

# EVALUATION OF WATER-QUALITY BY CHLOROPHYLL AND DISSOLVED OXYGEN

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## ABSTRACT

**T**his paper focuses on the impact of Chlorophyll and dissolved Oxygen on water-quality. Kalar Kahar and Rawal lakes were selected for this research. A Spectrophotometer was used for determination of Chlorophyll a, Chlorophyll b, Chlorophyll c and Pheophytin pigment. Dissolved Oxygen was measured in situ, using dissolved-oxygen meter. The  $\delta^{18}\text{O}$  of dissolved Oxygen, like concentration, is affected primarily by three processes: air-water gas exchange, respiration and photosynthesis;  $\delta^{18}\text{O}$  is analysed on isotopic ratio mass-spectrometer, after extraction of dissolved Oxygen from water-samples, followed by purification and conversion into  $\text{CO}_2$ . Rawal lake receives most of the water from precipitation during monsoon-period and supplemented by light rains in December and January. This water is used throughout the year for drinking purposes in Rawalpindi city. The water-samples were collected from 5, 7.5, and 10 meters of depth for seasonal studies of physiochemical and isotopic parameters of water and dissolved Oxygen. Optimum experimental conditions for  $\delta^{18}\text{O}$  analysis of dissolved Oxygen from aqueous samples were determined. Stratification of dissolved Oxygen was observed in Rawal Lake before rainy season in summer. The water-quality deteriorates with depth, because the respiration exceeds the photosynthesis and gas exchange. The concentration and  $\delta^{18}\text{O}$  of dissolved Oxygen show no variation with depth in 1998 winter sampling.

Kalar Kahar lake gets water from springs, which are recharged by local rains on the nearby mountains. It is a big lake, with shallow and uniform depth of nearly 1.5 meters. A lot of vegetation can be seen on the periphery of the lake. Algae have grown on the floor of the lake. Water-samples were collected from the corner with large amount of vegetation and from the center of the lake for dissolved Oxygen and Chlorophyll measurements. Chlorophyll result shows that Kalar Kahar Lake falls in Eutrophic category of Chlorophyll concentrations. Dissolved Oxygen ranges 0.3 to 9.1 mg/l with minimum at the morning and

maximum at 16.00 hours of the day. These alarming dissolved Oxygen results show that fish can not survive in these conditions.

*Key words:* Dissolved Oxygen, Chlorophyll, isotopic ratio mass spectrometer, water-quality.

## INTRODUCTION

**C**hlorophyll 'a' is a blue-green microcrystalline solid, while Chlorophyll 'b' is green black microcrystalline solid. Chlorophyll 'a' is of universal occurrence in the green plants; Chlorophyll 'b' occurs in higher plants and green algae. All plant-life contains the primary photosynthetic pigment Chlorophyll 'a'. Chlorophyll concentration is an indirect estimation of the biomass and the photosynthesis-rate of the primary producers. According to Sakamoto and Vollenweider, lakes can be classified as Eutrophic lakes, with Chlorophyll 'a' concentration 5-140  $\text{mg/m}^3$ , Mesotrophic lakes with 1-15  $\text{mg/m}^3$  and Ologotrophic lakes with 0.3-2.5  $\text{mg/m}^3$ . Chlorophyll has been used to ameliorate bad breath, as well as to reduce the odors of urine, feces and infected wounds. Good dietary sources of Chlorophyll include dark green leafy vegetables, algae, chlorella, wheat-grass and barley grass. Supplements of Chlorophyll as powder, capsule, Tablet, and drinks are also available. Several kinds of Chlorophyll have been discovered. All green plants contain Chlorophyll 'a' and, for planktonic algae, it constitutes about 1 to 2 % of the dry weight<sup>1,2</sup>.

Data on King County Lake (Lake Washington and Lake Sammamish) elucidate that dissolved Oxygen concentrations may change dramatically, with depth, particularly as thermal stratification persists. Oxygen is added to the water via diffusion through wind-mixing and produced in the top portion of the lake during photosynthesis. Respiration also consumes dissolved Oxygen. Oxygen depletion is greatest near the bottom of the lake, where settled organic matter decomposes. Water temperature influences the amount of gas that water can hold. As water becomes warmer, it becomes saturated more easily with Oxygen, meaning it can hold

less of the dissolved gas. The amount of algae present can control the dissolved oxygen-concentration and the pH, as well as the amount of nutrients. Algae produce Oxygen during daylight hours but use up Oxygen during the night, in respiration, and when they die, sink, and decay. These same processes basis the changes in lake pH<sup>3</sup>.

