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Environmental Mercury Contamination By A Caustic-Chlorine Industry located At Ganjam, Odisha And Its Ecological Implications.

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Highlights

- The effluent and solid waste of M/S Jayashree Chemicals Pvt. Ltd is deadly toxic and contains elemental mercury, much above compared to permissible limits.
- The effluent significantly changed the physico-chemical properties of water available in the surrounding environment.
- The effluent significantly affected the growth and photosynthetic efficiency of the experimental alga tested.
- The pigments like, total chlorophyll, total pheophytin and carotenoid content significantly decreased with the increase in toxicant concentration.
- No recovery was observed in the exposed alga after 15days of exposure.
- However prolonged exposure to toxicant free medium helped that alga to recover from stress.

Abstract

The effluent and solid waste of M/S Jayashree Chemicals Pvt. Ltd is deadly toxic and contains elemental mercury, much above compared to permissible limits. The effluent significantly changed the physico-chemical properties of water available in the surrounding environment. The effluent was deadly toxic. The maximum allowable concentration was 0.44% effluent concentration and 4.15% effluent was dangerously toxic for alga to survive. The effluent significantly affected the growth and photosynthetic efficiency of the experimental alga tested. The pigments like, total chlorophyll, total pheophytin and carotenoid content significantly decreased with the increase in toxicant concentration. Little increment of chlorophyll content was noted at sub-lethal concentration. Partial recovery was noted when the alga was exposed to maximum allowable concentration. No recovery was observed in the exposed alga after 15days of exposure at higher concentrations. However prolonged exposure to toxicant free medium helped that exposed alga to recover from stress. Dilution of the effluent can be a probable solution but the enrichment of mercury in the algal body leading to bio-enrichment. Due to bio-enrichment of mercury in the algal body with time, there is every possibility of residual mercury impacting the alga, ultimately killing the alga.

Key words: Chlor-alkali industry, effluent, mercury, BGA, Growth, Pigment, Chlorophyll, Pheophytin, Carotenoid.

Introduction

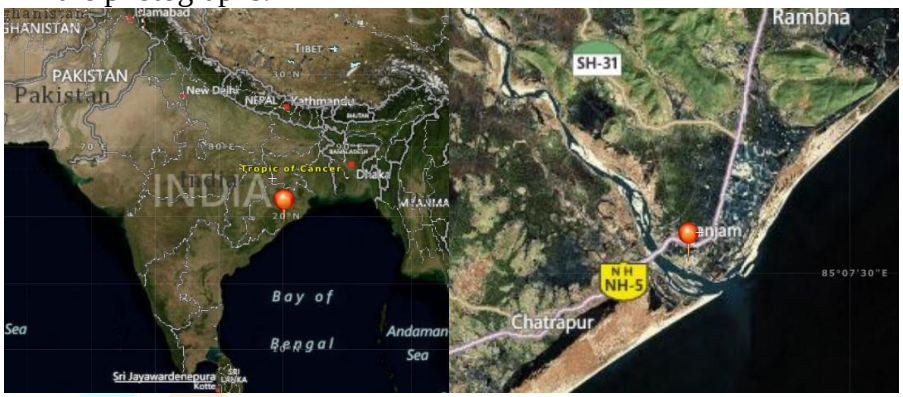
Environmental pollution by industrial wastes is not a new phenomenon. Industries release its process wastes into three environmental segments like air, water bodies and landmasses. Industry is responsible for creating a fantastic range of new chemicals every year all of which eventually find their way into the environment. Chemical industry in India has grown up phenomenally since independence. There is to-day about 4050 chemical factories in India. They release large quantities of chemicals in the form of gas, liquid and solid wastes, into the environment. Many of these chemicals are toxic and create pollution problem. The problem of toxic hazard has already reached alarming proportions in this country and is bound to grow with increasing industrialization. Pollution has become an acute problem in some developed and developing countries. India is the largest manufacturer of pesticides (killer chemicals used in agriculture) in the whole of

South Asia and Africa. As many as 139 organic chemicals, heavy metals like zinc, lead, chromium, copper, mercury and various other compounds are used in the manufacture of dyes only. Sundaresan *et al*. (1983) have given the growth of industries dealing with toxic chemicals and generating toxic and hazardous wastes during 1950s, 1970s and 1980s, in India. Industries which are known to produce potentially toxic and hazardous wastes are pesticides, dyes and pigments, organo-chemicals, fertilizers, non-ferrous metals, steel and Chlor-alkali plants.

