use the leaves of the plant as effective in curing the intestinal disorder, liver and spleen disturbance, piles, heart, diabetes, gynecological problems and anemic condition is found in Atharva Veda. It forms the RBC Cells of the blood and cures the anemic condition. Nature has given many types of medicinal plants to cure the different types of diseases as reported by Chopra et al. (1956), Singh et al. (1983) and Aimbasta et al. (1986). The whole plant of *Hydrocotyl asiatica* has the medicinal value to cure the mental and physical disability has been reported by Singh & Ahmed (1999). The plant *Aloe* which has the great medicinal value to cure many types of human diseases, belongs to family Liliaceae. The present communication is an attempt to gather the medicinal value of plant being used by vast population since long, before the academic world for its further detailed study and specific use.

The plant collected from the garden is a perennial herb of 60-90 cm height. The green leaves of the plant are 30-45 cm long, 2.5 - 7.5 cm wide and 1.25 cm thick. The leaf is wide at the base and pointed at the apex, which bears small spines on margin at short intervals. It has adventitious root system and bunch of leaves develops at the base of stem. The old plants develop an inflorescence from the middle of the bunch, which bears reddish green flowers at the end of winter season (Fig. 1).

The plant is identified as medicinal plant used to cure the different types of diseases by old people. The pulp of leaf of the plant has aloein glucoside, a light yellow coloured crystalline water soluble glucoside, imodin, resin, galic acid and scented oil. The patient can be prevented from surgery by using pulp of *Aloe* regularly especially for the pain of heart, the disease known as angina. Different medicinal plants like *Holarrhena antidysentrica* (Panda et al. 1991) and *Nelumbo nucifera* (Mukherjee et al. 1955) effectively cure dysentery when used regularly.

The *Aloe* is also effective in high blood pressure. When pulp of the plant is used it downs the cholestrol percentage in blood and cures fully when regularly used for one or two years. When the

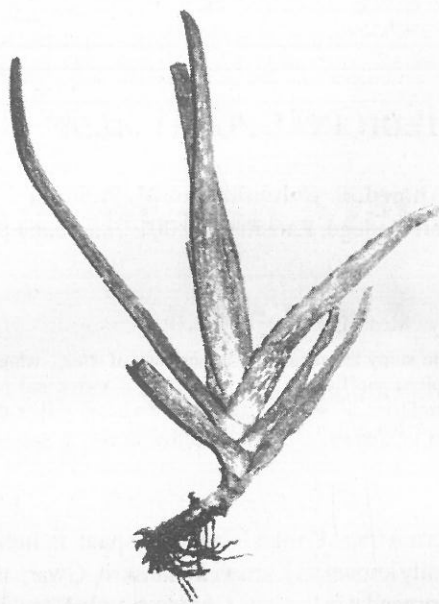


Fig. 1: The plant of *Aloe*.

pulp of *Aloe* is used regularly for a longer duration with neem fruit it cures the weakness of sensory organs and maintains the young age. The pulp of *Aloe* is useful for women in curing the irregular flow of blood and also the excess flow of blood. It is also used as antifertility herbal medicine. The herbal medicinal plants like *Catharanthus roseus* (Benjamin et al. 1990, Singh et al. 1992) and *Ocimum santum* (Singh & Agrawal, 1992) having the chemical constituents like alkaloids act as antiasthmatic and antiinflammatory activity against many diseases. The use of root, stem and leaves of *Achyranthus aspera* has been reported to cure many diseases of human beings (Singh & Ahmed 2000).

The plant is also used in spleen disturbance where the RBC cells decrease and WBC increase in blood causing anemia. The RBC cells are increased in blood and cure anemia when pulp of *Aloe* is used regularly for 15 days.

Pandey (1978) reported that the pulp is used in fever, enlargement of liver, spleen, gonorrhoea, constipation, menstrual suppression, piles, jaundice and rheumatic disorders. The juice of leaf is given as a remedy for intestinal worms in children.

In homoeopathy the medicine Aloe Socotrina, prepared from the *Aloe* species, has a wide range of medicinal value to cure many diseases of human beings. It is frequently used for the treatment of diarrhoea, dysentery and piles. It is also a valuable remedy in uterine haemorrhage and prolapsus of the uterus (Bhanja 1979, Ghosh 1995).

