# Effect of Pulp and Paper Mill Effluents on Phytoplankton (Duck Weed)

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**Abstract:** To assess the effect of pulp paper mill effluents on phytoplankton, duckweed (*Spirodela polyrriza*) were exposed to 100, 75, 50 and 25% concentration of the effluents for 7, 14, 21 and 28 days exposure. Data demonstration that duckweed showed significant accumulation of metal (Cu, Fe and Zn) which were dependent on concentration and exposure duration. A maximum accumulation of metal was recorded in higher concentration of the effluent. Impact of different concentration of pulp and paper mill effluents on the total chlorophyll content and biomass exposed duckweed plant for varying days was also studied. Reduction of total chlorophyll content and biomass of duckweeds was recorded at higher effluent concentration and prolonged duration of treatment.

***Key word:*** *Bio indicator, feasible, hemogenized, potential indicator, unimpact condition.*

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## I. Material and Methods:

In order to study the effect of different concentration of paper mill effluent on phytoplankton *Spirodela polyrrhiza* (L.)S chleider (duckweed) was selected. It belongs to family Lemnaceae and is a small floating fresh water plant with much reduced dorsiventral thalloid shoot and has adventitious roots with red colour beneath.

The duckweeds were collected from natural fresh water ponds nearby K.S. Saket College Campus. The stock of same sized young and healthy specimen of duckweed, was maintained as large hydroponic culture (with 55 Hoagland's Solution) in 15 litre capacity glass aquarium under laboratory condition and acclimatized with 16 hours of illuminating photoperiod using fluorescent tube light (115 µ mol $M^{-2}S^{-1}$ intensity)and 8 hours dark period at 28+2 $^0$C for four week.

Following composition of Hogland's nutrient medium was used according to EPA (1975).

| Compounds | Stock solution (g/l) | Ml. of stock solution to make 1 litre of nutrient medium (mg/l) |
|---|---|---|
| $Ca(NO_3)_2\ H_2O$ | 118.08 | 10 |
| $KNo_3$ | 50.25 | 10 |
| $Mg\ SO_4 7H_2O$ | 24.08 | 10 |
| $KH_2\ PO_4$ | 13.61 | 1 |
| $H_3\ BO_3$ | 2.86 | 1 |
| $MnSO_4\ H_2O$ | 1.54 | 1 |
| $ZnSO_4\ 7H_2O$ | 0.22 | 1 |
| $CuSO_4\ 5H_2O$ | 0.88 | 1 |
| EDTA (no salt) | 2.5 | 1 |
| $FeSO_4\ 7H_2O$ | 2.5 | 1 |

The experiment was laid out following complete randomized design (CRD). The settled and filtered industrial effluent (100%) were diluted to 75%, 50%, 25%, of original effluent concentration using deionized water as diluted. The experiment was carried out in plastic trough (4 litre capacity of equal volume with same surface area) in which the healthy acclimatized plants were kept with 3 litres of each effluent concentration (100%, 75%, 50%, 25%) separately with paper mill effluent.

Plant samples was periodically harvested after 7, 14, 21 and 28 days of exposure and washed thoroughly with deionised water. Following estimations were made with or without effluents exposure to plant.

**1. Metal Content:**The freshly harvested and washed plant sample were dried in over at $105^0$C for 24 hours and digested the plant powder in $HNO_3$. $HclO_4$ (4:1 v/v) at $80^0$C. The metal (Cu, Fe and Zn) content (as µg/g dw of plant) in these digested samples, were determined by a Perkin Elmer 2380 Atomic Absorption at CDRI Lucknow.

**2. Total Chlorophyll:** Total chlorophyll content as mg/g fresh weight (fw), was measured in 80% chilled acetone extract of plant leaves, by the method of Arnon (1949) with slight modification 1 gm of freshly harvested and washed plant leaves were homogenised in 20 ml of 80% acetone with a pinch of $MgCO_3$ and centrifuged the content at 3000 rpm for about 10 min then the supernatant was decanted in flask and made up of final volume of extract to 25 ml by further adding the 80% acetone. Absorbance was ready by Chemito UV-2000 spectrophotometer at 663 nM and 645 nM using 80% acetone as blank. The total chlorophyll content was calculated by using formula.

Total chlorophyll (as mg/g fw) = $\frac{20.2\ (A_{645}) + 8.02\ (A_{663})xv}{1000xw}$

**3. Biomass:** Biomass was determined on dry weight basis (as g dw) by drying the freshly harvested single plant in an over at $105^0$C for 24 hours. The weight of derived plant (in g) was recorded as the biomass of plant.

## II. Observation and Results:

Duckweed is a small free floating aquatic plant belonging to Lemnaceae family. Duckweed is well known for high productivity and high protein content in temperate climate. They are green and have a small size (1-3mm). They also have short but dense root (1-3cm) duckweed plant grown in colonies that, in particular growing condition form a dense and uniform surface mat.

In order to assess the effect of pulp and paper mill effluent on phytoplankton exposed to 100, 75, 50 and 25% effluent concentration at varying exposer duration (7, 14, 21 and 28 days). Metal accumulation, biomass, total chlorophyll, pH, TDS, BOD and COD contents were analysed from exposed plants of Duck weeds.