## SAMPLING SITES

**R**awal and Kalar Kahar lakes, and Humak, Tarlai and Gandaf ponds were selected for samplings.

### Rawal Lake

Rawal dam was constructed in 1960 across Kurrang river near Islamabad. It is 33.54 m high and 213 m long. The storage capacity is 58.6 million cubic meters. The Rawal lake receives most of the water from precipitation during the monsoon period (August-September), and supplemented by light rains in December and January. The stored water is used throughout the year for drinking purposes in Rawalpindi City. Water-samples were collected in July & December 1998 and July & December 1999, for extraction of dissolved Oxygen. 400 µl of saturated solution of mercuric chloride per one liter of water-sample was used as an preservative<sup>4</sup>. The samples were taken from: surface, 5, 7.5 and 10 meters depth.

### Kalar Kahar Lake

Kalar Kahar lake gets water from springs, which are recharged by local rains on the nearby mountains. It is a big lake, with shallow and uniform depth of nearly 1.5 meters. A lot of vegetation can be seen on the periphery of the lake. Algae has grown on the floor of the lake as shown in Figure - 1. Water-samples were collected from (i) the corner with

large amount of vegetation and from (ii) the centre of the lake. The samples were also collected for the measurement of Chlorophyll.

### Humak and Tarlai Ponds

Humak and Tarlai ponds, near Islamabad, are not very big in size. These are roughly 20 m<sup>2</sup>, with no outlet. These are filled by surface runoff when there is rainfall. The water in the ponds evaporates with time. The water-samples were collected for estimating dissolved Oxygen and Chlorophyll measurement.

## INSTRUMENTS

*Dissolved Oxygen* (by digital DO<sub>2</sub> meter-model 9071, Jenway, UK)

*pH* (by pH meter, CD 62, WPA, UK)

*EC and temperature* (by digital EC meter LF 191, WTW, Germany)

*Chlorophyll concentration* (by Visible and U V Spectrophotometer)

*δ<sup>18</sup>O of dissolved Oxygen in water and δ<sup>18</sup>O & δ<sup>2</sup>H of water-samples* (by Isotopic ratio mass-spectrometer).

## METHODS

### Chlorophyll

*Chlorophyll Sample Collection, Preservation and Filtration:*

0.5-2.0 liter of water-sample is often convenient for measurement of Chlorophyll in the biomass. Smaller amount can be used for denser population. Water-samples should be measured as soon as possible after collection. The constrains should be placed on filtering of the water-samples that are to be used for the extracted analysis. From the time of collection to the measurement, the sample should be stored in the dark, on ice. Whole water-sample can be held up to 2 weeks in the dark at 4 °C.



Figure-1: Algae Is Seen On The Floor Of The Kallar Kahar Lake (Sun-Set View)

**Evaluation of Water-Quality by Chlorophyll and Dissolved Oxygen**

Used opaque bottle, because even exposure to light during storage will alter the Chlorophyll values. Samples on the filters taken from water having pH 7 or higher may be placed in airtight plastic bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly, to prevent Chlorophyll degradation.

Water-sample was filtered through glass-fiber filters (Whatman GF/A). Filtration assembly was attached with suitable source of reduced pressure. Glass-fiber filter containing green pigment should be immediately analysed for Chlorophyll. Placed the glass-fiber filter in beaker and added pure acetone for

approximately 3 ml acetone to the one cuvette and measured the optical density at 750 nm and adjusted the zero. Similarly, clarified extract was added to the second cuvette and the absorbance measured at same wavelength. Same measurements were then repeated at 664 nm. Extract was then acidified with 0.1ml 0.1M HCl. Gently agitated the acidified extract and measured the optical density at 750 and 665 nm within 90s. Subtracted the 750 nm value from the reading before (OD 664 nm) and after acidification (OD 665 nm). Following formulas was used for the calculation of Chlorophyll a and Pheophytin a.

$$\text{Chlorophyll a, mg/m}^3 = \frac{26.7 (664_b - 665_a) \times V_1}{V_2 \times L}$$

$$\text{Pheophytin a, mg/m}^3 = \frac{26.7 [1.7 (665_a - 664_b) \times V_1]}{V_2 \times L}$$

where:

$V_1$  = Volume of extract, L  
 $V_2$  = Volume of sample, m<sup>3</sup>  
 L = Width of cuvette (cm)

and

664<sub>b</sub>, 665<sub>a</sub> = Optical densities of extract before, and after acidification respectively

The value 26.6 is the absorbance correction and equals A x K  
 where  
 A = Absorbance coefficient for Chlorophyll a at 664 nm = 11.0, and  
 K = Ratio expressing correction for acidification

Chlorophyll extraction. The sample was then crushed by glass rod. Filtered this extract through cellulose nitrate filter paper. Same procedure was repeated until the glass-fiber filter was absolutely clear from Chlorophyll. The extract was diluted to the desired volume. Sample was then transferred to the amber glass bottles and wrapped with black paper, to avoid exposure to light and stored at 4°C<sup>5,6,7</sup>.

