



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 Caustic-chlorine is deadly toxic and contains elemental mercury, much more than the permissible limits..
- The mercury content was high at discharge point and decreased with increase in distance along the effluent flow canal.
- The effluent changed the physico-chemical properties of environmental water.
- The effluent significantly affected the growth of the experimental alga tested.
- The effluent showed dual behavior of stimulation at sub-lethal concentration and inhibition of growth at higher concentration of effluent.

Abstract

The study indicated that the surrounding area of Jayashree Chemicals Pvt. Limited, Ganjam, Orissa a caustic soda plant released huge amount of mercury into the environment in the past 4-5 decades. Evaporated mercury from the Mercury cell house contaminated the surrounding biota leading to elevated mercury levels beyond prescribed limit was the major concern during analysis period. The effluent of a Caustic-chlorine industry (M/S Jayashree Chemicals Pvt. Ltd., Ganjam) showed significant variation and difference in action at different concentrations is clearly evident from tables and figures explained in the result chapter. The highest concentration (concentration Z, 3.8% Effluent) showed typical mercurial toxic symptoms, beyond which survival of the alga became extremely difficult and at times, impossible under laboratory controlled conditions. The alga, *Westiellopsis prolifica*, Janet is more tolerant and less sensitive to toxicants. The analysis of variance ratio tests and correlation coefficient analysis carried out for all parameters studied indicate clearly that the toxicant, effluent containing mercury, is extremely toxic to blue-green alga. At higher concentration, all most all the growth parameters studied showed declining trend in exposed cultures, when compared to control cultures. At lower concentration of effluent (0.45% effluent), all the growth parameters showed an increasing trend in the exposed culture, when compared to control culture. Hence, it can be concluded that the toxicant (mercury contained) showed dichotomous behavior. At lower concentrations, the toxicant is growth regulatory and at higher concentration, the toxicant is growth inhibitory. The observed changes in the exposed cultures when compared to control cultures were only due to the toxicant, effluent. Hence, it can be concluded that the damage caused in the exposed system, at higher concentration, was only due to the toxicant and most important component of the effluent being mercury. Care should be taken to treat the effluent before discharge in to natural environments.

Key words: Caustic-chlorine industry, Mercury, Effluent, Cyanobacteria, dry weight, pigment.

Introduction

Today the main reason of environmental pollution is the over confidence of man who thought that just because of his brain he is the master of nature. The mad rat race between the developed countries and developing countries leads to rapid progress in agriculture, industry, transportation and technology. Such activities of man had created adverse effects on all living biota in the system. Environmental pollution by industrial wastes is not a new phenomenon. Industries release its process wastes into three environmental segments like air, water bodies and land masses. Pollution of surrounding biota through the discharges of effluents and solids wastes from chlor-alkali industries have been amply demonstrated (Hartung & Dinaman, 1974; Shaw *et al.*, 1986; Hall *et al.*, 1987). Gardner *et al.* (1978) analyzed sediment, plants and animals from a salt marsh contaminated by a chlor-alkali plant. Reports of mercury dispersion and contamination in Yatsushiro Sea (Kudo & Miyahara, 1984), Lake Superior region (Glass *et al.*, 1986) and Northern Minnesota lakes are available. Shaw *et al.* (1985 & 1986a, b) reported the residual mercury accumulation in different biotic systems available in and around a Chlor-alkali industry. The monitoring and assessment of mercury pollution in the vicinity of a chlor-alkali plant has been done by Sahu (1987) and Shaw (1987). There is no doubt that Chlor-alkali industries are seriously polluting the surrounding environments. But comparatively very few reports are available on the toxicity and toxicological effects of chlor-alkali industrial wastes on different biotic systems. Shaw *et al.* (1988) and Rath (1991) studied the toxicity of the effluent of a chlor-alkali industry containing mercury on blue-green algae and different biota available in the contaminated sites. The addition of mercury via effluent and solid waste 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 industry changed its mercury cell technology to Membrane technology from 2012 onwards where no mercury is used in the process. The present study aims first at understanding the impact of mercury as a waste on the physico-chemical property of the effluent and natural waters. Second, experimental study of effluent on the growth of a cyanobacterium under laboratory controlled conditions.

Materials & Methods

Location of the industry:

The chlor-alkali industry M/S Jayashree Chemicals Pvt. Ltd., is situated at Ganjam, on the Bank of Rushikulya estuary about 1.5km 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 $84^{\circ} 53'E$ longitude and $19^{\circ} 16'N$ latitude.

Field analysis: The physico-chemical analysis of the water and effluent sample was carried out following the protocols of APHA (1985). The pH of the samples was measured by portable pH meter (Systronics) Temperature was recorded by minimum-maximum thermometer. Dissolved oxygen was measure by modified Winkler's method (APHA, 1985) on spot. The samples were brought in glass bottles in ice box to the laboratory for analysis.