Heavy metal contamination caused by either natural processes or by human activities is one of the most serious eco-toxicological problems. Since, photosynthetic plants function as the primary and principal entry point of heavy metals into the food chain leading to animals and ultimately to man. These heavy metals enrich, bioaccumulate, and biomagnify in the food chain and ultimately significant amount of these heavy metals are found in animal body. Heavy metal ions in small quantities are required for various physiological process and the normal functions of cells in plants and animals. Elevated levels of such metal ions are generally toxic and cause major damage to cells. In addition to the utilization of metal ions as essential elements, adjustment of intracellular levels of free ions by binding to macromolecules or other mechanisms is indispensable if cells are to protect themselves against excessive metal ions or changes in levels of such ions in the environment. Two different classes of cytoplasmic molecules participate in binding of metal ions and thus, in resistance to metal ions have been identified in various plant and animal cells. Extensive literature on the pollution of surrounding biota through the discharges of effluents and sludges from chlor-alkali industries is available (Suckcharoen, 1979 & 1980; Lodenius, 1980; Suckcharoen & Nourteva, 1982; Lodenius & Tulisalo, 1984 and Shaw *et al*., 1986). Studies on loss of mercury into the environment from the chlor-alkali industry were carried-out by Flewelling (1979), Bouveng (1968), Ross (1974) and Shaw *et al*., (1986). Turney (1970) reported contamination of river Detrait by mercury discharges from a Chlor-alkali plant similar studies were also conducted by Matida and Kumada (1969) on the river Agano of Japan on the Ohre River and Carlos (1979) on the Jab River of Papua, New guinea. Hattula *et al* (1978) indicated a severe pollution of the lake Paijane, which had received in past, the waste discharge from a caustic soda factory. Lodenius (1981) reported the mercury fallout in terrestrial vegetation around three Finnish Chlor-alkali works. Lodenius and Tulisalo (194) investigated the geographical spread of mercury around a chlor-alkali factory. Reports of mercury dispersion and contamination of Yatsushiro Sea, Lake Superior region (Glass *et al*., 1986), and eight northern Minnesota lakes (Sorensen *et al*., 1990) were available. Dylewski and Bradecks (1976) formulated methods of diminishing the contamination of natural environment by mercurial effluents and sludges of a chlor-alkali plant. Thangappan (1972) investigated on the pollution and its prevention, by chlor-alkali plants. One of the major users of metallic mercury is the chlor-alkali industry, in which chlorine and caustic soda (NaOH) are simultaneously produced by the electrolysis of brine solutions using a flowing cathode of metallic mercury. The sodium ion (Na^+) which amalgamates with mercury at the cathode is converted to NaOH in presence of water and the released mercury is recycled into the cell. Mercury emissions from chlor-alkali plants were assumed to consist mostly of Hg vapour as elemental (metallic) mercury (Hg^0) and bivalent Hg (Hg II as $HgCl_2$). About 65% of total chlorine production in the USA makes use of the mercury cells process (George, 1977) instead of diaphragm process. Mercury cells account for over 80% of chlorine / caustic soda production in Japan and the European countries (OECD report, 1974). In India at the beginning of 1984 the total installed capacity for caustic soda was 9, 09,900 tonnes per annum of which 88% came from mercury cells and the rest from diaphragm cells. In 1983, the total quantity of caustic soda made from mercury cells was 5, 14,700 tonnes (64.2% of capacity). It is estimated that the rate of mercury consumption is 394 gm/ton of caustic against the targeted value of 350 g Hg/ton, it is 90 g Hg/tonne in industrialized countries (Sundaresan, 1991).

The chlor-alkali industry M/S Jayashree Chemicals Pvt. Ltd., is situated at Ganjam, on the Bank of Rushikulya estuary about 1.5 km. Away from the sea, Bay of Bengal, on the East and 30 km. North of Brahmapur city on the south-eastern side of India at 84^0 53'E longitude and 19^0 16'N latitude. The industry was established in 1962 and started manufacturing caustic soda, liquid chlorine and hydrochloric acid by using a sheet of elemental mercury as a mobile cathode for the electrolysis of brine water (saturated sodium chloride solution) since August 1967. In the process of manufacture of chemicals, the factory discharges the effluent containing mercury and chlorine, into the estuary and deposits solid waste (brine mud, enriched with mercury) on the adjacent land areas. This addition of mercury was the primary contamination. The secondary contamination occurs through the chimney into the atmosphere and its fall out by the process of precipitation. All these discharges collectively caused a major environmental threat to crop production and also to fisherman engaged in fishing both in the river and also in the estuary. The important point to note is this industry has sufficiently dumped mercury in to the environment along with the liquid effluent discharged from the industry. In addition, the vaporized mercury from the cell house during operation by way of

evaporation, subsequent transportation and precipitation in the surrounding area is equally important which adds to the load of mercury in the area. The precipitation of mercury in the surrounding, the leached mercury from the solid wastes of the industry, the availability of mercury in the effluent which joins the river and the use of this river water for irrigation led to enrichment of mercury in the crop fields. This mercury affected the crop plants and in addition the non-target systems like the inhabitants of the crop fields e.g. blue-green algae (BGA) suffer the most. These tiny organisms are the inhabitants of the crop fields fix atmospheric nitrogen and increase the fertility of the crop fields and acts as biofertilizer suffer the most. Even there are crop fields at present where no BGA grows. The industry under study, its effluent canal and the solid waste dumping sites has been shown in the photographs.



(Photo-1 a, b: .Arc GIS explore Photograph showing India Map and location of M/s Jayashree Chemicals (P) Ltd. Located at Ganjam, Odisha, India.)

(Photo-2a, b. Showing Jayashree Chemicals Pvt. Limited and its waste channel)

The present piece of work was designed to analyze the effluent of the industry, geographical distribution of mercury in and around the industry; residual mercury in select plants collected from the contaminated area at Ganjam, Odisha and an experimental study related to detoxification of mercury contained waste / effluent by environmental chemicals and secondary metabolites of some select plants in the laboratory for toxicity testing and detoxification study in presence of blue-green alga.

Materials & Methods

Brief description of the test organism: *Westiellopsis prolifica*, Janet, the alga shows true branching with distinct prostrate and erect filaments and possesses a single row of cells. This resembles with other algal species of the family Stigonemataceae.

Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) and adopted by Sahu (1987) was most suitable basic culture for the growth of the test organisms. It was used as the basic culture solution in all the experiments in the present study. The experimental algal cultures were grown under controlled conditions of light and temperature inside a culture room. The culture flasks were kept in series on a culture rack, of glass plate with iron frame. Light was provided by means of white fluorescent tubes, connected at the backside of glass plate of each rack, which illuminates the upper glass surface at the intensity of 2400±200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and was maintained at 28± 2^0C. The culture flasks were regularly hand shaken twice a day to avoid clumping of the cells as well as their adhesion to the wall of the conical flasks. Dry weight of the alga in the culture flasks was estimated centrifuging in a refrigerated centrifuge (High speed centrifuge, Remi) at 8000 rpm for 10 minutes. The algal pellet was transferred to a pre-weighed glass cover slip. It was dried in an oven at 60^0C for 24 hours, cooled in desiccators and the final weight of the glass cover slip was recorded in a single pan electric balance (Dhona). The data were expressed as mean of 5 samples ± standard deviation in mg / 100 ml culture. The pigment contents of both control and exposed alga were estimated and calculated following the method described by Vernon (1960) and Davies (1976). Measurement of mercury in the samples followed

the basic principle of Wanntorp and Dyfverman (1955), which has undergone substantial modification in the light of recent development. The process is described in Analysis Methods for development of Mercury with Mercury Analyser MA 5800 A issued by ECII, 1981. All the obtained data was statistically computed and levels of significance verified.

Results

The effluent of the chlor-alkali industry was carefully collected and brought to the laboratory in glass containers and analyzed. After analysis of the mercury content present in the effluent samples brought in different containers were mixed and mean mercury concentration was estimated. The mixed effluent was sterilized by UV irradiation and stored in closed containers, to avoid biomethylation of mercury by microbes. A graded series of concentrations of the mixed effluent (now called as effluent) was prepared in different culture flasks along with the culture medium. Unialgal, pure axenic cultures of *Westiellopsis prolifica*, Janet was inoculated in an inoculation chamber (aseptic). The survival percentage and lethal concentration values were determined after 15 days of exposure. From the toxicity study and toxicity curve, the lethal concentration values were determined.

The above table indicated the lethal concentration and percent survival values deduced from toxicity study. This alga could tolerate up to 0.45% of the effluent concentration, where hundred percent survivals and no death was recorded. This concentration was selected and named as "A" concentration for the entire period of experimentation. The second selected concentration was 1.61% of the effluent, where 50% mortality and 50% survivability was marked and named as "B" concentration. The final and third selected concentration was 3.82% of the effluent, where 90% mortality and 10% survivability was marked and named as "C" concentration.

Table showing the percent survival (PS) value, lethal concentration (LC) value and effluent concentration.

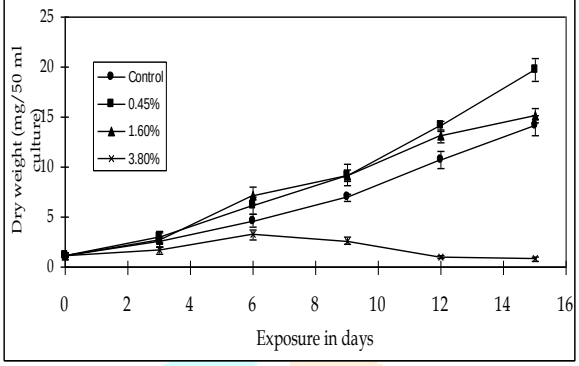
Lethal concentration value (LC)	Effluent concentration (%)	Percent Survival value (PS)
LC_o (A)	0.45	PS_{100}
LC_{10}	0.66	PS_{90}
LC_{50} (B)	1.61	PS_{50}
LC_{90} ©	3.82	PS_{10}
LC_{100}	4.15	PS_0
MAC	0.44	PS_{100}

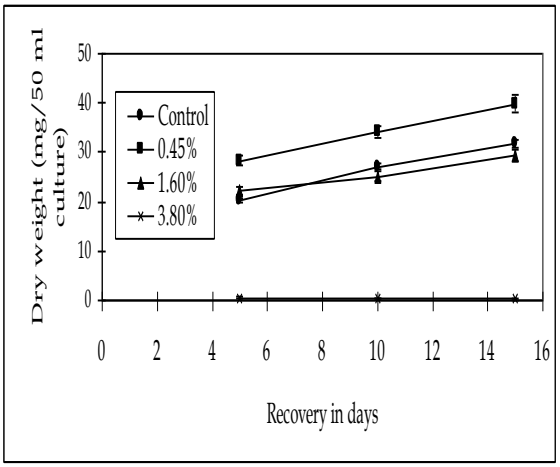
MAC was selected to study the effect of the effluent on the organism without killing the organism. The second selected concentration was 1.61% and the third selected concentration was 3.82% of the effluent where 50% and 90% mortality was observed, respectively. These three concentrations were selected to study the effect of the mercury contained effluent on the physiological and biochemical parameters of the exposed alga and the observed effects were compared with the control alga. The Table also indicated that with the increase in the effluent concentration, the percent survival decreased and the percent mortality increased. The PS values showed the existence of a negative correlation and LC values showed a positive correlation. With the increase in exposure period, the lethal concentration values decreased, showing a negative correlation. Experiments were conducted exposing the blue-green alga, only for 15 days. This alga showed maximum exponential growth up to 12 days and then the growth was stabilized and after 15 days of exposure, the declining trend in growth started. Once the exposed alga was transferred to toxicant free medium, in recovery studies, with fresh nutrient medium the growth rate was vitalized for another period of 15 days. After which, the aged alga need to be homogenized and recharged. Hence, experiments were planned to complete within 30 days. Fifteen days of exposure and 15 days of recovery was planned to test whether 15 day exposed alga could recover within the same period of recovery.