*Spectrophotometric determination of Chlorophyll:*

Spectrophotometer was used for determination of Chlorophyll a, Chlorophyll b, Chlorophyll c and Pheophytin pigment. Checked the optical density of the two cells (1 cm path length each) using acetone. Transferred

*Determination of Chlorophyll a, b, and c (trichromatic method)*

Transferred extract to a 1-cm cuvette and measured optical density (OD) at 750, 664, 647, and 630 nm. Used the optical density readings at 664, 647, and 630 nm to determine Chlorophyll a, b, and c, respectively. The OD reading at 750 nm is a correction for turbidity. Subtracted this reading from each of the pigment OD values of the other wavelengths before using them in the equation below.

Calculate the concentrations of Chlorophyll a, b, and c in the extract by inserting the corrected optical densities in the following equations:

a)  $C_a = 11.85 (OD_{664}) - 1.54 (OD_{647}) - 0.08 (OD_{630})$   
 b)  $C_b = 21.03 (OD_{647}) - 5.43 (OD_{664}) - 2.66 (OD_{630})$   
 c)  $C_c = 24.52 (OD_{630}) - 7.60 (OD_{647}) - 1.67 (OD_{664})$

where

$C_a$ ,  $C_b$ , and  $C_c$  = concentrations of chlorophyll a, b, and c, respectively, in mg/L, and  
 OD<sub>664</sub>, OD<sub>647</sub>, and 630 = corrected optical densities at the respective wavelengths.

After determining the concentration of pigment in the extract, calculated the amount of pigment per unit volume as follows:

$$\text{Chlorophyll a, mg/m}^3 = \frac{C_a \times \text{extract volume, L}}{\text{Volume of sample, m}^3}$$

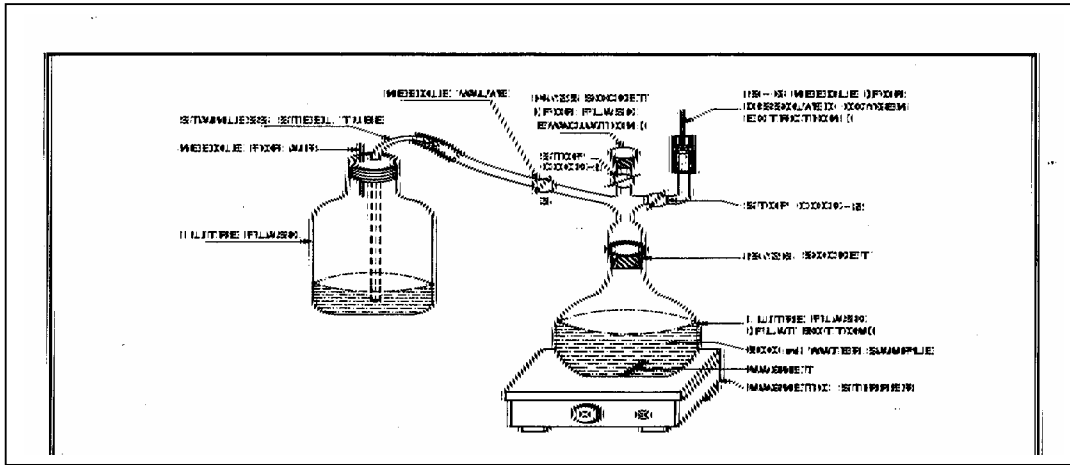


Figure - 2: Dissolved Oxygen Extraction Assembly from Water Samples

**Dissolved Oxygen**

Value of Dissolved Oxygen were measured in situ, using Jenway dissolved Oxygen meter, model 9071.

*Extraction and  $\delta^{18}O$  Analysis of Aqueous Dissolved Oxygen:*

The extraction assembly, designed and fabricated locally, is shown in Figure - 2. It consists of one liter flat-bottom flask, with 19/26 socket. An adapter is fitted in this socket. This adapter has two ends. One end has 14/23 socket for the evacuation of flask and second end is attached with one liter sample-bottle through a needle valve. The flask assembly and adapter is placed over a magnetic stirrer. One-liter flask, with adapter, is evacuated for the removal of air. Stopcocks towards evacuation system are closed and the needle valve is opened slowly. When approximately 800 ml of water is poured into the flask, the needle valve is closed. The water is stirred with magnetic stirrer. The line-valve towards rotary vacuum pump is closed and the stopcock towards purification traps is opened

slowly, the gas starts flowing in the line. All the other stopcocks of the line are opened, one by one, so that gas can flow through all three traps and in finally collected in 50 ml flask containing molecular sieves at liquid nitrogen temperature. At the end, the magnetic stirrer is switched off, the ampoule is closed and detached from the line.