The plant is being used since long by the tribal population of Jharkhand. The detailed study on chemical constituents of the plant and its ecology is under progress. Attempts are being made for commercial production of the plant too. The study on the pollutional impact of coal mining on the plant is also under way.

Grateful thanks are due to Dr. K. K. Nag, Prof. & Head, Department of Botany, Ranchi University, and Dr. D. P. Gupta retired Prof. of Botany, Ranchi University for continuous encouragement.

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Environmental Pollution and Management of Wastewaters by Microbial Techniques

By G.R. Pathade and P.K. Goel

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TESTICULAR TOXICITY IN ALBINO RAT (WISTAR STRAIN) TO ORAL ADMINISTRATION OF SODIUM ARSENITE AND ASCORBIC ACID

K. Shanthi and R. Jaganathan

P.G. and Research Department of Zoology, Kongunadu Arts and Science College,
Coimbatore-641 029, Tamil Nadu, India

ABSTRACT

Sodium arsenite was administered orally to male albino rat to test the biochemical changes and the changes in histological picture of testis. It brought about significant decrease in glycogen and total protein. Alteration in cholesterol and alkaline phosphatase revealed that sodium arsenite has adversely affected the structural and functional integrity of testicular tissues. Vitamin C, given along with Na_3AsO_3 , due to its antioxidant property nullified the effect of the latter.

INTRODUCTION

The toxicity of arsenite has long been of concern due to frequent use of arsenicals in herbicides, insecticides, rodenticides, paints, pigments and wood preservatives. Arsenic occurs in the wastage derived from the production of several metals and as by-product of fossil fuel usage (Boxley et al. 1981). Environmental arsenic exposures have drawn attention primarily because of diseases resulting from ingestion of water containing this element.

Though toxicity of Na_3AsO_3 to male and female animals, in general, has been reported (Lee et al. 1985, 89) there are only a few attempts to study the reproductive toxicity of arsenicals. Hence an attempt was made to study the long term sub-lethal effects of Na_3AsO_3 on biochemical and histological aspects in testis of albino rats.

MATERIAL AND METHODS

Pure bred male albino rats weighing 150-250 g were procured from Tamil Nadu Agriculture University, Coimbatore and housed in polypropylene cages. They were fed with laboratory animal feed (Hindustan Lever Ltd., Bangalore) and water *ad libitum*.

Technical grade disodium hydrogen arsenite with maximum purity was procured from Merck. LD50 value for 24 h was found to be 5 mg/kg body weight using probit analysis after Finney (1952). Sub-lethal concentration of Na_3AsO_3 ranging from 2 to 5 ppm were given to individual groups of rat orally daily for a period of 60 days. On the 61st day the animals were sacrificed and testis was removed surgically and weighed. Standard methods were used to determine glycogen (Folin & Wu 1920) total protein (Lowry et al. 1951), cholesterol (Parekh & Jung 1970), GOT and GPT (Reitman & Frankel 1957) and ALP (Kind & King 1954). The results were statistically evaluated using Student's 't' test. For histopathological studies small pieces of testes were fixed in Bouin's fluid. After routine processing, paraffin sections were cut at 5 μ and stained with haematoxylin and eosin (Bancroft & Stevens 1977) for microscopic examination.

RESULTS AND DISCUSSION

When the rats were treated with different concentrations of Na_3AsO_3 , the glycogen and total protein

content of the testis was found to decrease gradually with increasing concentration of the toxicant (Table 1). Cholesterol content and the enzyme alkaline phosphatase were found to show a steady increase with increasing concentration of Na_3AsO_3 . However, the enzymes GOT and GPT reacted in a different way. There was a gradual increase in the level of enzyme activity up to a concentration of 3.5 ppm Na_3AsO_3 , but beyond that they showed a declining tendency.

The decreased level of protein and glycogen in the present study may be correlated with retardation of spermatogenesis as suggested by Dixit & Bhargava (1983), Murugan et al. (1985), Malini et al. (1985), Vachhrajani (1988) and Patil et al. (1998).

Table 1: Effect of sodium arsenite on biochemical changes in the testis of albino rat.