In the control set, a gradual and steady increase in optical density was marked. The optical density value measured at 530 nm, increased from 0.024±0.002 to 0.095±0.008 within 15 days, which indicated normal growth of the alga in the culture flasks. At 0.45% of the effluent concentration (A), the optical density of the exposed culture flasks increased significantly at all exposure periods, when compared to the control set. The optical density increased from 0.024±0.002 to 0.135±0.02 after 15 days of exposure. When the exposed alga was transferred to toxicant free nutrient medium, significant increase was recorded in the recovery period. At 1.6% of the effluent concentration (B set), the optical density value increased up to 9th day of exposure and then the optical density value declined steadily, when compared to the control value. The effluent exposed alga was transferred to toxicant free nutrient medium to study the extent of recovery. It was observed that the exposed alga could recover significantly. At 3.8% effluent concentration (LC_{90}), no increase in O. D. value was marked. Comparatively, the optical density value significantly declined, when

compared to control set, A set and B set. An initial insignificant increase followed by decrease in optical density value was marked up to 9th day of exposure. At higher exposure period, the optical density value remained steady. No recovery was recorded, when the exposed alga of the 'C' set was transferred to toxicant free medium for 15 days. Fig. 2A and 3 indicated changes in dry weight of the control and effluent exposed alga at different exposure and recovery periods. In the control set, significant growth of the alga was recorded. The dry weight increased from 1.16±0.22mg to 14.14±1.06mg within 15 days of exposure, showing significant growth of the alga.

Fig.2A. Showing changes in dry weight of control and exposed alga at different effluent concentrations and different exposure and recovery periods.





The control set alga, when recharged with fresh nutrient medium, still better growth was recorded during recovery period. At 0.45% effluent concentration, excellent growth of the alga was observed, when compared to the control set. The dry weight increased from 1.16±0.22mg to 19.66±1.14mg within 15 days of exposure. When compared to the control set, 39% increase over the control value was recorded (Fig. 2). At 1.6% of the effluent concentration, on 6th day of exposure 53.9% increase in dry weight over the control value was recorded. With the increase in exposure period, the percent increase, decreased significantly and 6.8% increase in dry weight was recorded on 15th day of exposure. The 'B' set exposed alga was transferred to toxicant free medium for recovery studies. An initial recovery followed by significant decrease in the dry weight was marked. On 15th day of recovery, 7.3% decrease in the dry weight was recorded. When we observe the data of Fig. 3, the dry weight increased from 15.11±0.73mg to 29.32± 1.32mg within 15 days but when we compare the obtained value with the control set, decrease in dry weight was marked (Fig. 2).

Fig. 3. Showing percent change in dry weight of exposed alga at different concentrations of the effluent of the chlor-alkali industry, at different exposure periods when compared to control.

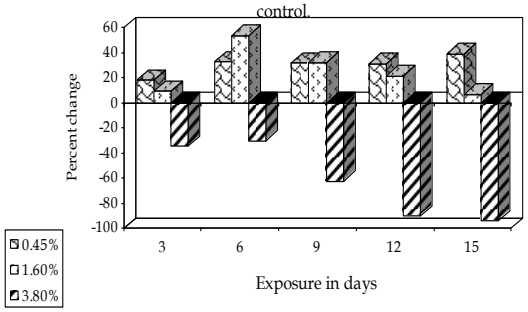


Fig. 4.Showing changes in total chlorophyll content in control and effluent exposed alga at different concentrations of the effluent and different exposure and recovery periods.

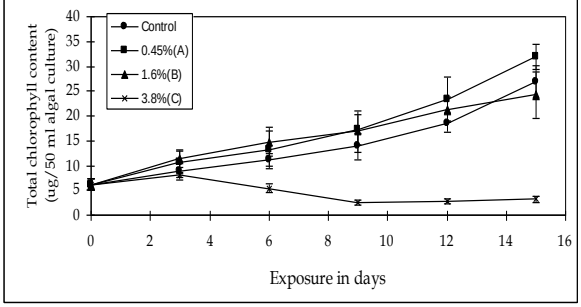


Fig. 5. Showing percent change in total chlorophyll content in effluent exposed alga at different exposure periods when compared to control.

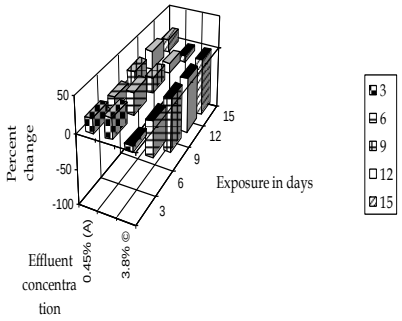
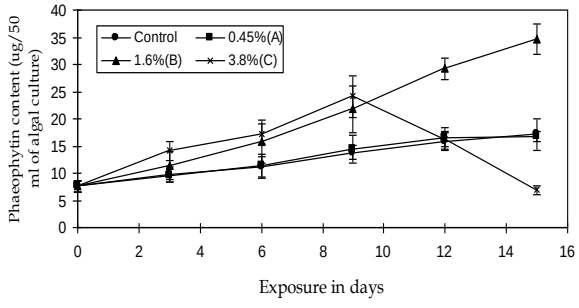


Fig. 6. Showing changes in phaeophytin content in control and effluent exposed alga at different concentrations of the effluent and at different exposure and recovery periods.



