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Impact of mercury and its removal from contaminated environment by biological agents

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Abstract
The present study was designed to study the impact of mercury contained effluent of chlor-alkali industry on Cyanobacteria, certain aquatic plants and the possible detoxification by Cyanobacteria in the environment. Different concentration of the effluent was prepared and homogeneous suspension of Cyanobacteria i.e Westiellopsis Prolifica Janet and certain aquatic plants were inoculated to find out the growth and decontamination of mercury contaminated effluent. It was found that at higher concentration (i.e. 3.8% Hg) bleaching of the filament was marked and at lower concentration (i.e.0.45%) stimulation of growth was observed. Inhibition of growth was also recorded at higher concentration of mercury where as Insignificant stimulation of growth have been recorded at low concentration. Similarly increase in chlorophyll content was recorded at lower concentration whereas decrease in chlorophyll content was marked at higher concentration. Photosynthetic rate was also increased at lower concentration and it was decreased at higher concentration as compared to the control value. During the experiment it has been observed that Cyanobacteria can absorb and releases the mercury into the environment from mercury contaminated effluent.

Keywords: Chlor-alkali industry, Cyanobacteria, Mercury, pollution, Detoxification, aquatic plants.

Introduction
Mercury in the environment has many uses but when present in excess amount, it becomes harmful to flora and fauna [2]. Mercury is considered as one of the most environmental pollutants, noted for causing health disasters in Minamata bay, Japan in the year of 1953-1960 [3] .Mercury is rarely found in nature in the form of pure and liquid metal rather with in compounds and inorganic salts. Broadly there are two sources from which mercury enters into environment. First, Mercury present in the earth's crust gets released into the environment by physical or chemical erosion and degassing. The second source of mercury in the environment is due to human activity, particularly from coal-fired power stations and incinerators. Mercury is also released as a result of mining of gold (where Mercury is used to form an amalgam before being burnt off) .Dental amalgam is also a potential source of mercury as it contain up to 50% elemental Mercury . Mercury containing beauty creams, hair treatment and other cosmetic product may releases the significant quantity of Mercury to the environment. The most significantly mercury available in the environment are due to chlor-alkali industries .Mercury pollution of the environment has created some serious hazard for mankind. The most significant incident of the toxicity of this metal from the scientific epidemiological point of view have been those in Japan in Minamata(1953-60) and Niigata(1965); which were caused by industrial release of mercury and its compounds into the Minamata bay and the Agano river respectively [4]. Once this metal enters into the body of human being it neither degrade nor excreted out from the body, It simply accumulate inside the body of human being and creates disorder in metabolic activities.. So there is need to remove this metal from the environment before it reaches to natural system. By using Cyanobacteria and certain aquatic plants, experiments were conducted in laboratory to find out which organism is best for removal of Mercury metal from the contaminated environment.

2. Materials and methods
We have collected effluent from three different locations of discharged channel of Jayashree chemical pvt. Ltd (A Chlor-alkali industry) Berhampur, Ganjam, Odisha and mixed in a large glass bottle. It was shaken to settle down the suspended particles. Supernatant was collected and three different concentration of solution was prepared by using culture media and were expressed in terms of percent (v/v). The three different concentrations of effluents are of Concentration X (0.45%), Concentration Y (1.6%), Concentration Z (3.8%) were selected from the algal bioassay.
By using Mercury analyzer it was found that concentration X contains 3.8 µg of mercury, concentration Y contains 10.20 µg of mercury and concentration Z contain 24.7 µg of mercury. 50 ml from each sample was taken and transferred into 24 different 100 ml conical flask. Conical flasks were sterilized with UV rays for 10 minutes before taking the samples and then they were inoculated with 1ml of homogeneous sample of specific Cyanobacteria and aquatic plants. They were incubated for 15 days at 24^0 C ± 2^0C .In all samples a few parameters like removal of mercury, chlorophyll content and photosynthesis rate of the organism were measured after 15 days of incubation. These activities were shown in the below table (table-1) which shows the impact of these selected aquatic organisms on mercury dynamics.

Table 1: Action of aquatic organisms on effluent containing Mercury.