**Metals**

The periodic accumulation of metals viz., copper, iron and zinc (as µg/g dry weight, dw) in duckweed from different concentrations of industrial effluents at various exposure durations are shown in Table (IV, 23, 24, 25) and Fig. (22, 23, 24). From the result, it is quite clear that the maximum amount of metal accumulated form 100% effluent concentrations after 28th day exposure of phytoplankton (duckweed) than other days (7, 14, 21) of exposure. The result was recorded in following descending order as 2770 (Fe) > 999 (Zn) >550 (Cu).

**(i) Copper (Cu):**Maximum and minimum amount of Cu was accumulated from 100 and 25% effluent concentrations after 28th day exposure of duckweeds than the other days of exposure. It was recorded 550µg/g dw as maximum and 341 µg/g dw as minimum values.

**(ii) Iron (Fe):** The maximum (2770 µg/g dw) and minimum (2205 µg/g dw) was accumulated from 100% and 25% effluent concentrations after 25th day exposure of duckweed plants than other days (7, 14, 21) of exposure.

**(iii) Zinc (Zn):** The maximum amount of Zn was accumulated from 100% effluent concentrations after 28th days (7, 14, 21) of exposure. It was minimum from 25% if effluent concentration of the maximum days (28) of exposure. Zn value 999µg/g dw and 731 µg/g dw were recorded as maximum and minimum values respectively.

**Chlorophyll:**

The decrease in total chlorophyll contents as(as mg/g fresh weight, fw) of phytoplankton (*Spirodela polyrrhiza*, Duckweed) after exposure of different concentrations of industrial effluents of pulp and paper mill at varying exposure durations are shown in Table 1.

The maximum inhibition of total chlorophyll contents of phytoplankton was observed as compared to control values in 100% effluent concentrations after 28th days than other days (7, 14, 21) of exposure. It was minimum (0.242 mg/g fw) in the same day of exposure and with same concentration of industrial effluents.

**Biomass:** The changes in biomass (as g dry weight; dw) of phytoplankton (duckweed) after exposure of pulp and paper mill effluent concentrations (100, 75, 50, 25%) at varying exposure durations (7, 14, 21, 28 days) are shown in Table 2.

The maximum decrease in biomass of phytoplankton (duckweed, *Spirodela polyrrhiza*) was observed as compared to control values in 100% effluent concentration at 28 days of exposure than other days (7, 14, 21) of exposure. The biomass values were recorded minimum (0.315 g dw) in same industrial effluent concentration and in the same days of exposure.

**Table 1: Effect of different concentrations of pulp and paper mill effluents on total chlorophyll (mg/g fw) of phytoplankton (*Spirodela polyrrhiza,* Duckweed) at various exposure durations:**

| Effluent concentrations | Exposure durations | | | |
|---|---|---|---|---|
| | 7 days | 14 days | 21 days | 28 days |
| Control | 0.970 (0.00) | 0.978 (0.00) | 0.985 (0.00) | 0.994 (0.00) |
| 25% | 0.771* (20.52) | 0.649* (33.64) | 0.577* (41.42) | 0.546* (45.07) |
| 50% | 0.726* (205.15) | 0.534* (45.40) | 0.486* (50.66) | 0.426* (57.14) |
| 75% | 0.625* (35.57) | 0.405* (58.59) | 0.356* (63.86) | 0.307* (69.11) |
| 100% | 0.572* (41.03) | 0.334* (65.85) | 0.292* (70.36) | 0.242* (75.65) |

(Value in parentheses represent percent inhibition in Total Chlorophyll *p<0.01, significant)

**Table 1: Effect of different concentrations of pulp and paper mill effluents on biomass (g.fw) of phytoplankton (*Spirodela polyrrhiza,* Duckweed) at various exposure durations:**

| Effluent concentrations | Exposure durations | | | |
|---|---|---|---|---|
| | 7 days | 14 days | 21 days | 28 days |
| Control | 0.0425 (0.00) | 0.0438* (0.00) | 0.0451 (0.00) | 0.0462 (0.00) |
| 25% | 0.0403* (5.18) | 0.0391* (7.76) | 0.0386* (8.65) | 0.0380* (9.74) |
| 50% | 0.0395* (7.06) | 0.0383* (9.59) | 0.0375* (11.09) | 0.0362* (13.64) |
| 75% | 0.0368* (13.41) | 0.0366* (13.47) | 0.0353* (15.96) | 0.0336* (18.61) |
| 100% | 0.0342* (19.53) | 0.0341* (19.18) | 0.0327* (21.64) | 0.0315* 23.81 |

(Value in parentheses represent percent inhibition in biomass *p<0.01, significant).