The ampoule containing the purified dissolved aqueous Oxygen sample is attached at  $CO_2$  preparation system. Then the sample is introduced in the evacuated line. The valve towards rotary pump side is closed and the circulatory pump is switched on. The U-trap is immersed in a flask containing liquid nitrogen. Carbon dioxide prepared in the line is frozen in U-trap and non- condensed gases are removed by opening the valve. The liquid nitrogen flask was replaced with Freon slush at  $-65^\circ C$  temperature. The carbon dioxide frozen in U-trap sublimates and is recollected in another evacuated ampoule at liquid nitrogen temperature. Non-condensed gases trapped in the frozen carbon dioxide are released on sublimation. The sample ampoule is detached from the line and  $^{18}O$  is analyzed on mass

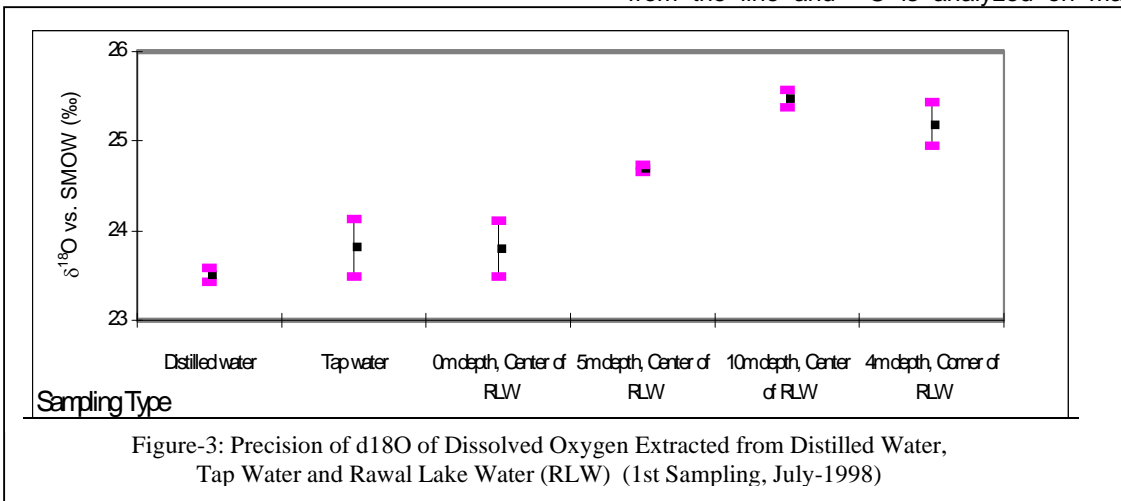


Figure-3: Precision of  $\delta^{18}O$  of Dissolved Oxygen Extracted from Distilled Water, Tap Water and Rawal Lake Water (RLW) (1st Sampling, July-1998)

**Table-1. Experimental conditions for  $\delta^{18}\text{O}$  analysis of dissolved oxygen.**

| <b>A. For extraction of dissolved <math>\text{O}_2</math></b>   |   |
|---|---|
| 1. Water sample taken in plastic bottles at the site  | 1 liter   |
| 2. Preservative   | Mercuric chloride   |
| 3. Evacuation time of one liter pyrex glass flask   | 1 minute  |
| 4. Water sample filled in the pyrex flask   | 800 ml  |
| 5. 5 gm molecular sieve (5 $^{\circ}$ A) heating (200 $^{\circ}$ C) time  | 1 minute  |
| 6. Moisture removal temperature for two traps   | -80 $^{\circ}$ C  |
| 7. $\text{CO}_2$ removal temperature for one trap   | Liquid air  |
| 8. Dissolved Oxygen adsorption time on molecular sieve  | 30 minutes  |
| 9. Dissolved Oxygen desorption time from molecular sieve  | 6 minutes   |
| <b>B. For conversion of aqueous dissolved <math>\text{O}_2</math> into <math>\text{CO}_2</math> for <math>\delta^{18}\text{O}</math> analysis</b> |   |
| 1. Reduction furnace, cylinder type within the silica furnace was made by   | Graphite furnace with spiral platinum wire, inside and around |
| 2. Reaction temperature provided by external heated furnace   | 700 $^{\circ}$ C  |
| 3. Reaction time  | 10 minutes  |
| 4. Gas circulatory pump speed   | 1.3 liters per minute   |
| 5. Non-condensed gases removal time   | 2 minutes   |
| 6. $\text{CO}_2$ sublimation time   | 3 minutes   |
| 7. $\text{CO}_2$ is purified by using   | -60 $^{\circ}$ C slush  |

