MATERIALS AND METHODS
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COLLECTION OF SAMPLES:
Samples were collected in plastic cans. Before collecting the sample, the cans were washed thoroughly with tap water and then were rinsed several times with the pond water to be collected. Surface water only was collected from the ponds, for experimentation.

SAMPLE COLLECTION FOR IDENTIFICATION OF CHLOROCOCCALEAN ALGAE:
To identify the chlorococcalean algae, from the pond water samples were collected in the following manner: A vial of about 50 ml was tied to the bottom of plankton net. Through the net 10 litre of surface water was filtered from each of the ponds. Formalin (4%) or Lugol’s iodine solution was added as preservative to this sample. Volume of this vial-collected sample was made up to 100 ml. These preserved samples were observed afterwards for the presence of chlorococcalean algae at convenient time.

TREATMENT OF ALGAE TO DIFFERENT CONCENTRATIONS OF HEAVY METALS:
In all the cases, pond water, in which the algae were present, had been taken as the medium for the experiment. Variation in physico-chemical characters between pond waters can not be ruled out. However, considering the more dominant effects of the experimental elements, taken presently, within the concentration range of 0.01 ppm to 100 ppm, the effect of difference, in physico-chemical characters of pond water has been taken to be non-significant.

STANDARD SOLUTION OF ELEMENTS:
Five elements viz: copper, zinc, lead, nickel and cobalt, selected to observe their effects on chlorococcalean algae, were taken either as sulfate or nitrate salts these salts were then converted to chlorides. Solutions of 100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm of these elements were prepared from their salts as follows:
1. **Copper:** 3.93 g CuSO$_4$·5H$_2$O was dissolved in 200 ml of distilled water. 1.5 ml concentrated HCl was then added to it and was then diluted to 1000 ml with distilled water. This was a solution of 1000 ppm of copper.

2. **Zinc:** 2.46 g ZnSO$_4$ was dissolved in 200 ml of distilled water. 1.5 ml concentrated HCl was then added to it and was then diluted to 1000 ml with distilled water. This was a solution of 1000 ppm of zinc.

3. **Lead:** 1.59 g Pb(NO$_3$)$_2$ was dissolved in 200 ml of distilled water. 1.5 ml concentrated HCl was then added to it and was then diluted to 1000 ml with distilled water. This was a solution of 1000 ppm of lead.

4. **Nickel:** 4.95 g Ni(NO$_3$)$_2$·6H$_2$O was dissolved in 200 ml of distilled water. 1.5 ml concentrated HCl was then added to it and was then diluted to 1000 ml with distilled water. This was a solution of 1000 ppm of nickel.

5. **Cobalt:** 4.94 g Co(NO$_3$)$_2$·6H$_2$O was dissolved in 200 ml of distilled water. 1.5 ml concentrated HCl was then added to it and was then diluted to 1000 ml with distilled water. This was a solution of 1000 ppm of Cobalt.

**Treatment to Algae:**

Glass jars of four liter capacity were taken for experiment. 3 liters of pond water was taken in each of the jar. To this water, standard solution of heavy metals were added to make the concentration of the solution to 100 ppm, 10 ppm, 1 ppm, 0.1 ppm, and 0.01 ppm respectively. To obtain above concentrations following procedure was followed:

For 100 ppm- 300 ml standard solution was added to 3 liters of pond water.
For 10 ppm- 30 ml standard solution was added to 3 liters of pond water.
For 1 ppm- 3 ml standard solution was added to 3 liters of pond water.
For 0.1 ppm- 0.3 ml standard solution was added to 3 liters of pond water.
For 0.01 ppm- 0.03 ml standard solution was added to 3 liters of pond water.

To experiment with the water from different ponds a set of six jars were prepared for each pond. In each of the 3 liters of pond water was taken. One of the jars was maintained as control. Remaining five jars were prepared, one each with
100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm of the heavy metal. The glass jars were fitted with air bubblers (Plate 7). Bubbling was done at the lowest speed. This was done to keep the algae floating, because neither keeping the sample in jars resulted in settling of the algae.

Before applying the heavy metal solution, 5 ml of water was taken to observe it as control. Thereafter, 5 ml water sample was taken from each of the jars, on alternate days. This alternate day collection of sample was continued till complete disappearance of algae in the sample. To count the algae one drop (0.05 ml) of the sample was put on the slide and was covered with 18x18mm² cover glass. Then different species of chlorococcal algae were counted in 10 focus area. Density of different species of chlorococcal algae was then estimated using the following formula.

Diameter of the observation field (focus area) = 0.336 mm

\[
\text{Radius (r) } = \frac{0.336}{2} = 0.168 \text{ mm}
\]

Area \((\pi r^2)\) of one focus = \(3.14 \times (0.168)^2\) = 0.088 mm²

Ten focus area = 0.088 x 10 = 0.88 mm²

Area of one Cover slip = 18x18 mm² = 324 mm²

Number of chlorococcal algae under one Cover slip area (0.05 ml)

Total No. of individuals present in 10 focus area \(\times\) area of Cover slip (324 mm²) = \(\frac{\text{Area of 10 focuses (0.88 mm²)}}{\text{Area of one Cover slip (324 mm²)}}\)

\[\text{No of Algae ml}^{-1} = \text{No. of algae in 0.05 ml} \times 20\]

**SUCCESSIVE DAY VARIATION IN DENSITY OF CHLOROCOCCAL ALGAE WITH DIFFERENT CONCENTRATIONS OF SALTS:**

Change in density of algae was recorded on successive days, after the exposure of the algae to heavy metal salts in jars. Due to time required it was not possible to record the density on each successive day hence density was recorded with a gap of 1 or 2 days. Change density was followed till the days complete
disappearance of all the species. Change in density was recorded separately for all the species will all the salts and all the pond water selected for studies.

**DAYS OF DISAPPEARANCE FOR ALGAE:**

Reaction, in the form of survival or death, of the chlorococcalean algae, towards presently taken concentrations (100, 10, 1, 0.1 and 0.01 ppm) of metals had always been exhibited in the forms of death, sooner or later, of all the chlorococcalean forms. Death has been recorded as complete disappearance of the algae from the experimental jars. This complete disappearance, with different concentrations of metals had been estimated separately for the algae from each of the ponds, selected for present studies. Disappearance of the algae for each of the ponds was recorded either metal wise or concentration wise. The original data being much lengthy, disappearance of algae has been presented as average number of days of disappearance, metal wise or concentration wise.

**METAL WISE:** - Average No. of days of disappearance of algae have been calculated for different concentrations of elements, element wise viz: cobalt, copper, lead, nickel and zinc for each species and form of chlorococcalean algae for each of the ponds.

**CONCENTRATION WISE:** - Average No. of disappearance days had been calculated for similar concentration of different elements viz: 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm separately for each species and form of the algae from the different ponds. With the help of these observations, comparative toxicity (high to low) of different metals at different concentrations of elements with different algae have been estimated.

**STATISTICAL ANALYSIS:**

**COEFFICIENT OF CORRELATION (r):** - Karl Pearson's coefficient of correlation had been calculated between: (i) Different forms of chlorococcalean algae and different concentrations of heavy metals, pond wise and (ii) different species and form of algae recorded monthly at different ponds. For computation of coefficient of correlation, a COSTAT software computer programming had been used.