Treatment	Control	2.0 ppm	2.5 ppm	3 ppm	3.5 ppm	4.0 ppm	4.5 ppm	5.0 ppm
Glycogen mg/g								
S ₁	9.000 ± 0.100	9.400 ± 0.100	8.500 ± 0.264	8.100 ± 0.200	7.300 ± 0.360	7.100 ± 0.100	6.800 ± 0.100	6.700 ± 0.100
S ₂	9.000 ± 0.100	9.250 ± 0.200	8.900 ± 0.264	8.700 ± 0.264	8.100 ± 0.173	7.800 ± 0.100	7.623 ± 0.200	7.100 ± 0.200
Total protein mg/g								
S ₁	200.600 ± 0.100	183.200 ± 0.400	171.500 ± 0.300	164.300 ± 0.600	151.600 ± 0.300	143.200 ± 0.400	135.400 ± 0.300	122.300 ± 0.500
S ₂	200.600 ± 0.100	195.200 ± 0.500	192.300 ± 0.400	188.600 ± 0.100	185.300 ± 0.500	182.200 ± 0.300	178.300 ± 0.300	175.700 ± 0.200
Cholesterol mg/g								
S ₁	18.500 ± 0.100	19.300 ± 0.400	19.800 ± 0.100	20.600 ± 0.300	23.200 ± 0.800	25.100 ± 0.100	25.600 ± 0.800	26.700 ± 0.200
S ₂	18.500 ± 0.100	18.900 ± 0.173	18.900 ± 0.200	19.300 ± 0.300	19.500 ± 0.300	20.300 ± 0.700	20.500 ± 0.200	27.800 ± 0.400
GOT IU/L								
S ₁	26.600 ± 0.100	26.200 ± 0.400	26.200 ± 0.700	31.300 ± 0.300	35.600 ± 0.400	33.200 ± 0.300	29.500 ± 0.400	27.800 ± 0.400
S ₂	26.600 ± 0.100	26.500 ± 0.700	26.500 ± 0.100	28.300 ± 0.200	30.500 ± 0.346	29.300 ± 0.300	28.600 ± 0.700	27.100 ± 0.173
GPT IU/L								
S ₁	27.200 ± 0.800	29.500 ± 0.400	29.500 ± 0.300	36.700 ± 0.200	41.300 ± 0.435	37.500 ± 0.100	33.400 ± 0.800	31.600 ± 0.100
S ₂	27.200 ± 0.800	28.600 ± 0.300	28.600 ± 0.100	31.600 ± 0.200	35.800 ± 0.100	33.200 ± 0.600	29.700 ± 0.200	29.100 ± 0.700
ALP IU/L								
S ₁	171.500 ± 0.400	178.300 ± 0.300	178.300 ± 0.200	192.500 ± 0.300	198.700 ± 0.200	205.200 ± 0.200	211.400 ± 0.200	210.400 ± 0.100
S ₂	171.500 ± 0.400	175.300 ± 0.400	175.300 ± 0.300	183.800 ± 0.100	185.500 ± 0.400	193.400 ± 0.400	196.600 ± 0.200	196.800 ± 0.100

S₁ = sodium arsenite; S₂ = sodium arsenite + vitamin C; Values are expressed as mean ± SD.

Cholesterol content was found to increase gradually with increasing concentration of Na_3AsO_3 . Cholesterol is known to function as a precursor molecule for the synthesis of steroid hormone (Biswas & Dob 1967, Preidkalns & Weber 1968). The results of the present study show that the increase in cholesterol is due to its non-utilization as suggested by Aruldas et al. (1986), Shah et al. (1987) and Bhatnagar & Soni (1990).

It is of interest to note that the alteration in enzyme profile is associated with histopathological changes in the testes of the rats exposed to Na_3AsO_3 . Increase in the enzyme level was correlated with the histopathological changes in the testes. Similar observations have been made by some earlier workers. Geeta et al. (1977) have reported increased ALP activity as a reflection of sperm resorption, Chattopadhyay (1998) with carcinogenesis and Mishra et al. (1998) with testicular injury, spermatogenesis, hormone biosynthesis and steriodogenesis. However, the enzymes GOT and GPT were found to increase up to 3.5 ppm treatment and then decreased with increasing concentration, a typical response to overcome the stress.