At 3.8% effluent concentration, the dry weight increased, insignificantly up to 3.22±0.52mg on 6th day of exposure and then with the increase in exposure period, the dry weight of the alga in exposed flasks declined

and 1.01±0.14mg was recorded on 15th day of exposure. No recovery was marked, when the exposed alga was transferred to toxicant free medium. A maximum of 94.1% decrease in dry weight, when compared to the control value was marked and the percent decrease reached maximum to 99.1% on 15th day of recovery (Fig. 3). The correlation coefficient indicated the existence of non-significant correlation between the dry weight vs. effluent concentrations. The correlation coefficient analysis conducted between the dry weight vs. exposure period at different effluent concentration, indicated the existence of significant positive correlation in control (r=0.982, P≤0.01), A set (r=0.981; P≤0.01) and B set (r=0.993, P≤0.001) and non-significant negative correlation (r=-0.927, P=NS) in the C set. The correlation coefficient calculated between residual mercury concentration vs. dry weight, showed positive and significant correlation in A set (r=0.853, P≤0.05) and in B set (r=0.945, P≤0.01) and non-significant negative correlation in the C set (r=-0.105, P=NS). Fig. 4 showed the changes in total chlorophyll content in control and effluent exposed alga at different exposure and recovery periods. The chlorophyll content increased from 6.2±1.1 to 26.8±3.4µg/50 ml culture within a period of 15 days, in the control set. Whereas, at 0.45% effluent concentration (A), the total chlorophyll content significantly increased with the increase in exposure period and the value of each exposure period was more than the control value. Both the control set and A set showed the existence of a significant positive correlation. On 12th day of exposure 27.2% and on 15th day of exposure 19% increase was marked when compared to the control value. At 1.6% effluent concentration (B), the total chlorophyll content increased significantly, when compared to the control set & A set up to 6th day of exposure. The total chlorophyll content increased with the increase in exposure period up to 15th day of exposure and also in the recovery period. But the values were less than the control values. On 15th day of exposure 9.7% decrease in the parameter was recorded. In recovery studies, we observed an initial recovery by 22.1% then the percent decrease, increased and a maximum of 13.6% decrease was recorded on 15th day of recovery (Fig. 5). At 3.8% effluent concentration, an initial insignificant increase followed by decrease on total chlorophyll content was recorded. On 15th day of exposure, 88.1% decrease in total chlorophyll content when compared to the control value was marked. When the exposed alga (C set) was transferred to toxicant free media, an insignificant recovery by 7.1% was recorded on 15th day of recovery (Fig. 5). The two way analysis of variance ratio test conducted for the total chlorophyll content data indicated the existence of significant difference between rows and columns.

Fig. 7. Percent change in phaeophytin content in effluent exposed alga at different exposure periods when compared to control.

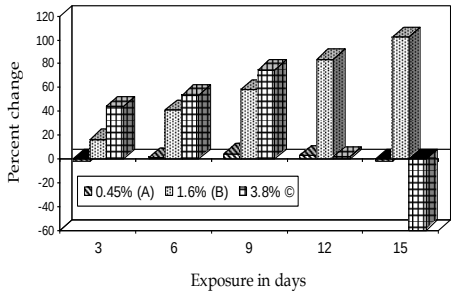


Fig. 8: Showing changes in carotenoid content in control and effluent exposed alga at different effluent concentrations and at different exposure and recovery periods.

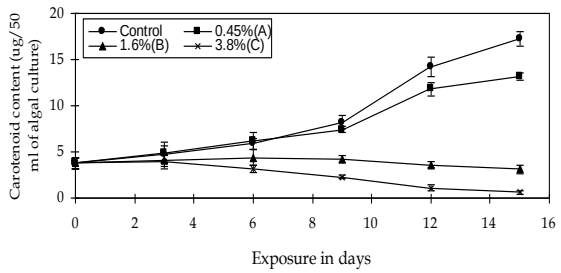
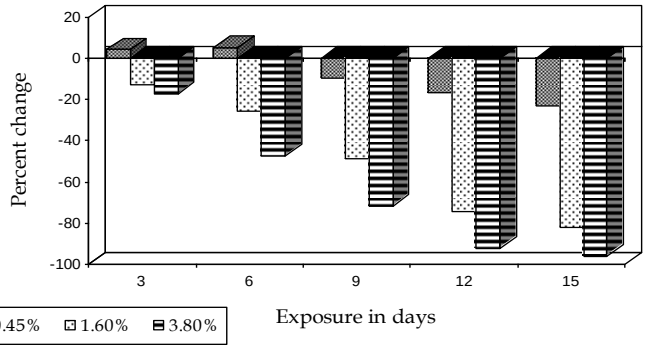


Fig.9: Percent change in carotenoid content in effluent exposed alga at different exposure periods when compared to control.