Experimental procedure:

Each time the effluent before use was thoroughly hand-shaken and the suspended particles were allowed to settle. The decanted clear supernatant was used for the experiments. Graded concentrations of the effluent were prepared using the culture medium as diluent and were expressed in terms of per cent (v/v). Three concentrations of the effluent viz. 0.45%, 1.6% and 3.8% were selected from the algal bioassay and were named as treatment 'A', 'B' and 'C' respectively in all the experiments. Treatment 'A' was the maximum allowable concentration (MAC), treatment 'B' was LC₅₀ and treatment 'C' was LC₉₀ deduced from toxicity test. Fifty milliliter each from the three concentrations of the effluent amended with nutrient medium and normal culture medium as control, were taken in 100 ml Borosil / Corning conical flasks stoppered with non-absorbent cotton plugs. They were irradiated with UV for 10 minutes and then inoculated with 1 ml of homogeneous suspension of the alga to each flask. For each concentration of the effluent and control, eight sets of flasks were taken and each one of them was again replicated either 3 times or 5 times, as per the requirement of the experiment. Estimation of different parameters was made after the 3rd, 6th, 9th, 12th and 15th days of exposure. The algal cultures were centrifuged in a refrigerated centrifuge at 20°C and 5000 rpm, for 10 minutes. The algal mass in the pellet was thoroughly washed in double distilled water and centrifuged again before any estimation. After 15 days of exposure, three sets of flasks from each treatment and control, were centrifuged, the algal residues were thoroughly washed in double distilled water, centrifuged again and were re-suspended in freshly prepared sterilized culture medium (50 ml in each 100 ml flask).

In order to maintain proper concentration of the effluent during the period (15 days) of exposure, the medium in all the treatments was changed at 5 (five) days interval under aseptic conditions, except in case of nitrogen estimation studies. In nitrogen estimation study, the exposed solutions were not changed but at 5 day

interval, the exposed solutions were recharged with fresh limited toxicant only. Allen & Arnon's (1955) nitrogen free medium with trace elements (Fogg, 1949) was found most suitable for the growth of the alga and used as the basic culture solution throughout all the experiments. Growth was estimated by measuring the dry weight of the alga after centrifuging the culture in a refrigerated centrifuge at 20°C and 5000 rpm for 10 minutes. The algal pellet was then transferred to pre-weighed glass weighing bottle. The weight in bottle with algal mass was dried in oven at 70°C for 48hrs cooled in desiccators and the weight was recorded in a single pan balance. For determination of residual mercury the algal pellet was collected and digested in a Bethge's apparatus with 10 ml of acid digestion mixture (1:3 Conc. H₂SO₄ and HNO₃). The amount of mercury present was estimated in a cold vapour atomic absorption 'Mercury Analyser' (MA 5800, ECIL (1981), India). The amount of mercury in the samples was computed from the standard graph. The residual mercury contents were expressed in µg of mercury per 50 ml algal culture. All the obtained data was statistically analyzed.

Results

The different characteristics of the collected effluent from the effluent channel and from the storage tank were as follows:

Table-A.2. Physico-chemical Properties of the effluent collected from the effluent channel immediately living the industry:

Sl. No	Parameter (mg/liter)	Data mean of 5 estimations ± S.D.	Sl. No	Parameter (mg/liter)	Data mean of 5 estimations ± S.D.
1	Temperature (°C)	30.2 ± 1.5	7	BOD	31.3 ± 1.8
2	pH	9.8 ± 0.2	8	COD	321.4 ± 9.2
3	Alkalinity (as CaCO ₃)	258.2 ± 2.4	9	Suspended solids	146.3 ± 34.5
4	Hardness (as CaCO ₃)	481.8 ± 17.4	10	Total nitrogen	5.4 ± 0.7
5	Chlorinity	1127.6 ± 48.4	11	Total phosphorus	0.23 ± 0.08
6	Dissolved oxygen	3.1 ± 0.6	12	Total mercury	3.12 ± 0.13

The temperature of the effluent at the discharge point was high and ranged between 28.5°C to 31.8°C. The pH of the effluent was alkaline and the value recorded was 9.8±0.2. The alkalinity was 258.2±12.4 as CaCO₃ in mg l⁻¹. The hardness of the effluent sample was 481.8±17.4 as CaCO₃ in mg l⁻¹. The Chlorinity was 1127.6±48.4mg l⁻¹. The dissolved oxygen content was low and ranged within 3.1±0.6 mg l⁻¹. The BOD and COD values were 31.3±1.8mg l⁻¹ and 321.4±9.2mg l⁻¹, respectively in the effluent collected from the discharge point. The suspended solids were low but significant and the value was 146.3±34.5 mg l⁻¹. A small amount of 5.4±0.7mg.l⁻¹ total nitrogen was also noted to be present in the effluent. An insignificant amount of phosphorus was recorded in the effluent. However, a significant amount of mercury was recorded in the effluent discharged from the industry. An amount of 3.12±0.13 mg of mercury l⁻¹ was noted in the effluent sample (Table-A.2). The temperature of the effluent at the mid point was low when compared to delivery point and ranged between 29.8±1.2°C. The pH of the effluent was alkaline and the value recorded was 9.8±0.2, no change on pH was recorded when compared to delivery point. The alkalinity was 254.2±6.8 as CaCO₃ in mg l⁻¹. The hardness of the effluent sample was 406.2±14.2 as CaCO₃ in mg l⁻¹. The Chlorinity was 1098.6±22.9mg l⁻¹. The dissolved oxygen content was little high when compared to discharge point and ranged within 3.3±0.2mg l⁻¹. The BOD and COD values were 31.8±1.6mg l⁻¹ and 301.4±12.8mg l⁻¹, respectively in the effluent collected from the mid point and no significant difference was marked with the distance. The suspended solids were low but significant and the value was 106.5±24.3mg l⁻¹. A small amount of 3.4±0.4mg l⁻¹ total nitrogen was also noted to be present in the effluent, which was less than earlier value. An insignificant amount of phosphorus was recorded in the effluent. However, a significant decrease in the amount of mercury was recorded in the effluent at mid point when compared to the delivery point from the industry. An amount of 2.68±0.25 mg of mercury l⁻¹ was noted in the mid point effluent sample (Table-A.3).