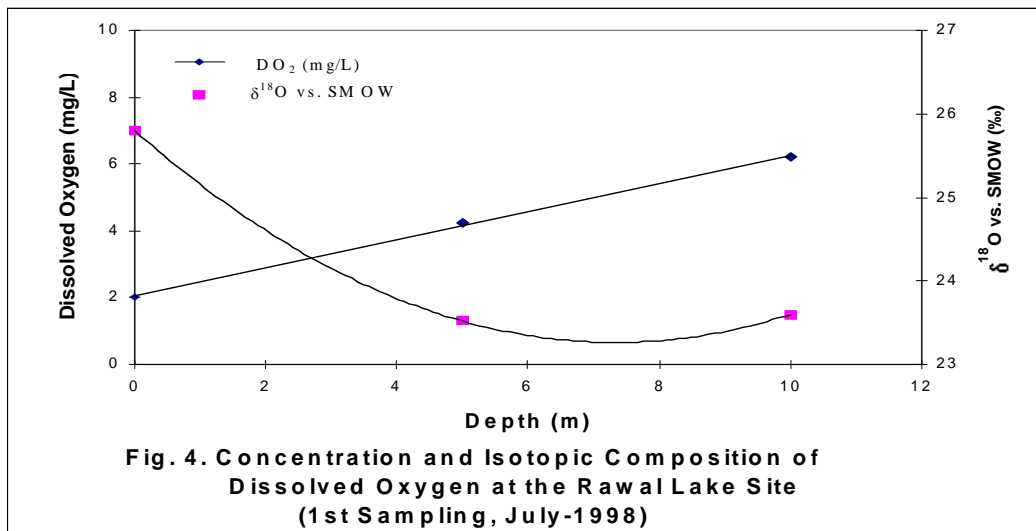
spectrometer<sup>8</sup>. Optimum experimental conditions for  $\delta^{18}\text{O}$  analysis of dissolved Oxygen of aqueous samples are described in Table-1 and the precision of  $\delta^{18}\text{O}$  of dissolved Oxygen extracted from distilled water, tap water and Rawal lake water (RLW) are depicted in Figure-3.

## RESULTS AND DISCUSSION

### Rawal Lake

The EC values of Rawal lake vary from 306  $\mu\text{s}/\text{cm}$  to 462  $\mu\text{s}/\text{cm}$ . The pH varies from 6.65 to 7.84, temperature varies from 15.6 $^{\circ}$ C to 32.2 $^{\circ}$ C.  $\delta^{18}\text{O}$  values of dissolved Oxygen from Rawal lake have variations from 24.15 ‰ to 29.5 ‰ and indicate that gas-exchange dominates photosynthesis and respiration<sup>9,10</sup> at the surface of water, as the dissolved

Oxygen has values close to 24.2‰ both in July and December (Figure - 4, 5). However in December, the EC, temperature, dissolved Oxygen concentration and  $\delta^{18}\text{O}$  of dissolved Oxygen have no variations with depth. In July, dissolved Oxygen concentration decreases and  $\delta^{18}\text{O}$  increases with depth. Respiration dominates over photosynthesis at 5 meters and 10 meters of depth, as dissolved Oxygen is undersaturated and  $\delta^{18}\text{O}$  is greater than 24.2‰. This lake receives most of the water from heavy precipitation in Monsoon period (August-September) and some contribution in December/January. The water remains standing throughout the year and is subjected to strong evaporation, as evident from isotopic data (Figure - 6, 7). In July, the water is at the lowest level and by that time microbiological activity becomes dominant at large depths.



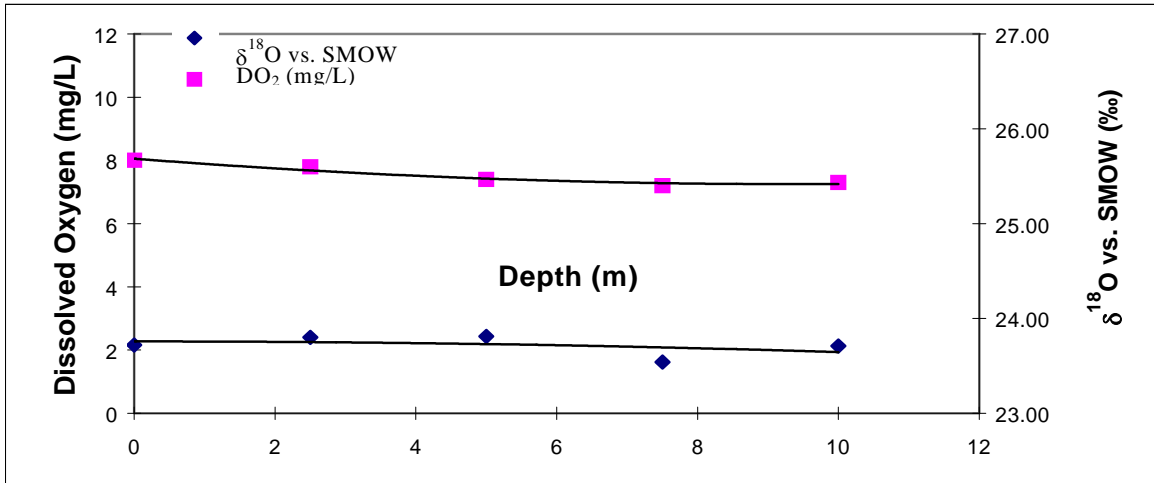


Figure-5: Concentration and Isotopic Composition of Dissolved Oxygen at the Rawal Lake Site (2nd Sampling, Dec.-1998)

