Research Paper



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IMPACT OF CADMIUM ON THE BIODIVERSITY OF ALGAE, PROTOZOA, BACTERIA AND FUNGI GROWN IN OXIDATION PONDS

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Studies were carried out on the toxic effects of cadmium on the sewage quality and microbial diversity of oxidation ponds. It was found that wastewater treatment efficiency was affected by the presence of cadmium. On day 16, the level of BOD reduction recorded was 95.7%, 92.1%, 79.7%, 38.3% and 34.8% and the level of phosphate reduction recorded was 68.2%, 65.9%, 54.5%, 42.0% and 31.8% in control, 0.5 mg/L, 1.0 mg/L, 5.0 mg/L and 10.0 mg/L of cadmium treated oxidation ponds respectively. The level of pH, DO, and catalase enzyme activity were decreased and the level of TDS, BOD and phosphate concentrations were increased as the concentration of cadmium was increased in the oxidation pond. Microbial density recorded was maximum in control and 0.5 mg/L of cadmium treated oxidation ponds compared to other ponds treated with higher concentrations of cadmium. The algal species belonged to the genus Anacystis and the species Chlorella vulgaris showed more tolerance, whereas the species Zygnema pectinatum, Scenedesmus acuminatus and Euglena viridis were found to be more sensitive to cadmium toxicity. Among protozoans, the species of the genus Spasmostoma and Onchomonas showed more tolerance and the species Podophrya fixa, Pelomyxa palustris and the species belonged to the genus Thylacomonas were showed more sensitivity to cadmium toxicity.

Keywords: Cadmium, BOD, Algae, Protozoa, Bacteria, Fungi

INTRODUCTION

Cadmium is an element that occurs naturally in the earth's crust and is one of the most toxic metal (Marcantonio *et al.*, 2007). It is a pollutant of industrial origin and was the cause of the disease Itai-Itai (Forstner and Wittmann, 1983). The allowable limit of cadmium in drinking water is about 0.05 mg/L and a maximum permissible level for the effluent is about 2 mg/L (Seth and Pandey, 1983). Heavy metals such as Ni, Zn, Cu, Co, Mn, Cd, As, Hg, Pb, etc., constitute a major source of environmental pollution in mining, metallurgical and manufacturing industrial sectors. In the aquatic environment, these heavy

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metals bring about major changes in the biodiversity of organisms which are exposed to it.

Oxidation pond is a kind of the biological treatment of sewage. The biological process in oxidation pond mainly involves an interaction between bacteria, algae and other organisms. The bacteria in the pond decompose the biodegradable organic matter and release CO₂, ammonia and nitrates (Hosetti et al., 1985). These are utilized by the algae, together with sunlight and photosynthetic process releases oxygen, enabling the bacteria to breakdown more waste and accomplish reduction in BOD levels (Pearson et al., 1987). Oxidation pond is found to be most suitable for the treatment of domestic and hospital sewage. It comprises different groups of organisms like bacteria, algae, fungi, viruses, rotifers, protozoa, nematodes, insects, crustacean larvae, etc. These organisms coexist and compete each other for food and other requirements (Nair, 1997). Pollutants like cadmium can change the normal function of the oxidation pond and affect organisms. Present studies aimed to evaluate the toxic effects of cadmium (as CdCl₂) on the physico-chemical and biological parameters including the biodiversity of algae, protozoa, bacteria and fungi grown in oxidation pond.

METHODOLOGY

The experiment was designed according to the procedure of Patil *et al.* (1986). The heavy metal salt, cadmium (as $CdCl_2$) was used for this study. Five sets of plastic container in sixlet of uniform size of 15 L capacity were taken and filled with 10 L of raw sewage collected from sewers of Mangalore City Corporation. Sewage in the first set of plastic containers without cadmium

treatment was taken as control. The second, third, fourth and fifth set of plastic containers were treated with 0.5 mg/L, 1.0 mg/L, 5.0 mg/L and 10 mg/L of CdCl₂, respectively which were selected from range finding tests. To all the oxidation ponds, seed sample containing algae, protozoa, bacteria and fungi which was collected from natural oxidation pond was introduced in equal quantities and all the ponds were kept in open sunlight.

The experiments were carried for 16 days. Observations were made by collecting 150 mL of water sample from each pond on every fourth day. The samples were analyzed for pH, TDS, DO, BOD and phosphate by following the standard methods prescribed in APHA (1995). Catalase activity was determined according to the method adopted by Sridhar and Pillai (1969). Algal counts were made by Lacky's drop method using lugol's iodine solution and protozoa were enumerated by using plankton counting chamber called Sedgewick-Rafter cell (S-R cell) (Trivedy et al., 1998). Total bacterial count in the sewage was made by counting the colonies grown in Nutrient Agar media. Escherichia coli were enumerated by using MacConkey Agar media. Salmonella sp. and Staphylococcus aureus were enumerated by using Brilliant Green Agar media and Mannitol Salt Agar media respectively. Pseudomonas aeruginosa was enumerated by using Cetrimide Agar media. Fungal sp. was enumerated by using Czapeck Dox Agar media.