In the present study, phytoplankton (*Spirodelapolyrrhiza*, Duckweed)showed accumulation of metals in different effluent concentrations at varying exposure durations. This accumulation was dependent on effluent concentrations and exposure durations and varied considerably from concentration to concentration as well as time of exposure. The metal accumulation enhanced significantly as metal contents increased in effluent concentration from 25% to 100% and at prolonged treatment duration from $7^{th}$ day to $28^{th}$ day. However, the steady state metal concentrations in test plants were obtained after third week (21 days) of exposure. The metal accumulation by duckweeds under the influence of metal chelators and pH has been reported by Singh *et al.*, (2002). However, the substantial accumulation of these metals by the aquatic plants may be due to the presence of tufts of profuse and fine root systems and well developed adventitious roots of the plants. Aquatic plants also accumulate metals due to occurrence certain metal binding complexes. Some workers have also explained the ability of aquatic plants to bio-accumulate metals due to occurrence of certain metal binding complexes. Free floating and rootless plants take up metals from the water medium by roots and / or leaves. Some workers have also demonstrated that the aquatic angiosperms extracts nutrient and heavy metals mostly from the water medium. There is evidence of increasing surface area and number of exposed metals binding sites in plants which ultimately increase the metal uptake capacity of plants. Metals presumably become complexes to the anionic sites associated with peptic substances with the plant cell wall, so called Donnan-free space. The active mechanisms for metal ion transport involves a kind of electrogenic pump supported by energy deriving from a metabolic process (e.g. $NADH_2$), and passive uptake dependent on pH, solid phase concentration, complex-forming ligands and ionic strength of water medium. Both these passive and active uptake are based on diffusion of metals through free space (e.g. pores) and by dissolution into the lipid layer. The absorbed (uptake) metals are translocated and accumulated to different plant parts, and consequently follows the scropetal water flow as

upward transport of these uptake ions in the xylem of plants. The mechanical harvesting of these metals-loaded plants may be the best method for abatement of metal pollution (Bajpai and Kumar, 2008).

Data given in Table 1 & 2 show that the effluent concentrations and exposure durations impart significant alterations in total chlorophyll contents and biomass of exposed aquatic plants. At higher effluent concentrations, total chlorophyll content decreased significantly on prolonged exposure durations in case of exposed duckweeds. The significant decrease in total chlorophyll contents may be possibly due to metal toxicity as well as under the influence of abiotic environment and uneven pH of exposure medium. The metal toxicity is supposed to reduce chlorophyll biosynthesis by reacting with SH group of α-aminolevulinic dehydratase. The inhibition of total chlorophyll content may be due to the toxic constituent-induced inhibition of electron transfer mechanism in photosystem-II (Abu and Randall, 1989).

The biomass of aquatic macrophytes was also sensitive to industrial effluents. The loss in biomass may be accomplished with the inhibition of chlorophyll contents of the duckweeds and toxicity imposed due to pollution stress caused by paper mill effluents.

## References:

[1] APHA (1998).Standard methods for the examination of water and waste water 24th Ed. Washington DC.

[2] Abu-ghran, Z.H. and C.W. Randall (1989).A proposed model for the anaerobic metabolism of short fatty acid in enhanced biological phosphorus removal. *J. Water Pollun. Contr. Fed.* 61: 1729.

[3] Bajpai, M. and R. Kumar.(2008). Nature of Alkins pulp and paper mill effluent of Kanpur district (U.P.).*SCITECH,* 3 (2): 60-62.

[4] Brinkhurst, R.O. (1966). The tubificide (Oligochacta) of polluted water, *Int. Verei.Theo. and Limno.Verno.,Verha*, 16: 854-859.

[5] Campbell, R.C. (1974). Statistics of biologists, Cambridge Univ. Press, London.

[6] Edmondson, W.T. ed (1993). Ward and Whipple's Fresh water biology 2nd ed. John Wily & Sons, N.Y.

[7] Hellawell, J.M. (1986). Biological indicators of fresh water pollution and environment management, Elsevier, London.

[8] Joy, C.M. (1990). Toxicity testing with fresh water algae in river Periyar (India) Bull. Environ. ContamToxicol., 45: 915-922.

[9] Keith, L.H. Telliard, W.A. (1979). Priority pollutants-A perspective view. Environ. Sc. Technol. 13: 416-423.

[10] Korner, S., J.E. Vermaat and V. Siemen. (2003). The capacity of duckweed to treat waste water. *J. Environ. Qual.* 32: 1583-1590.

[11] Nies, D.H. (1999). Microbial heavy metal resistance, Applied microbiology, Biotechnolgy. 51: 730-750.

[12] Pennak, R.W. (1989). Fish invertebrates of the U.S. Protozoa to mollusca John Wily & Sons INC.

[13] Reddy, M.R., Jivendra and S.C. Jain (1981).Paper mill effluent for sugarcane irrigation IAWPC Tech. Ann. 8: 129-196.

[14] Singh, A., Agrawal, S.B., Rai, J.P.N. and Singh (2002).Assessment of paper and pulp mill effluent on growth, yield and nutrient quality of wheat Abassi, A. (1985). Occurrence, toxicity and treatment of lignin in pulp and paper effluents.*J. Environ.*, 65: 01-08.

[15] Whitley, L.S. and Sikora, R.A. (1970).The effect of three common pollutant on the respiration rate tubificid worm *Wat. Pollut.Control*.Fed, 42, R, 57-R 56.