Table-A.3. Physico-Chemical Properties of the effluent collected from the effluent channel at mid point:

Sl. No	Parameter (mg/liter)	Data mean of 5 estimations \pm S.D.	Sl. No	Parameter (mg/liter)	Data mean of 5 estimations \pm S.D.
1	Temperature (°C)	29.8 \pm 1.2	7	BOD	31.8 \pm 1.6
2	pH	9.8 \pm 0.2	8	COD	301.4 \pm 12.8
3	Alkalinity (as CaCO ₃)	254.2 \pm 6.8	9	Suspended solids	106.5 \pm 24.3
4	Hardness (as CaCO ₃)	406.2 \pm 14.2	10	Total nitrogen	3.4 \pm 0.4
5	Chlorinity	1098.6 \pm 22.9	11	Total phosphorus	0.21 \pm 0.11
6	Dissolved oxygen	3.3 \pm 0.2	12	Total mercury	2.68 \pm 0.25

The temperature of the effluent at the mid point was low when compared to delivery point and ranged between 28.2 \pm 1.2°C. The pH of the effluent was alkaline and the value recorded was 9.1 \pm 0.1, no change on pH was recorded when compared to delivery point. The alkalinity was 246.4 \pm 12.2 as CaCO₃ in mg l⁻¹. The hardness of the effluent sample was 385.9 \pm 12.6 as CaCO₃ in mg l⁻¹. The Chlorinity was 962.5 \pm 22.6mg l⁻¹. The dissolved oxygen content was little high when compared to discharge point and ranged within 3.4 \pm 0.4 mg l⁻¹. The BOD and COD values were 39.1 \pm 3.9mg l⁻¹ and 352.4 \pm 18.6mg l⁻¹, respectively in the effluent collected from the mid point and no significant difference was marked with the distance. The suspended solids were low but significant and the value was 86.3 \pm 5.2mg l⁻¹. A small amount of 1.2 \pm 0.3mg.l⁻¹ total nitrogen was also noted to be present in the effluent, which was less than earlier value. An insignificant amount of phosphorus was recorded in the effluent. However, a significant decrease in the amount of mercury was recorded in the effluent storage tank when compared to the delivery point and mid point from the industry. An amount of 2.04 \pm 0.11mg of Hg l⁻¹ was noted in the mid point effluent sample (Table-A.4).

Table-A.4- Physico-Chemical Properties of the effluent collected from the storage tank.

Sl. No	Parameter (mg/liter)	Data mean of 5 estimations \pm S.D.	Sl. No	Parameter (mg/liter)	Data mean of 5 estimations \pm S.D.
1	Temperature (°C)	28.2 \pm 1.2	7	BOD	39.1 \pm 3.9
2	pH	9.1 \pm 0.1	8	COD	352.4 \pm 18.6
3	Alkalinity (as CaCO ₃)	246.4 \pm 12.2	9	Suspended solids	86.3 \pm 5.2
4	Hardness (as CaCO ₃)	385.9 \pm 12.6	10	Total nitrogen	1.2 \pm 0.3
5	Chlorinity	962.5 \pm 22.6	11	Total phosphorus	0.18 \pm 0.08
6	Dissolved oxygen	3.4 \pm 0.4	12	Total mercury	2.04 \pm 0.11