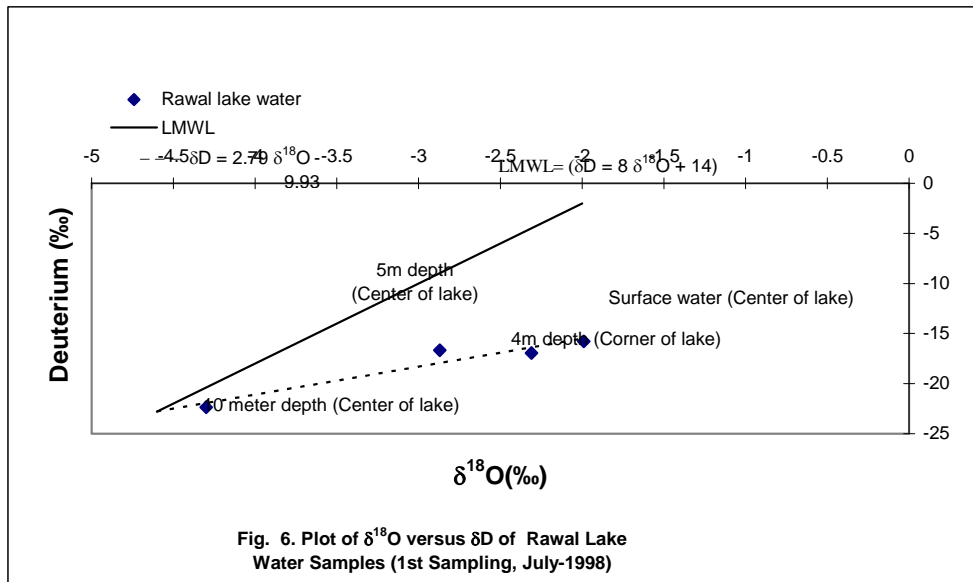


Fig. 6. Plot of  $\delta^{18}\text{O}$  versus  $\delta\text{D}$  of Rawal Lake Water Samples (1st Sampling, July-1998)

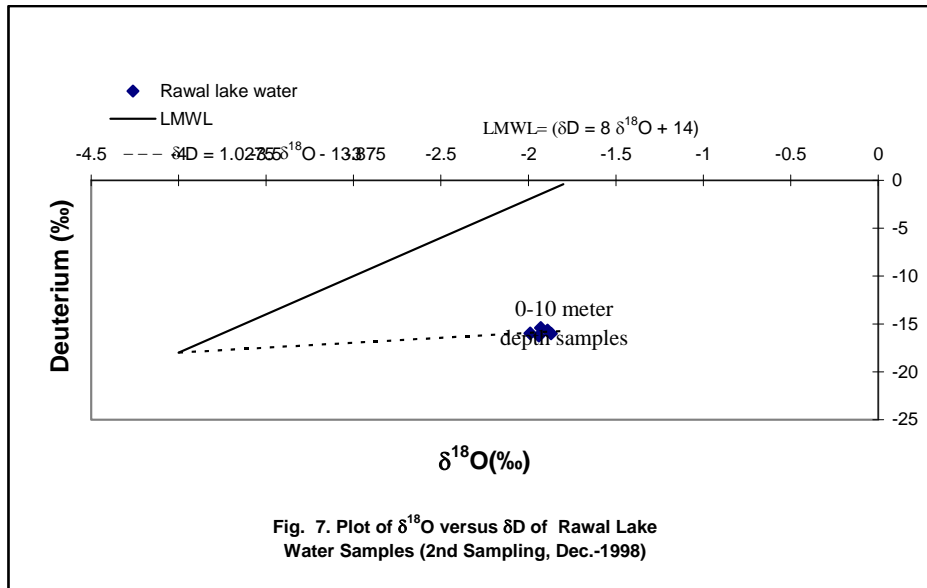


Fig. 7. Plot of  $\delta^{18}\text{O}$  versus  $\delta\text{D}$  of Rawal Lake Water Samples (2nd Sampling, Dec.-1998)

**Kalar Kahar Lake**

EC varies from 3,100  $\mu\text{s/cm}$  to 3,410  $\mu\text{s/cm}$ . Temperature range from 15.8  $^{\circ}\text{C}$  to 17.7  $^{\circ}\text{C}$ . Dissolved Oxygen concentration varies from 11.1 mg/l to 14.9 mg/l.  $\delta^{18}\text{O}$  values of 19.15 ‰ to 20.87 ‰ indicate that photosynthesis dominates respiration and exchange-rate. The