Aquatic Plants/Cyanobacteria	Control and different Concentration of effluent	Mercury concentration in the medium at the time of inoculation(µg)	Amount of mercury absorbed from the medium, after 15 days	Chlorophyll content (µg/50 ml)of culture after 15 days	Photosynthetic rate (µl of O_2 evolved/50ml/hour) after 15 days
1.Nostoc species	Control, 00	0	----	24.4	286.4
	Concentration X(0.45%)	3.8	0.6	17.4	299.8
	Concentration Y(1.60%)	10.2	1.2	6.5	178.4
	Concentration Z(3.8%)	24.7	2.9	4.2	56.8
2.Oscillatoria species	Control, 00	0	----	28.6	311.4
	Concentration X(0.45%)	3.8	0.8	24.4	319.2
	Concentration Y(1.60%)	10.2	1.9	19.3	185.6
	Concentration Z(3.8%)	24.7	3.6	7.8	28.4
3.Anabaena species	Control, 00	0	----	27.2	311.8
	Concentration X(0.45%)	3.8	3.7	29.8	395.6
	Concentration Y(1.60%)	10.2	9.9	25.6	285.2
	Concentration Z(3.8%)	24.7	23.6	9.4	65.4
4.Chlorella species	Control, 00	0	----	18.9	134.5
	Concentration X(0.45%)	3.8	0.4	7.4	138.6
	Concentration Y(1.60%)	10.2	0.8	3.8	70.4
	Concentration Z(3.8%)	24.7	1.1	0.9	18.2
5.Westiellopsis species	Control, 00	0	----	26.8	365.6
	Concentration X(0.45%)	3.8	3.7	31.9	431.3
	Concentration Y(1.60%)	10.2	10.1	24.2	301.6
	Concentration Z(3.8%)	24.7	24.4	13.2	142.2
6.Eichornia species	Control, 00	0	----	36.4	262.4
	Concentration X(0.45%)	3.8	3.7	31.2	241.2
	Concentration Y(1.60%)	10.2	10.1	29.4	176.4
	Concentration Z(3.8%)	24.7	24.6	18.5	88.9

3. Results

In case of **Nostoc** species, a maximum of 0.6 µg of mercury was absorbed from concentration X out of 3.8 µg. 1.2 µg of mercury was absorbed from concentration Y out of 10.2 µg and 2.9 µg of mercury was absorbed from concentration Z out of 24.7 µg. In concentration X photosynthetic rate was increased, in concentration Y, photosynthetic rate was decreased and in concentration Z, photosynthetic rate was still decreased. It was also found that the Chlorophyll content decreased from 24.4 µg/50 ml (control) to 17.4 µg / 50 ml at concentration X and 6.5 µg/50 ml at concentration Y and 4.2 µg/50 ml at concentration Z.

In case of Oscillatoria species, a maximum of 0.8 µg of mercury was absorbed from the medium at concentration X. Similarly a maximum of 1.9 µg of mercury was absorbed from the medium at concentration Y and a maximum of 3.6 µg of mercury was absorbed at concentration Z. The chlorophyll content was decreased from 28.6 µg/50 ml to 24.4 µg/50 ml at concentration X and 19.3 µg/50 ml at concentration Y and 7.8 µg/50ml at concentration Z. The photosynthetic rate increased from 311.4 µl O_2 evolved/50ml culture/hour) to 319.2 µl at concentration X. It has been noticed that the photosynthetic rate becomes decreased to 185.6 µl at concentration Y and 28.4 µl at concentration Z.

In case of Anabaena species, a max .of 3.7 µg of mercury have been absorbed from the concentration X similarly a max of 9.9 µg of mercury absorbed from the concentration Y and a max. of 23.6 µg of mercury have been absorbed from the concentration Z.The chlorophyll content increased from 27.2 µg/50 ml to 29.8 µg/50ml at concentration X and decreased to 25.6 µg/50 ml culture at concentration Y and 9.4 µg/50ml culture at concentration Z. The photosynthetic rate increased from 311.8 µl O_2 evolved/50 ml culture/hour) to 395.6 µl at concentration X and decreased to 285.2 µl at concentration Y and further decreased to 65.4 µl at concentration Z.

In case of Chlorella, a max of 0.4 µg, 0.8 µg, 1.1 µg of mercury have been absorbed from the concentration X, Y and Z respectively. Similarly Chlorophyll content was decreased

from 18.9 µg/50ml to 7.4 µg/50 ml to 3.8 µg/50 ml to 0.9 µg/50 ml at concentration X, Y and Z respectively. The photosynthetic rate increased significantly from 134.5 µl to 138.6 µl at concentration X and decreased to 70.4 µl at concentration Y and still decreased to 18.2 µl at concentration Z.

In case of Westiellopsis species, a maximum of 3.7 µg, 10.1 µg, 24.4 µg of mercury have been absorbed from the concentration X, concentration Y and concentration Z respectively . The chlorophyll content increased from 26.8 µg/50 ml to 31.9 µg/50 ml at concentration X, then decreased to 24.2 µg /50 ml culture at concentration Y and again decreased to 13.2 µg/50 ml culture at concentration Z. The photosynthetic rate increased significantly from 365.6 µl to 431.3 µl at concentration X and significantly decreased to 301.6 µl at concentration Y and decreased to 142.2 µl at concentration Z.