Changes in phaeophytin content in control and effluent exposed alga at different exposure and recovery periods has been presented in Fig. 6. The phaeophytin content increased steadily with the increase in exposure period and recovery period in the control set, showing the existence of a positive correlation between exposure period and phaeophytin content. At 0.45% effluent concentration (A), the phaeophytin content showed an insignificant variation, when compared to the control alga. The pigment content increased

at few exposure periods and decreased on 3rd and 15th day of exposure. During recovery studies, the rate of depletion of the pigment though increased, but the values were significantly less than the control set (Fig. 6). In the B set, interestingly the phaeophytin content increased from 7.6±1.1 to 34.6± 2.8µg/50 ml of algal culture within 15 days of exposure period. The 15th day of exposure value was more than double of the respective control value, where 102.3% increase was recorded. But during recovery period the pigment content decreased interestingly and on 15th day of recovery, 43.9% decrease was noted (Fig. 7). In the C set, the phaeophytin pigment increased with the increase in exposure period up to 9th day of exposure, then the phaeophytin content decreased significantly and 59.7% decrease was recorded on 15th day of exposure. When the exposed alga was transferred to toxicant free medium 85.8%, 93.3% and 85.1% decrease, when compared to the control value was recorded on 5, 10 and 15th day of recovery, respectively. The two way analysis of variance ratio test conducted on the changes of phaeophytin content indicated the existence of significant difference between columns and no significant difference between rows. The correlation coefficient analysis conducted between effluent concentration vs. phaeophytin content indicated the non-existence of significant correlation (P=NS) at all exposure periods except on 3rd day of exposure, where a positive and significant (r=0.990; $P \leq 0.01$) correlation was marked. The correlation coefficient analysis between days of exposure vs. phaeophytin content indicated the existence significant positive correlation in the control set (r=0.996, $p \leq 0.001$), A set (r=0.986, $P \leq 0.001$) and B set (r=0.994, $P \leq 0.001$) and negative non-significant correlation in the C set (r=-0.080, P=NS). The correlation coefficient analysis conducted between residual mercury concentration vs. phaeophytin content indicated the existence of significant positive correlation in the A set (r=0.927, $P \leq 0.01$) and B set (r=0.964, $P \leq 0.01$) and non-significant (P=NS) positive correlation the C set. The carotenoid pigment content increased from 3.8±0.6 to 17.2± 0.8µg/50 ml of algal culture within 15 days of exposure in the control set (Fig.8). When the control set was transferred to toxicant free medium for another period of 15 days, the rate of carotenoid content biosynthesis reduced in the control set. However, the value reached to 76.8±1.3µg/50 ml of algal culture. At 0.45% of effluent concentration (A), an initial insignificant increase by 4.3% and 5.1% on 3rd & 6th day of exposure and consequent decrease by 9.8%, 16.9% and 23.3% on 9, 12 and 15th day of exposure, when compared to the control value was marked, respectively. The exposed alga was transferred to toxicant free medium for recovery studies. A partial recovery was recorded in the A set (Fig.8). At 1.6% effluent concentration, the carotenoid content increased up to 6th day of exposure, and then the carotenoid content significantly declined, when compared to the control value. All the observed values were significantly less than the control values and a maximum 81.9% decrease was noted on 15th day of exposure. When the exposed alga was transferred to toxicant free medium, no recovery was marked. Rather the carotenoid content further depleted and 85.6%, 86.3% and 85.4% decrease was recorded on 5th, 10th and 15th day of recovery, respectively (Table-9). At 3.8% effluent concentrations, the carotenoid content significantly declined at all exposure period, showing the existence of a negative correlation. The percent decrease of the pigment content increased with the increase in exposure period and 96.3% decrease was recorded on 15th day of exposure. No significant recovery was marked in the recovery studies, when the exposed alga was transferred to toxicant free medium (Fig.9). The correlation coefficient analysis conducted between residual mercury concentration vs. changes in carotenoid content indicated the existence of significant positive correlation (r=0.842; $P \leq 0.05$) in the A set, non-significant negative correlation (r=-0.597, P=NS) in the B set and significant negative correlation (r=-0.926, $P \leq 0.01$) in the C set. The correlation coefficient analysis conducted between exposure period vs. carotenoid content indicated the existence of significant positive correlation (r=0.957, $P \leq 0.01$) in the control set, in A set (r=0.968, $P \leq 0.01$), negative non-significant correlation (r=-0.549, P=NS) in the B set and significant negative correlation (r=-0.974; $P \leq 0.01$) in the C set. The correlation coefficient analysis between carotenoid content vs. effluent concentration indicated non-significant negative correlation on 3, 12 and 15 days of exposure and significant ($P \leq 0.05$) negative correlation on 6th and 9th day of exposure. Significant changes in the exposed spectra were marked, when compared to the control spectrum. The gradual depletion of peak height of chlorophyll and phaeophytin was marked and at the highest concentration of the effluent, these pigment peaks at 649, 665, 655 and 666nm depleted significantly indicating destruction of chlorophyll and phaeophytin in exposed blue-green alga. No shift of peak in the pigments was marked. The ratio values significantly increased (ratio value between the highest peak/lowest peak at the range of 640-670nm), when compared to the range value of 1.5 to 1.8. This higher peak ratio value indicated the presence and effect of toxicant in the medium causing extensive damage to the exposed system under study.