23.43  $\text{mg/m}^3$ , to 33.33  $\text{mg/m}^3$  as shown in Table-2.  $\delta^{13}\text{C}$  value shows that inorganic carbon source is from rock sediments (Table-3). Temperature ranges from 27.30  $^{\circ}\text{C}$  to 32.60  $^{\circ}\text{C}$  at point-2 (east side), from 27.20  $^{\circ}\text{C}$  to 32.60  $^{\circ}\text{C}$  at point-4 (west, vegetation side) and is observed maximum at 16:10 hours of

| Sampling Point     | Sampling Date | Chlorophyll a in the presence of Pheophytin a ( $\text{mg/m}^3$ ) | Chlorophyll a, b, c, trichromatic method ( $\text{mg/m}^3$ ) |      |      |
|--------------------|---------------|---|--|------|------|
|                    |               |   | a  | b    | c    |
| KK-1 (Surface)     | 29-12-1999    | 27.39   | 27.89  | 6.57 | 0.59 |
| KK-1 (3 feet deep) | 29-12-1999    | 33.33   | 34.33  | 2.77 | 2.13 |
| KK-2 (Surface)     | 29-12-1999    | 23.43   | 24.2   | 1.36 | 0.25 |
| KK-2 (3 feet deep) | 29-12-1999    | 30.03   | 30.97  | 2.2  | 0.6  |

| Sampling Point     | Sampling Date | $\text{DO}_2$ (mg/L) | $\delta^{18}\text{O}$ vs. SMOW (‰) | $\delta^{13}\text{C-CO}_2$ (Dissolved in water) vs. PDB (‰) | $\delta^{18}\text{O-CO}_2$ (Dissolved in water) vs. SMOW (‰) |
|--------------------|---------------|----------------------|------------------------------------|---|--|
| KK-1 (Surface)     | 29-12-1999    | 11.1                 | 20.25                              | -2.83   | 41.49  |
| KK-1 (3 feet deep) | 29-12-1999    | 11.1                 | 20.87                              | -3.71   | 41.8   |
| KK-2 (Surface)     | 29-12-1999    | 14.9                 | 19.39                              | -2.78   | 41.54  |
| KK-2 (3 feet deep) | 29-12-1999    | 14.9                 | 19.15                              | -2.75   | 41.36  |

| Sampling Point      | Sampling Date           | E. C. $\mu\text{s/cm}$ | Temp. $^{\circ}\text{C}$ | $\delta^{18}\text{O}$ vs. SMOW (‰) | $\delta^2\text{H}$ vs. SMOW (‰) |
|---------------------|-------------------------|------------------------|--------------------------|------------------------------------|---------------------------------|
| Humak Pond (Center) | 15-12-1999              | 1129                   | 8                        | -3.50                              | -22.64                          |
| Humak Pond (Center) | 22-12-1999 (after rain) | 445                    | 14                       | -1.45                              | +0.62                           |
| Humak Pond (Bank)   | 22-12-1999              | 415                    | 15.2                     | -1.26                              | +2.2                            |
| Terlai Pond         | 22-12-1999              | 468                    | 10.3                     | -2.06                              | +1.3                            |
| Gandaf Pond         | 24-12-1999              | 166                    | 15.6                     | 2.48                               | +18.46                          |

| Sampling Point      | Sampling Date | $\text{DO}_2$ (mg/L) | $\delta^{18}\text{O}$ vs. SMOW (‰) | $\delta^{13}\text{C-CO}_2$ (Dissolved in water) vs. PDB (‰) | $\delta^{18}\text{O-CO}_2$ (Dissolved in water) vs. SMOW (‰) |
|---------------------|---------------|----------------------|------------------------------------|---|--|
| Humak Pond (Center) | 15-12-1999    | 12.1                 | 21.62                              | -14.24  | 27.07  |
| Humak Pond (Center) | 22-12-1999    | 13.7                 | 19.83                              | -7.4  | 38.2   |
| Humak Pond (Bank)   | 22-12-1999    | 16.2                 | 20.68                              | -8.28   | 38.47  |
| Terlai Pond         | 22-12-1999    | 8.8                  | 18.31                              | -8.86   | 38.23  |
| Gandaf Pond         | 24-12-1999    | 13                   | 22.91                              | -8.6  | 42.89  |

photosynthesis is evident from the presence of Chlorophyll in the ponds and Kalar kahar lake. The Chlorophyll concentration has a range of

the whole day experiment (August 31, 2001). Dissolved Oxygen concentration varies from

| Table - 6: Chlorophyll Concentrations in Pond Water |               |  |   |       |       |
|---|---------------|--|---|-------|-------|
| Sampling Point                                      | Sampling Date | Chlorophyll a in the presence of pheophytin a (mg/m <sup>3</sup> ) | Chlorophyll a, b, c, trichromatic method (mg/m <sup>3</sup> ) |       |       |
|   |               |  | a   | b     | c     |
| Humak Pond (Center)                                 | 15-12-1999    | 300.39   | 301.27  | 128.9 | 28.69 |
| Humak Pond (Center)                                 | 22-12-1999    | 246.88   | 252.35  | 48.15 | 0.4   |
| Humak Pond (Bank)                                   | 22-12-1999    | 338.95   | 345.76  | 75.01 | 1.19  |
| Terlai Pond   | 22-12-1999    | 248.93   | 255.43  | 35.74 | 1.29  |
| Gandaf Pond   | 24-12-1999    | 4.62   | 4.74  | 0.63  | 0.37  |