In case of Eichornia species, a maximum of 3.7 µg, 10.1 µg, 24.6 µg of mercury have been absorbed in concentration X, Y, and Z respectively. The chlorophyll content decreased significantly in three concentration i.e from 36.4 µg / 50 ml culture to 31.2 µg/50 ml culture at concentration X and 29.4 µg/50 ml culture at concentration Y and 18.5 µg/50 ml culture at concentration Z. The photosynthetic rate decreased from 262.4 µl to 241.2 µl at concentration X and decreased to 176.4 µl at concentration Y and decreased to 88.9 µl at concentration Z.

The different results related to Concentration of Effluent versus Absorption of mercury, total Chlorophyll content and photosynthetic rate of Westiellopsis species are shown in figure 1, 2 and 3.

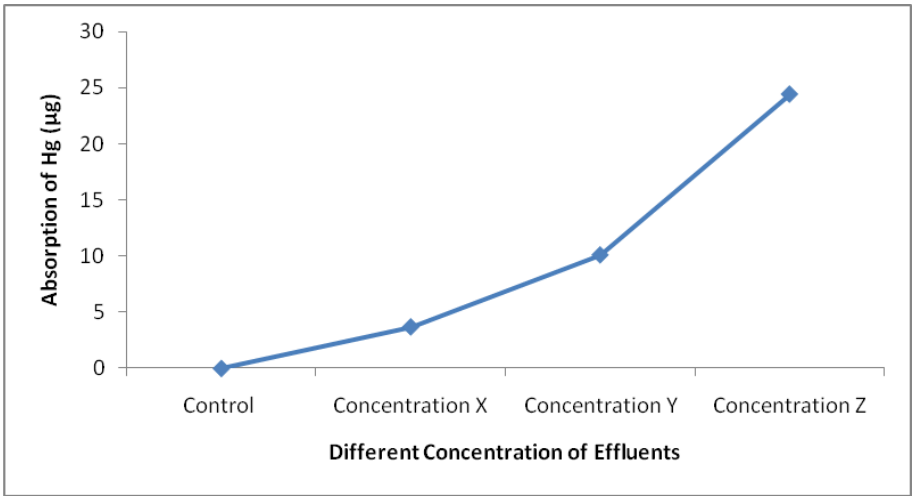

Fig 1: Showing changes in absorption of Mercury by Westiellopsis (sp.) from different concentration of effluents after 15 days of incubation. Concentration of Control=0; Concentration X=0.45%; Concentration Y =1.60%; Concentration Z=3.8%

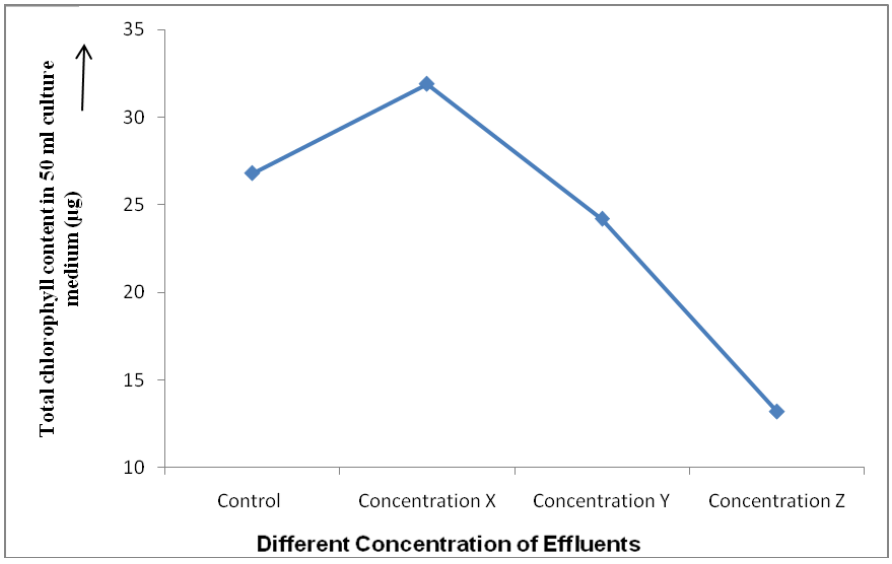

Fig 2: Showing the chlorophyll content in westiellopsis species in different concentration of effluent after 15 days of incubation. Concentration of Control=0; Concentration X=0.45%; Concentration Y =1.60%; Concentration Z=3.8%