Discussion

Microbes play an important role in the movement of mercury in nature, especially in the soil, sediments and aqueous environments. Microbes play a crucial role in methylation of mercury in to its organic derivatives in natural environments. The main result of microbial action on mercury seems to be its volatilization, whether it involves reduction of the mercuric ion or methyl or phenyl mercury compounds to volatile Hg^o, or whether it involves conversion of the mercuric ion to dimethyl mercury or of the phenyl mercuric ion to diphenyl mercury. The mercuric ion (Hg^{2+}) may be methylated by bacteria and fungi to give methyl mercury [(CH_3) Hg^+], which is water soluble. Some bacteria may further methylate methyl mercury and convert it to dimethyl mercury, which is volatile and escapes into the air. Upon weathering, mercuric sulphide (cinnabar, HgS) is converted to mercuric sulphate and becomes disseminated in soil and water. Bacteria, fungi, and humic acid reduce Hg^{2+} and cause a wider range of distribution. Methyl mercury, as well as phenyl mercury, may again be enzymatically reduced to volatile Hg^o by bacteria. This causes detoxification of soil. Phenyl-mercury, which is usually anthropogenic in origin, may be reduced by soil bacteria and converted to diphenyl mercury. Biogenic H_2S may convert the mercuric ion to HgS, again under anaerobic conditions. Accumulation of heavy metals in agriculture soils has become a major concern for food crop production. Of these metals, mercury is recognized as one of the most hazardous elements, which is not essential for plant growth. Since mercury is known to be easily taken up by plants and translocated within the plant (Sahu, 1987), a clear understanding of its bioavailability to plants is essential for reducing the mercury entry into the food chain with potentially harmful effects on human health. It is well known that mercury concentrations in plant tissues are directly related to the concentration of plant available mercury in soil (Shaw, 1987). Wiener *et al.*, (2012) studied the risks of mercury in yellow perch a species important in trophic transfer of methyl mercury in the Laurentian Great Lakes region. Wu and Wang (2012) studied the accumulation, sub-cellular distribution and toxicity of inorganic mercury and methylmercury in marine phytoplankton. Mercury exerts its toxicity by binding with sulphydryl groups and producing oxidative stress. Different algae employ different physiological strategies and exhibit different sensitivities to mercury exposure. It is known that phytoplankton use at least three strategies to alleviate mercury toxicity. The first strategy is metal exclusion, which assumes that a higher metal tolerance is related to a smaller metal-reactive cell surface (different and / or fewer ligands per cell surface units). The second strategy is mercury evaporization. For example some algae produce dissolved gaseous mercury to reduce metal internalization. The third strategy is metal intercellular sequestering (Davies, 1976), which assumes that metal binding with some sulfur-rich complexes averts an attack on sensitive sites within the cells. Wu and Wang (2012) out right rejected the above ideas as little experimental evidence was not available to them. It has been reported that some algae can evaporate mercury and also can accumulate mercury in their tissues and in the process decontaminate mercury contaminated environments. The present study indicated very interesting information. The impact of mercury in the toxicant on the study object clearly indicated the dual nature of mercury. Many workers including Gorden and Prouse (1973), Dustan *et al*. (1975) and Gaur & Kumar (1981) have observed a stimulation of algal growth by crude oils and hydrocarbons. Kashyap & Gupta (1981), Rath *et al*. (1986), Sahu (1987), Sahu *et al*. (1988) and Shaw *et al*. (1988) observed stimulation by different mercurial compounds however the mechanism of growth stimulation cannot easily be explained. Such stimulation might be due to the presence of some growth regulating compounds (Gorden and Prouse, 1973) and / or trace elements (Hufford, 1971). The effluent of the chlor-alkali industry may only contain an insignificant or negligible amount of trace elements or growth regulating compounds in it. Hence we do not agree at this stage with the reports of Hufford (1971) supported by Gorden and Prouse (1973) and Dustan *et al*. (1975). Some workers suggested the uptake and interference of the toxicant with the cellular biochemicals which might be producing some growth regulating chemicals or chemicals which can induce stimulation in growth as the possible mechanism for the growth stimulation (Sahu, 1987). O'Brian & Dixon (1976) suggested the uptake and metabolization of oil constituents as the probable mechanism of growth stimulation in exposure to oil. However, Gaur & Kumar (1981) showed their inability to explain the mechanism of growth stimulation. Unlike the reports of Gaur & Kumar (1981), here, we have observed an increase in the final yield following effluent treatment. Since, an increase in the final yield in the effluent treated alga was observed in the present study, the possibility of stimulation either by the absorbed metal or by some other mechanisms looks more appropriate than the metabolization of the effluent with mercury. The only speculation left, to account for the enhancement; by Gaur & Kumar (1981) was the likely presence of some growth regulator(s), which might have influenced climax of the test alga. This type of speculation is not acceptable at this stage and is not valid for this type of effluent treatment, where most of the fractional constituents at higher concentrations, are independent poisons/toxicants and in combination might show antagonistic or synergistic effects. The peculiar behavior of the algal organisms under stress to avoid the

stress is an interesting feature in toxicological studies. Due to exudation, the medium might be changing or the exuding chemicals might be reacting with mercury and the other chemicals of the effluent forming a hard cyst, which must be providing an adhering surface for the heavy metal. The cyst might be restricting the heavy metal's entry into the cell, due to the formation of a barrier. It has been reported that with the increase in exposure period, the mercury concentration increased in exposed algae (Sahu, 1987). The same authors opined that with the increase in residual mercury concentration the growth decreased significantly. In the present study, at higher exposure period, depletion in growth rate was observed. Growth rate studies by optical density method showed inconsistent data in exposed cultures. However, consistency was observed in the dry weight measurement studies. Dry weight has been considered by good number of workers as a parameter of growth. The change observed in optical density study exactly does not reflect the real changes induced by the pollutant, but an approximation can be made out of this data. Since growth is a summation of all cellular metabolisms, any inhibition of growth reflects toxic effects on a number of metabolic processes. Also, the use of growth rates allows one to observe, if the bioassay organism has the capability of recovery from the toxic effect, over extended periods of time. There is the disadvantage that as the algal cells increases in number, the concentration of the toxicant per cell decreases from the original value (Trevors and Vedelago, 1985). Walsh & Alexander (1980) demonstrated that algal species that were sensitive to pesticides in monoculture were less sensitive in the presence of resistant species, perhaps because, the resistant species grew quickly and absorbed the pesticide, thus, reducing its concentration in growth medium. Industrial waste caused a reversal in species numerical dominance in mixed algal cultures.