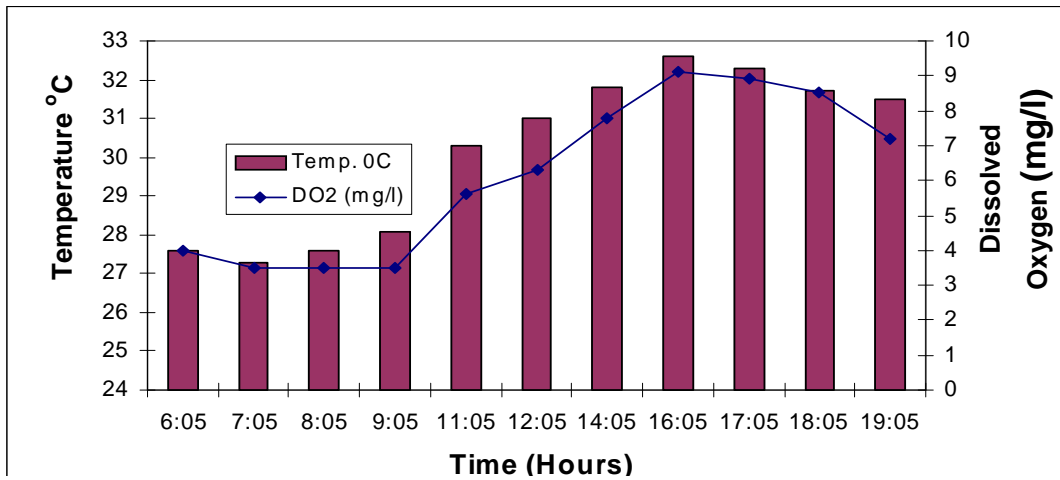


Figure - 8: Temperature and Dissolved Oxygen Behaviour in Different Day Hours at Kallar Kahar Lake (Sampling Point-2)

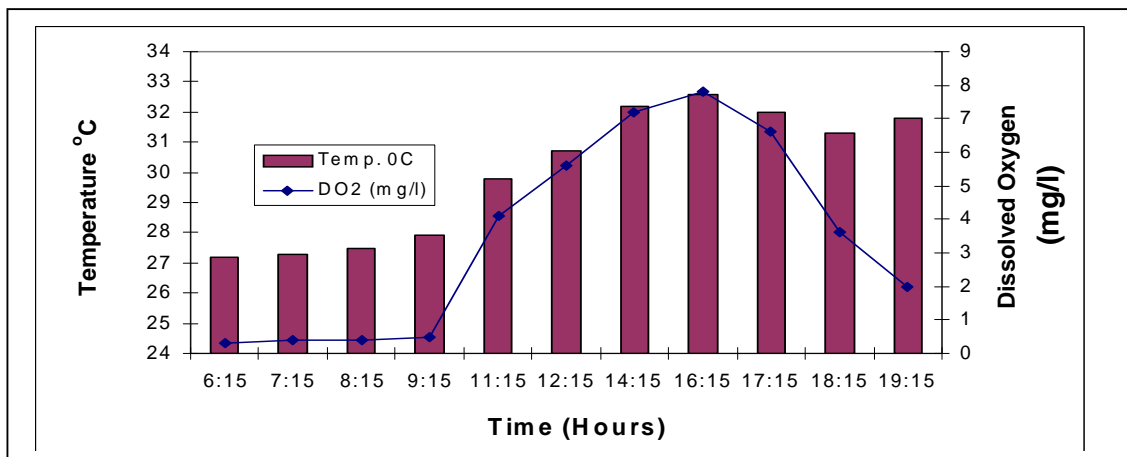


Figure - 9: Temperature and Dissolved Oxygen Behaviour in Different Day Hours at Kallar kahar Lake (Sampling Point-4)

3.50 mg/l to 9.10 mg/l at point-2 (east side), from 0.3 mg/l to 7.80 mg/l at point-4 (vegetation side) and is observed maximum at

16:10 hours of the whole day experiment (Figure - 8, 9).



**Humak, Terlai and Gandaf Ponds:**

EC varies from 166  $\mu\text{s/cm}$  to 1,129  $\mu\text{s/cm}$ . Temperature range from 8.0  $^{\circ}\text{C}$  to 15.6  $^{\circ}\text{C}$  as shown in Table-4. Dissolved Oxygen concentration has values of 8.8 mg/l to 16.2 mg/l.  $\delta^{18}\text{O}$  of dissolved Oxygen range from

19.83 ‰ to 22.91‰ endorsing the dominance of photosynthesis over respiration and gas-exchange processes (Table-5). The Chlorophyll has highest value of 338.95  $\text{mg/m}^3$  in Humak pond and lowest value of 4.62  $\text{mg/m}^3$  in Gandaf pond (Table - 6).

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