HISTOPATHOLOGY

Much work has been done on the damages inflicted on the testes of rats by pesticides with are indiscriminately applied to control plant pests (Copen 1964, Fabbrini et al. 1971, Dixit 1977, Marlow & Sullivan 1982, Chowdhury & Ravella 1986, Alabi & Whanger 1987, Vachhrajani et al. 1988, McClain et al. 1989, Akbarsha & Sivasamy 1998, Anand Laxmi 1998, Mishra et al. 1998 and Rao et al. 1998).

The Histological picture of the control testis shows clear seminiferous tubules, spermatogonial cells, spermatozoa and interstitial cells (Fig.1). Rats treated with 3ppm, 4ppm, and 5ppm Na_3AsO_3 showed clear pathological changes in the order, irregular seminiferous tubules with large space, retarded spermatogenesis, shrunken seminiferous tubules, inactive germinal epithelium, pycknotic nuclei, disintegration of basement membrane and total arrest of spermatogenesis (Figs. 2, 3 & 4). The degenerative changes observed may be attributed to the inhibition of gonadotrophins as suggested by Reddy et al. (1998).

Vitamin C, an antioxidant, given alone to the albino rat has not shown any marked changes. But when given to rats which have been treated with different concentrations of Na_3AsO_3 it has been noticed that Vitamin C has acted against the sodium arsenite and the deleterious effect of arsenic is countered by it in a majority of the cases.

The present findings may be useful in understanding the effect of toxicant like sodium arsenite in living systems and the protective role of Vitamin C in counteracting the damage caused by Na_3AsO_3 in particular and pollutants in general. There are a number of antioxidants available like super oxide

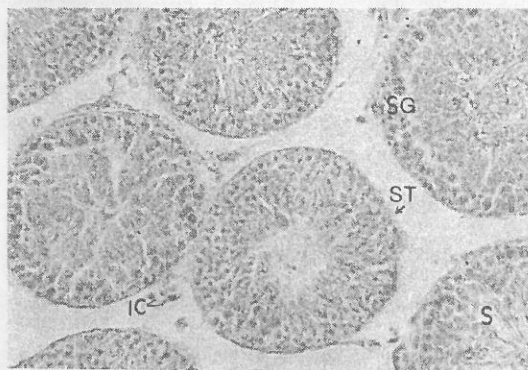
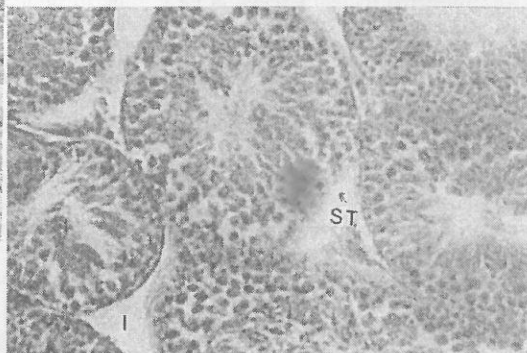


Fig.2: Section of testis of male albino rat treated with 3.0 ppm sodium arsenite. HE x 450. ST = Semini-ferous tubule (irregular); I = Interstitium.

Fig.1: Section of testis of control male albino rat. HE x450. IC = interstitial cells; ST = Seminiferous tubules; SC = Spermatogonia; S = Spermatheca.



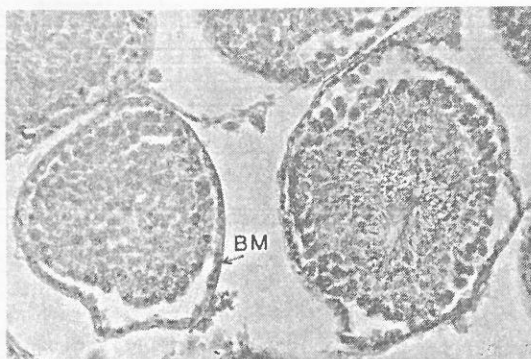
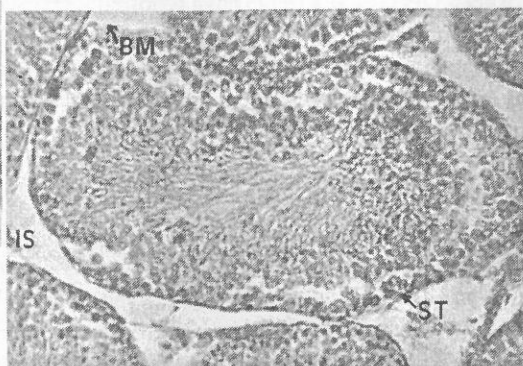


Fig.4: Section of testis of male albino rat treated with 5.0 ppm sodium arsenite. HE x 450. BM = Basement membrane (ruptured); IS = Intertubular space; ST = Seminiferous tubule (shrunken).