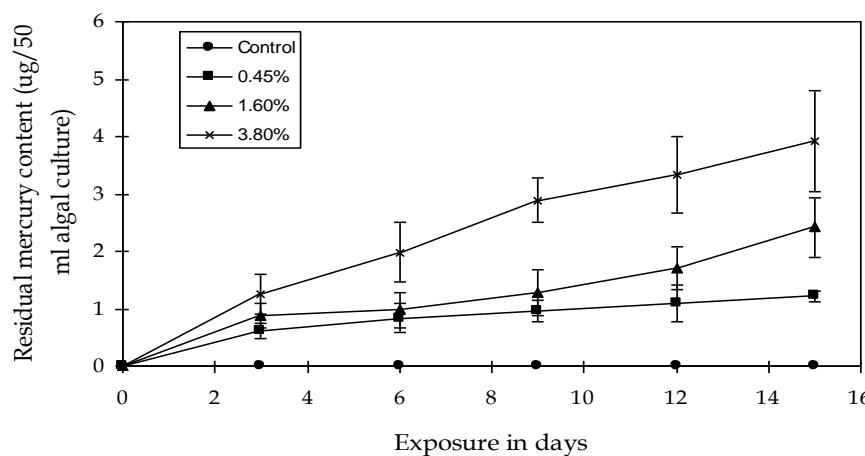
Out of the four stations selected for studying mercury dynamics in the estuary, station II, which is the junction point was found to be having the highest amount of mercury in comparison to other station. The range was 0.0176%mg l⁻¹ in the month of March mg l⁻¹. In the month of February with a mean value of 0.1690 \pm 0.1536 mg l⁻¹.Mercury levels at this station were found to be dependent on the levels of mercury in the effluent to some extent except in the month of January. Unlike water samples, levels of mercury were slightly higher at station I than at station III. when compared to 1986 mercury levels, in 1996 in station-I,18.37% increase; in station II,21.89% decrease, in station III, 135.42% increase; in station IV,34.62% increase and in the effluent channel, station -E,29.17% decrease in mercury concentration was recorded . Within 10 years time, significant increase in mercury level was recorded in station II and III and sediment mercury level in station I, III and IV (Table-A.1.). The decrease in mercury concentration in the effluent channel was due to periodic removal of sediments from the channel and dumping the wastes in a nearby site. Levels of mercury at other station were lower in comparison to station II. Out of the three station, station IV was found to contain the lowest amounts of mercury. Levels of mercury at station I and III were nearly identical with a tendency of little higher levels at station I than at station III, except in the month of April when the value was less than at station III. No particular trend of increase or decrease in the levels of mercury was noted at any of the station. However, all the three stations showed their minimum and maximum levels in the monsoon and pre-monsoon seasons, respectively. The levels of mercury during monsoon season at these stations were much less and very similar to each other. The trend of decrease or increase in the levels of mercury was also similar. Sediment analysis showed the presence of a remarkable quantity of mercury. Maximum amount of mercury was found in the sediment from the effluent channel. The levels of mercury fluctuated much, the maximum being mg kg⁻¹ dry weight in the month of July and the minimum mg Kg⁻¹ dry weight in the month of January. Out of the four station of the estuary, station II was the site of maximum mercury contamination similar to that of water. Here also the concentration of mercury fluctuated much. Maximum value was recorded in February (665mg kg⁻¹dry

weight) and minimum value in July (44.33mg kg^{-1} dry weight). Mean value was 181.69 mg kg^{-1} dry weights. No particular trend of increase or decrease was marked. However, lower concentrations of mercury were observed in the monsoon and the post monsoon season, i.e. from June to December. No relationship could be noticed between the concentration of mercury in sediment of this site and that of the effluent channel. However, it can be inferred that mercury was available in the contaminated area both in water and sediment. No particular trend of increase or decrease in the mercury concentration was noticed at any station during the entire period. However, the levels of mercury were lower in the monsoon and the post monsoon season at all the three stations. The effluent which when released from the factory finds its way into the Rushikulya river estuary, was found to contain very high amount of mercury. Out of twelve analyses carried out in twelve months, only once, in the month of March, a lower concentration of mercury (0.0268 mg l^{-1}) was observed. Though the concentration in March was low, the value was in itself much higher than the permissible limit of 0.01mg l^{-1} . Maximum concentration of mercury, as recorded in the month of January, was to the tune of 1.5487mg l^{-1} . Concentration of mercury in the effluent was found to be much fluctuating having a mean value of $0.4474\pm0.0426\text{mg l}^{-1}$ in 1986. In 1996, the value depleted to $0.3894\pm0.0258\text{ mg.l}^{-1}$, where 12.96% decrease was recorded. The temperature of the effluent at the discharge point was high and ranged between 28.5°C to 31.8°C . The pH of the effluent was alkaline and the value recorded was 9.8 ± 0.2 . The alkalinity was 258.2 ± 12.4 as CaCO_3 in mg l^{-1} . The hardness of the effluent sample was 481.8 ± 17.4 as CaCO_3 in mg l^{-1} . The chlorinity was $1127.6\pm48.4\text{mg l}^{-1}$. The dissolved oxygen content was low and ranged within $3.1\pm0.6\text{mg l}^{-1}$. The BOD and COD values were $31.3\pm1.8\text{mg l}^{-1}$ and $321.4\pm9.2\text{mg l}^{-1}$, respectively in the effluent collected from the discharge point. The suspended solids were low but significant and the value was $146.3\pm34.5\text{mg l}^{-1}$. A small amount of $5.4 \pm 0.7\text{mg l}^{-1}$ total nitrogen was also noted to be present in the effluent. An insignificant amount of phosphorus was recorded in the effluent. However, a significant amount of mercury was recorded in the effluent discharged from the industry. An amount of $3.12\pm0.13\text{mg l}^{-1}$ was noted in the effluent sample. The temperature of the effluent at the mid point was low when compared to delivery point and ranged between $29.8\pm1.2^{\circ}\text{C}$. The pH of the effluent was alkaline and the value recorded was 9.8 ± 0.2 , no change on pH was recorded when compared to delivery point. The alkalinity was 254.2 ± 6.8 as CaCO_3 in mg l^{-1} . The hardness of the effluent sample was 406.2 ± 14.2 as CaCO_3 in mg l^{-1} . The Chlorinity was $1098.6\pm22.9\text{mg l}^{-1}$. The dissolved oxygen content was little high when compared to discharge point and ranged within $3.3\pm0.2\text{mg l}^{-1}$. The BOD and COD values were $31.8\pm1.6\text{mg l}^{-1}$ and $301.4\pm12.8\text{mg l}^{-1}$, respectively in the effluent collected from the mid point and no significant difference was marked with the distance. The suspended solids were low but significant & the value was $106.5\pm24.3\text{mg l}^{-1}$. Insignificant amount of $3.4\pm0.4\text{mg l}^{-1}$ total nitrogen content was available in the effluent, which was less than earlier value. An insignificant amount of phosphorus was recorded in the effluent. However, a significant decrease in the amount of mercury was recorded in the effluent at mid point when compared to the delivery point from the industry. An amount of $2.68\pm0.25\text{mg l}^{-1}$ was noted in the mid point effluent sample. The temperature of the effluent at the mid point was low when compared to delivery point and ranged between $28.2\pm1.2^{\circ}\text{C}$. The pH of the effluent was alkaline and the value recorded was 9.1 ± 0.1 , no change in pH was recorded when compared to delivery point. The alkalinity was 246.4 ± 12.2 as CaCO_3 in mg l^{-1} . The hardness of the effluent sample was 385.9 ± 12.6 as CaCO_3 in mg l^{-1} . The chlorinity was $962.5\pm22.6\text{mg l}^{-1}$. The dissolved oxygen content was little high when compared to discharge point and ranged within $3.4\pm0.4\text{mg l}^{-1}$. The BOD and COD values were $39.1\pm3.9\text{mg l}^{-1}$ and $352.4\pm18.6\text{mg l}^{-1}$, respectively in the effluent collected from the mid point and no significant difference was marked with the distance. The suspended solids were low but significant and the value was $86.3\pm5.2\text{mg l}^{-1}$. A small amount of $1.2\pm0.3\text{mg l}^{-1}$ total nitrogen was also noted to be present in the effluent, which was less than earlier value. An insignificant amount of phosphorus was recorded in the effluent. However, a significant decrease in the amount of mercury was recorded in the effluent storage tank when compared to the delivery point and mid point from the industry. An amount of $2.04\pm0.11\text{mg l}^{-1}$ of mercury was noted in the mid point effluent sample.