Photosynthetic pigments of the plant systems play a vital role in trapping solar energy. The pigments are known to participate in generation of energy for CO_2 fixation (Kashyap and Gupta, 1981). The chlorophylls have long been recognized as the primary light acceptors in plants and they are invariably present in every organism, which carries out photosynthesis with absorption of CO_2 and evolution of molecular oxygen. Shaw (1987) reported that with the waste treatment, the chlorophyll content increased to a great extent in lower concentrations and virtually no decrease in the level in higher concentration. Zingmark & Miller (1975) and De Filippis & Pallaghy (1976b) reported that heavy metals inhibit photosynthesis. De Filippis & Pallaghy (1976), Rai & Khatoniar (1980) and Rai *et al*. (1981b) reported that heavy metals reduce chlorophyll content. De *et al*. (1985) suggested that at 20ppm the Hg(II) lowered the chlorophyll by decreasing the synthesis of chlorophyll, as well as possibly by increasing the synthesis of chlorophyll as well as possibly by increasing chlorophyllase activity in *Pistia*. De Filippis and Pallaghy (1976) reported that all the heavy metal solutions inhibited the rate of chlorophyll synthesis in the cultures, with PMA and $ZnCl_2$ causing the greatest and least inhibition respectively. Conway (1978) found a significant lowering of pigment content in *Asterionella formosa* after addition of cadmium. Many studies on lead point to its weak toxic effect on photosynthesis, respiration and cell division of various algae. The algal cells lose their capacity to evolve oxygen in its absence (Cheniae and Martin, 1968). Shaw (1987) opined that when mercury has been reported to be toxic, an increase in the level of chlorophyll in the alga exposed to the effluent highly concentrated with mercury could hardly be explained. During the experimental period, bleaching of the effluent exposed algal filaments was marked at higher concentrations, when compared to control algal filaments. At lower concentration of the effluent, stimulatory growth was noticed, when compared to the control set. From the photograph, it was clearly visible that colour of the mass algal culture showed variation in colour at different concentrations of the effluent. At higher effluent concentrations, the bleached filaments remained as such with out showing any change. Interestingly, during recovery period, when the effluent exposed alga was transferred to effluent free medium, tiny coloured bead like structures appeared after 20 days of recovery. After 30days of recovery, further appearance of coloured beads were noted and ultimately, the entire bleached algae in the recovery culture turned coloured, indicating strongly that during exposure period, the exposed alga was not dead but by some mechanism avoided the stress period. Carotenoids play a vital role as a protector of photosynthetic tissues against photosensitized oxidation. The decrease in carotenoid content in algal cells exposed to heavy metal stress lead to a decrease in protection from the stress to the photosynthetic tissue. The ratio of carotenoid to chlorophyll has long been identified as a valuable parameter for defining environmental conditions unfavorable for algal growth. When the nutrients in the medium are exhausted or a toxicant is introduced into the medium, this ratio rises due to decrease in chlorophyll content. Chlorophyll and carotenoid contents were extrapolated for the measurement of growth of phytoplankton. It was reported that addition of mercuric chloride to the natural phytoplankton reduced chlorophyll content for which the growth was inhibited and which might have limited the primary production. The wide spread occurrence, as well as certain chemical properties of chlorophyll pigments *in vivo* suggest that these pigments play an active role in photosynthesis functioning as a photo enzyme and the mercurial compounds are toxic for the biosynthesis of chlorophyll pigments. The phaeophytin content of an

algal system is very important, since any unfavorable change in the environment inhabited by the alga is reflected through a change in phaeophytin level. Chlorophylls are known to be converted to phaeophytin as a consequence of exposure to weak acids, by replacement of Mg^{2+} with two atoms of hydrogen and thereby changing the spectral properties (Rao and Le Blanc, 1966 and Singh & Singh, 1984). Degradation to phaeophytin might be the first step towards break down of chlorophylls. This is clearly evident from the fact that increased levels of phaeophytin are found in toxicant exposed algae. At higher concentrations of toxicant phaeophytin are further broken down, showing a decrease in phaeophytin level (Sahu, 1987). The conclusion was obviously drawn from the fact that the only difference between the control and exposed cultures was the effluent applied at different concentrations selected during toxicity studies. No shift of peak was noted in the exposed cultures, which also indicated that the pigments of the exposed cultures did not show any variation in their physical and chemical properties or structure. During the experimental period, bleaching of the effluent exposed algal filaments was marked at higher concentrations, when compared to control algal filaments. At lower concentration of the effluent, stimulatory growth was noticed, when compared to the control set. From the photograph, it was clearly visible that colour of the mass algal culture showed variation in colour at different concentrations of the effluent. At higher effluent concentrations, the bleached filaments remained as such with out showing any change. Interestingly, during recovery period, when the effluent exposed alga was transferred to effluent free medium, tiny colored bead like structures appeared after 20 days of recovery. After 30days of recovery, further appearance of colored beads were noted and ultimately, the entire bleached algae in the recovery culture turned colored, indicating strongly that during exposure period, the exposed alga was not dead but by some mechanism avoided the stress period.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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