Fig.3: Section of testis of male albino rat treated with 4.0 ppm sodium arsenite. HE x 450. BM = Basement membrane.



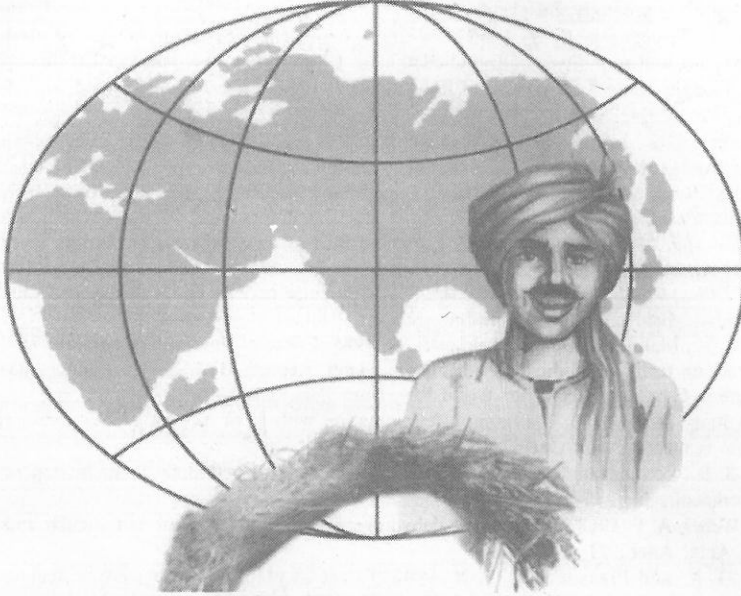
dismutase, catalase and glutathione peroxidase etc. It has been reported that some of the antioxidants like α -tocopherol are less effective when compared to ascorbic acid (Niki 1990). Therefore, Vitamin C is suggested for its role on minimizing the adverse effects of sodium arsenite.

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किसान...., हमारी उन्नति के साथी



विश्व व्यापी भविष्य की ओर अग्रसर

भारत में हरित क्रांति की अग्रदूत एवं देश को उर्वरकों के उत्पादन के मामले में आत्म निर्भर बनाने के लिए वर्षों तक कार्य करने के उपरांत इफको नाइट्रोजीनस व फास्फेटिक उर्वरकों के उत्पादन व विपणन के क्षेत्र में विश्व की अग्रणीय संस्था के रूप में उभर कर सामने आई है।

किसानों की खुशहाली के लिए कार्य करते हुए तीन दशक से भी अधिक समय का सफ़र तय करने के बाद इफको उर्वरकों के उत्पादन में विश्व की अद्वितीय संस्था बन चुकी है।

इफको की बहुआयामी योजना 'विजन 2000' पूरी हो चुकी है। यह योजना उत्कृष्टता के लिए किये गये अथक प्रयासों से पूर्ण की गई है। इस योजना के अंतर्गत आंवला, फूलपुर, कलोल

तथा कांडला संयंत्रों की क्षमता का विस्तार अनुमोदित लागत व निर्धारित समय के भीतर कर लिया गया है।

इफको ने अपने विकास के ऊँचे शिखर पर पहुँचने के लिए अब एक और पचवर्षीय योजना 'मिशन 2005' बनायी है। इस योजना के अंतर्गत उत्पादन क्षमता को बढ़ाने, नये संयंत्रों की स्थापना करने, विदेशों में संयुक्त उद्यम स्थापित करने, विविधीकरण करने व हर स्तर पर सहकारिता आंदोलन को सुदृढ़ करने का लक्ष्य रखा गया है।

इफको ने सामान्य बीमा क्षेत्र में प्रवेश करने के प्रयोजन से जापान की टोकियो मैरीन एण्ड फायर इंश्योरेंस कम्पनी लिमिटेड के साथ समझौता ज्ञापन पर हस्ताक्षर किये हैं।

इफको

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