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. 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

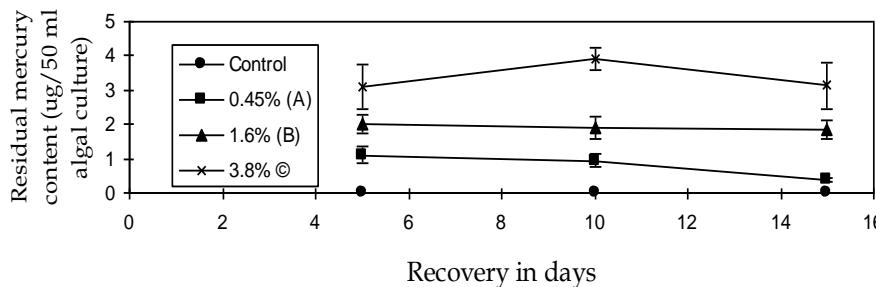
the increase in the effluent concentration, the percent survival decreased and the percent mortality increased. The PS values showed the existence of a negative correlation and LC values showed a positive correlation. With the increase in exposure period, the lethal concentration values decreased, showing 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 revitalized 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. Experiments were conducted to study the residual mercury accumulation at different exposure periods and excretion/removal of mercury during recovery period. In the control set, no residual mercury accumulation was recorded indicating absence of mercury in the background. At 0.45% effluent (A) concentration, the residual mercury increased from 0.62 ± 0.14 μg of mercury to 1.22 ± 0.09 μg of mercury/50 ml algal culture within 15 days of exposure. When the exposed alga was transferred to toxicant free medium, after 15 days of recovery, 0.38 ± 0.06 μg of Hg/50 ml culture was recorded. In the control set, no residual mercury accumulation was recorded indicating absence of mercury in the background. No mercury was detected by the mercury analyzer in the control set and at '0' day exposure (inoculation day). At 0.45% effluent (A) concentration, the residual mercury increased from 0.62 ± 0.14 μg of mercury to 1.22 ± 0.09 μg of mercury/50 ml algal culture within 15 days of exposure. When the exposed alga was transferred to toxicant free medium, after 15 days of recovery, 0.38 ± 0.06 μg of Hg/50 ml culture was recorded. On 5th day of recovery 9.02% mercury removal might be due to excretion was recorded. On 10th day and 15th day of recovery, 22.95% and 68.8% of mercury removal was recorded, respectively, in the exposed culture. In B set (1.6% effluent), higher rate of residual mercury accumulation/retention was marked, when compared to the A set.

Fig.1A. Showing changes in residual mercury content of control and effluent exposed alga at different effluent concentrations at different exposure periods.



Higher values were recorded at all exposure periods, when compared to the A set. A maximum of $2.42 \mu\text{g}$ of mercury/50 ml culture was recorded on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, a maximum of $1.84 \pm 0.28 \mu\text{g}$ of mercury/50 ml algal culture was recorded. A maximum of 23.97% decrease in the residual mercury level was marked on 15th day of recovery, when compared to 15th day exposure value (Fig.1A).