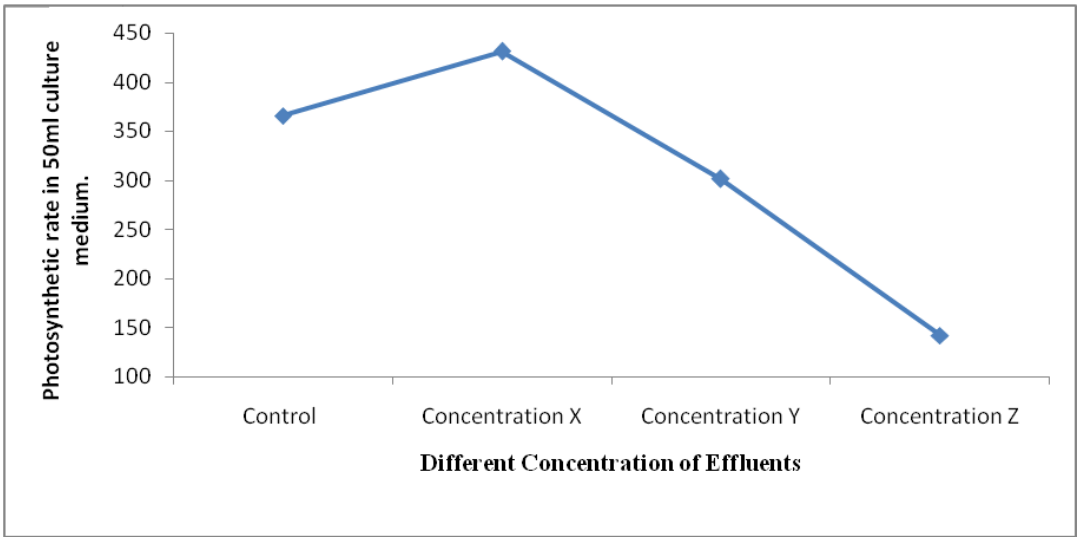

Fig 3: Showing the change in photosynthetic rate in Westiellopsis species in different concentration of effluent after 15 days of incubation. Concentration of Control=0; Concentration X=0.45%; Concentration Y =1.60%; Concentration Z=3.8%

4. Discussion

Metal contamination of soil is the one of the major environmental problems in the world. The in-situ phyto-remediation using plants to restore the contaminated soil is a promising technology to clean up polluted soil, which is less destructive, low cost and environmental friendly [8].Among various types of technology for removal of metal from the soil, phyto-remediation is regarded as a complete metal clean up technology and more and more appealing in recent decades [1, 9] .The same author also suggested that measures to improve metal uptake plant roots and translocation to shoot should be the major task in the future research. We agree with the above authors and also our opinion stands for selecting the suitable Cyanobacteria and aquatic plants for decontamination of the mercury contaminated environments. For decontamination studies suitable Cyanobacteria and aquatic plants were selected and inoculated in different concentration of effluent collected from the discharged channels of industry. The idea of selecting different concentration of effluent from toxicity testing was based on the idea that the inoculated organism should survive in the medium for the period of study and the mercury dynamics and fate of mercury can be studied [5].

The results which were obtained from above experiments, it is concluded that the Anabaena, Westiellopsis and Eichornia species were very useful for mercury decontamination purpose but the Westiellopsis species is the best to detoxify the contaminated environment by evaporation of mercury from the effluent.

Higher rate of detoxification was marked at lower concentration of mercury in the effluent. This results indicated the need of initial dilution of the effluent to a particular level where evaporation and adsorption became faster. To know the tolerance capacity of selected plants and Cyanobacteria in the mercury contaminated environment a trial experiment was conducted to study the involvement of transposons i.e Tn21 & Tn501 which is responsible for mercury resistance in bacteria. The probe could not identify the presence of transposons in Cyanobacteria. Hence observed tolerance in the Cyanobacteria was not genetic but due to slow acclimatization /adaptation. These Cyanobacteria could tolerate high concentration of mercury. This tolerance is probably due to environmental influence not due to genetic influence. This Cyanobacteria accumulates mercury from the effluent and simultaneously evaporate mercury either from the absorbed mercury or from the effluent. The double action of these Cyanobacteria was observed for the first time and also reported for the first time and generated much interest for using as a detoxifier of mercury contaminated environment.

5. Conclusions

As far as pollution of mercury is concerned, chlor-alkali industry is the main source of it. Hence to remove it, experiments were conducted by using Cyanobacteria and aquatic plants in the laboratory. Only Westiellopsis could remove significant amount of mercury from the three different concentration of the effluent. It has been found that significant increase in chlorophyll content was recorded in lower concentration (i.e. X) and significant decrease in chlorophyll content was marked in higher concentration (i.e. Z) and higher photosynthetic rate was recorded at all exposure period in X concentration when compared to the control value. In Y concentration, photosynthesis rate increased up to 12th day of exposure and then decrease. Significant depletion in Photosynthetic rate value was recorded in Z concentration. at all expose period, when compared to the control value. It has been also found that the tolerance of Cyanobacteria was not genetic but due to its slow acclimatization in the environment [6]. From the above results, it has been confirmed that at higher concentration it becomes lethal to the organism but at lower concentration it becomes growth stimulator [7] .So effluent should be treated properly before it is dumped into the environment. Protection and preservation of the environment is more important than short term benefits by industry for a better and pure environment for the future generation.

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