Fig.1B. Showing changes in residual mercury content of control and exposed alga at different effluent concentrations and different recovery periods.



With the increase in exposure period, the residual mercury increased showing the existence of a significant positive correlation. The correlation coefficient analysis indicated the existence of positive correlations ($r = 0.931, P \leq 0.01$) in the X set and ($r = 0.973, P \leq 0.01$) in the Y set. Regression analysis indicated the existence of non-significant correlation up to 6th day of exposure and significant correlation ($P \leq 0.05$) after 6th day of exposure and up to 15th day of exposure. In the C set (3.8% effluent concentration), the residual mercury accumulation was highest. With the increase in exposure period, the residual mercury accumulation increased showing a positive significant correlation ($r=0.987, P \leq 0.001$). A maximum of $3.92 \pm 0.88 \mu\text{g}$ of mercury/50 ml algal culture was recorded. During recovery period, no significant recovery was recorded. A maximum 20.66%, 0.25% and 19.89% mercury removal was recorded on 5, 10 and 15th day of recovery (Fig.1B). The data indicated that the effluent is deadly toxic and can affect the cyanobacteria present in crop fields.

Discussion

In recent decades, heavy metals or heavy metal contained waste pollution in crop field soil and water has become one of the most prominent environmental concern and issues (Li *et al*, 2019). Toxicological studies involve the science of poisons, their effects on living organisms, antidotes to neutralize the toxic effects and detection of the contamination and to find out remedial measure to combat the toxic effects of the toxicants. Toxicity is the ability of a chemical molecule or compound to produce injury once it reaches a susceptible site in or on the body of the organism. In toxicity testing the laboratory bioassay is generally the most favored test because experimental conditions can be controlled and the response of test organisms can be observed or monitored to a greater degree. Algae are more sensitive than animals to complex wastes such as industrial and municipal effluents. Rai *et al.* (1981) exposed *Chlorella vulgaris* to HgCl_2 , concentrations between 100 and 1000 $\mu\text{g}/\text{lt}$, for 3 weeks, and monitored survival and growth of the alga. LC_{50} for survival was at 400 $\mu\text{g}/\text{lt}$ of HgCl_2 and the growth rate was 92% of the control value at 100 $\mu\text{g}/\text{lt}$, the growth rate was 31% at 800 $\mu\text{g}/\text{lt}$ and there was no growth at 1000 $\mu\text{g}/\text{lt}$. There was a protective effect of calcium and phosphate in the medium and to a lesser extent of magnesium. Usually at very low concentration the compound becomes harmless and even acts as an essential substance for growth and development. So, the primary objective of toxicological testing is to obtain data on the dose-response characteristics of a chemical. These studies provide the primary data base from which estimates of risk to an identified population of organisms may be determined in connection with specific uses or disposal practices for a specific chemical. Normally, the objective of toxicological testing is to identify the nature of possible adverse effects and to relate response (or injury) to dosage, dose-response expressions to a toxic agent and the spectrum of observed effects. A liquid industrial waste may affect the algal growth in any of three ways: stimulation, inhibition and stimulation at lower concentrations but inhibition at higher concentrations (Walsh & Alexander, 1980; Sahu, 1987; Shaw, 1987, Rath, 1991 and Misra, 2006). The enhancements of growth, heterocyst frequency and nitrogen fixation at lower doses of furadon (0.75 $\mu\text{g}/\text{ml}$ of carbofuran) have also been reported. The present investigation did not agree with the above conclusions. But such stimulation in the growth cannot be easily explained at this stage of the study. Hufford (1971) attributed the reason for stimulation, for the presence of some growth regulating compounds and/or trace elements in the crude oil. Some suggested uptake and metabolism of the constituent as the probable mechanism for growth stimulation. Prasad & Prasad (1982) observed stimulation in the algal growth at low concentrations of Cd, Pb and Ni. Neither Cd, Pb and Ni has been reported to be essential micronutrients for algae (O'Kelley, 1974) nor the pure solution of these heavy metals are expected to contain any growth regulator. Thus, an ideal explanation for stimulation of growth at lower concentrations of the toxicant is yet to be ascertained. The present study indicated that the surrounding area of Jayashree Chemicals

Pvt. Limited, Ganjam, Orissa a caustic soda plant released huge amount of mercury into the environment in the past 4-5 decades. Evaporated mercury from the Mercury cell house contaminated the surrounding biota leading to elevated mercury levels beyond prescribed limit was the major concern during analysis period. The discharged mercury in the effluent of the industry contaminated the Rushikulya River and estuary lead to elevated mercury level in aquatic plants and animals. The sediment from the treatment tank and effluent channel contained a significant amount of mercury which was dumped in nearby places raised the residual mercury concentration in all types of plants (producers) and in animals (consumers). The status of mercury pollution in and around the industry at Ganjam, a significant problem up to 2006 was grim and grave. But due to change in technology in the industry, the mercury concentration declined significantly in the effluent channel. The decline in mercury level in water, sediment and effluent channel, solid waste dumping site, available plants and animals at present when compared to earlier reports is a positive sign for the area. But our concern rests on the future, as all mercury discharged from the industry ultimately entered in to Bay of Bengal by rain run off water, by leaching from the solid waste dumping sites, by leaching from the effluent stocking pond near the river basin. The residual mercury concentration values in water and sediments of the effluent channel, Junction point where the effluent canal joins Rushikulya River and Rushikulya estuary significantly varied in 2010 and 2011 when compared to 1985-86, 1995-96, where much higher values were reported. The residual mercury concentration values in water and sediments of the effluent channel, Junction point where the effluent canal joins Rushikulya River, Rushikulya estuary and Bay of Bengal significantly varied in 2008 when compared to 1985-86, 1995-96, 2002, where very insignificant amount of mercury was recorded in Station I to IV and from Station VI to Station-XV. The amount of mercury reported was either at non-detectable level or at insignificant level. Mercury concentration decreased in all the stations studied in 2010 and 11 when compared to 1885-86, 1995-96, 2002-03 and 2005-08., the reasons being- the industry stopped discharging mercury contained effluent of the chlor-alkali industry directly into River Rushikulya from later part of 2002 and early part of 2003. The effluent was diverted to one effluent stocking pond near the river basin. Prior to discharge the effluent was treated properly and the effluent was allowed to settle in settling tanks prior to discharge. The solid waste containing huge amount of residual mercury collected from the effluent channel was dumped in a plastic lined pond instead of dumping outside the industry premises and very nearer to the river bed. The solid waste is air dried and heated in a digester and the volatilized mercury was collected by condenser and recycled back. The industry changed the technology from Mercury Cell process to Membrane system, where no mercury was used obviously any mercury was discharged out. This is one of the most important aspects of mercury pollution studies in Rushikulya estuary, where the dangerous level of mercury in the environment depleted significantly. The effluent of a chlor-alkali industry (M/S Jayashree chemicals Pvt. Ltd., Ganjam) showed significant variation and difference in action at different concentrations is clearly evident from tables and figures explained in the result chapter. The highest concentration (concentration Z, 3.8% Effluent) showed typical mercurial toxic symptoms, beyond which survival of the alga became extremely difficult and at times, impossible under laboratory controlled conditions. The alga, *Westiellopsis prolifica*, Janet is more tolerant and less sensitive to toxicants. The analysis of variance ratio tests and correlation coefficient analysis carried out for all parameters studied indicate clearly that the toxicant, effluent containing mercury, is extremely toxic to blue-green alga. At higher concentration, all most all the growth parameters studied showed declining trend in exposed cultures, when compared to control cultures. At lower concentration of effluent (0.45% effluent), all the growth parameters showed an increasing trend in the exposed culture, when compared to control culture. Hence, it can be concluded that the toxicant (mercury contained) showed dichotomous behavior. At lower concentrations, the toxicant is growth regulatory and at higher concentration, the toxicant is growth inhibitory. The observed changes in the exposed cultures when compared to control cultures were only due to the toxicant, effluent. Hence, it can be concluded that the damage caused in the exposed system, at higher concentration, was only due to the toxicant and most important component of the effluent being mercury. Pollution occurs, due to careless, irresponsible discharge of the effluent in the vicinity of the industry and consequent discharge into River Rushikulya, enter into the paddy fields through irrigation, where the non-target organisms, like blue-green algae, inhabitant of the crop fields, suffer the most. These tiny beautiful organisms, fix atmospheric nitrogen, increase the fertility of the soil, acts as a biofertilizer, a substitute for chemical fertilizer is mostly affected at higher concentrations. The present investigation was designed to study the effects of the effluent of the Caustic-chlorine industry on the growth, photosynthetic efficiency and nitrogen fixing ability of the cyanobacterium, *Westiellopsis prolifica*, Janet. No significant visible morphological change was noticed in the exposed cyanobacterium. At higher concentration, bleaching of the filaments was marked. At higher recovery period (three months) and at lower concentration of the toxicant, the alga probably adopted to the toxicant and reappeared again, initially as tiny dot like particles and after some time full fledged organism with natural growth was marked. At higher concentration of the effluent, the alga could not revive, showing permanent

death of the exposed alga. The exposed alga, at higher concentrations could not recover, in recovery studies, which indicated that the toxicant causes permanent damage to the exposed system. Stimulation of growth was marked at lower concentration of the toxicant (0.45% effluent), as all growth parameters showed higher values, when compared to the control value. Inhibition of growth was recorded at higher concentrations of the toxicant (1.6% & 3.8%, concentrations), as all growth parameters showed significantly low values. Significant stimulation at low concentrations and significant inhibition at higher concentrations indicate the dichotomous behavior of the toxicant. Residual mercury was found inside the cyanobacterial cells and in between the space of the cell wall and mucilaginous sheath of the exposed alga. The dry weight value showed normal growth in the control set, showing a linear increase in dry weight values. At 0.45% effluent concentration, higher dry weight values were marked, when compared to the control value. In B concentration, initial increase up to 9th day followed by decrease in dry weight value, was marked. In concentration-C, the dry weight value significantly declined with the increase in exposure period. Significant variations were marked at different concentrations of the effluent. The typical behavior observed at higher concentration of the toxicant is significantly different from the behavior of the alga at lower concentrations. Hence, from the above results, we can conclude that the concentration of a chemical decides toxicity or toxic nature of the chemical. Hence, the effluent should be diluted for possible use as a stimulant in the crop fields. But we should not forget the bioconcentration of mercury and biomagnification of mercury in a food chain, in the ecosystem, which can be hazardous and an incident similar to Minamata Bay incidence or the incidence at Niigata prefecture may not be ruled out. The present day stimulatory effect of the toxicant showing higher growth rate, better nitrogen fixing capability will be a curse in future. Hence, proper care should be taken, while handling the issue at early phases. Protection and preservation of the environment is more important than short term benefits.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

References

Allen, M. B. and Arnon, D. I. (1955): Studies on nitrogen fixing blue-green algae, growth and nitrogen fixation by *Anabaena cylindrica*, Lemm. Pl. Physiol. Lancaster., 30, 366-372.

APHA(American Public Health Association)(1985): Standard methods for the examination of water and wastes. Environmental Protection Agency, USA.

ECIL (Electronic Corporation of India Limited) (1981): Analytical methods for determination of mercury with mercury analyser, MA 5800 A.

Fogg, G. E. (1949): Growth and heterocyst production in *Anabaena cylindrica*, Lemm. II. in relation to carbon and nitrogen metabolism. Ann. Bot. N. S., 13:241-259.

Gardner, W. S.; D. R. Kendall; R. R. Odom; H. L. Windom and J. A. Stephens (1978): Environ. Pollut., 15: 243.

Glass, G.E.; Edward N-Leonard; W.H. Chan and D.B.Orr (1986): J. Great Lakes Res., Internat. Assoc. Great Lakes Res, 12(1): 37.

Hall, A.; A. C. Duarte; M. T. M. Caldeira and M. F. B. Lucas (1987): The Science of the Total Environment, 64: 75.

Hartung, R. and B. D. Dinman (eds.) (1974): Environmental Mercury Contamination. Ann. Arbor. Science Publishers Inc., 349.

Hufford, G. L. (1971): The biological response of oil in the marine environment, a review. Background report, Washington, D. C., U. S. Coast guard Oceanographic Unit, Office of Research and Development, U. S. Coast guard Headquarters.

Kudo, A. and S. Miyahara (1984): Ecotoxicology Environmental Safety, 8:507.

Li, H., Liu, Y., Luo, Z., Zhou, Y., Hou, Z., Mao, Q., Zhi, D., Zhang, J., Yang, Y. and Luo, L. (2019): Effects of RM based passivator for the remediation of two kinds of Cd polluted paddy soils and mechanism of Cd(II) adsorption. Environmental Technology, DOI:10.1080/09593330.2019.1675772

Mishra, S. K. (2006): Effect of cadmium on the growth and nitrogen fixation of cyanobacterium. Ph.D. thesis. Berhampur University.

O'Kelley, J. C. (1974): Organic nutrients. In: Algal physiology and Biochemistry, W. D. P. Stewart (Ed.), pp. 610. Blackwell Scientific Publications, Oxford., 610-635.

Prasad, P. V. D. and P. S. D. Prasad (1982): Effect of cadmium, lead and nickel on three fresh water green algae. *Water, Air and Soil Pollut.*, 17, 263-268.

Rai, L. C.; J. P. Gaur and H. D. Kumar (1981): Phycology and heavy metal pollution. *Biol. Rev.* 56:99-151

Rath, S. C. (1991): Toxicological effects of a mercury contained toxicant on *Anabaena cylindrica*, Lemm. and its ecological implications. Ph. D. thesis, Berhampur University, Orissa, India.

Sahu, A. (1987): Toxicological effects of a pesticide on a blue-green alga. III. Effect of PMA on a blue-green alga, *Westiellopsis prolifica*, Janet. and its ecological implications. Ph. D. Thesis, Berhampur University, India.

Shaw, B. P. (1987): Eco-physiological studies of the waste of a chlor-alkali factory on biosystems. Ph. D. thesis, Berhampur University, Orissa, India.

Shaw, B. P., A. Sahu & A. K. Panigrahi, 1985: Residual mercury concentration in brain, liver and muscle of contaminated fish collected from an estuary near a Caustic-chlorine industry, *Curr. Sci.*, 54:810-812.

Shaw, B. P. & A. K. Panigrahi, 1986a: Uptake and tissue distribution of mercury in some species collected from a contaminated area in India: Its ecological implications *Arch. Environ. Contam. Toxicol.*, 15: 439-446.

Shaw, B. P., A. Sahu & A. K. Panigrahi, 1986b: Mercury in plants, soil and water from a Caustic-chlorine industry. *Bull. Environ. Contam. Toxicol.*, 36: 299-305.

Shaw, B. P., A. Sahu, S. B. Choudhury & A. K. Panigrahi, 1988: Mercury in the Rushikulya river estuary. *Mar. Poll. Bull.*, 19(5): 223-234.

Walsh, G. E. and S. V. Alexander (1980): A marine algal bioassay method: result with pesticides and industrial wastes. *Water, Air and Soil Pollution*, 13:45-55.

Wantorp, H. and A. Dyfverman (1955): Identification and determination of mercury in biological materials. *Arkiv. fur Kemi.*, 9(2), 7-12.