RESULTS

The data on the physico-chemical and biological parameters of the sewage samples both in the control and different concentrations of cadmium treated oxidation ponds on day 8 and 16 are presented in Table 1. The percentage reduction of BOD and Phosphate in control and ponds

Table 1: The Physico-chemical and Biological Parameters of the Sewage Sample Both in the Control and Different Concentrations of Cadmium Treated Oxidation Ponds on Day 8 and 16										
Days			8					16		
CdCl ₂	С	1	2	3	4	С	1	2	3	4
pН	8.0	8.2	7.9	7.6	7.4	8.9	9.0	8.4	8.2	8.0
TDS(mg/l)	590	650	655	655	665	710	700	730	785	800
DO(mg/l)	10	9.0	8.2	6.3	5.0	6.8	7.0	7.6	7.4	5.8
BOD(mg/l)	84	100	168	200	240	10	18.2	46.8	142	150
PO ₄ (mg/l)	3.6	4.5	5.0	6.0	6.7	2.8	3.0	4.0	5.1	6.0
Catalase*	30	35.32	20	11.97	10.36	22.45	23	8.94	10	2.42
Note: C = Control $1 = 0.5 \text{mg/l}, 2 = 1.0 \text{mg/l}, 3 = 5 \text{mg/l}, 4 = 10 \text{mg/l}; * Catalase units = Micromoles of hydrogen peroxide decomposed/100 ml of sample$										

treated with different concentration of cadmium are showed in Figure 1.



DISCUSSION

Physico-Chemical Parameters

The pH value of the raw sewage was 7.2 at zero hour increased to a maximum of 8.9 and 9.0 in control and 1.0 mg/L cadmium treated ponds respectively on day 16. The increase in pH value in the said ponds may be due to the increase of microbial growth. The Total Dissolved Solid (TDS) levels showed slight increase in all the ponds on day 16 which may also be due to the increase in the total microbial biomass. The Dissolved Oxygen (DO) showed decreasing trend and the Biological Oxygen Demand (BOD) showed increasing trend with the increase in cadmium treatment throughout the experimental period. The DO of the pond sewage was 3.0 mg/L at zero hour, increased to a maximum of 10.0 mg/L on day 8 in the control pond which may be due to the high photosynthetic activity by the algae.

The change of color of the sewage to yellow in the pond treated with highest concentration (10 mg/L) of cadmium indicates low level of algal growth due to the toxic effects (Patil *et al.*, 1986). The BOD value of sewage was 230 mg/L at zero hour, reduced to 10 mg/L in the control pond with the detention period of 16 days. Total phosphate level was decreased in control and in the ponds treated with lower concentrations of cadmium which indicates that phosphate was utilized more in these ponds due to the luxuriant growth of algae (Table 1).

Biological Parameters

The enzyme catalase detoxifies the hydrogen peroxide produced during oxidative metabolism of aerobic organisms. It was increased on day 8 in control and lowest metal treated ponds and lowest activity was recorded in the pond treated with highest concentration of cadmium. The inhibition of enzyme activity may be due to the formation of metal complexes and change in pH levels in the sewage. The level of catalase is used as a measure of water and wastewater quality (Gaddad et al., 1982).

The laboratory study showed that there was an exponential growth phase in control and 0.5 mg/L cadmium treated ponds. Anacystis sp. and Chlorella vulgaris showed more tolerance to the toxicity, whereas other species such as Zygnema pectinatum, Scenedesmus acuminatus and Euglena viridis were found to be more sensitive to cadmium treatment. Li (1978) carried research on Thalassiosira fluviatilis and reported that cell

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membrane was the main site of cadmium action and cadmium concentrations of 0.01-0.1 mg/L reduced the concentration of ATP and chlorophyll in that species. The comparison of protozoa growing in the control and treated ponds revealed that cadmium did not greatly affect some of the species. The species belongs to the genus Spasmostoma and Onchomonas showed more tolerance and some of the species like Podophrya fixa, Thylacomonas sp. and Pelomyxa palustris were recorded as sensitive protozoan to cadmium toxicity (Table 2).

The data on different bacteria and total fungi in sewage revealed that most of the pathogens were absent in the first 8 days and appeared on days 12 and 16. Pseudomonas aeruginosa and Fungal sp. were recorded more in the pond treated with lower concentrations of cadmium than in the control on day 12 and 16 which indicates that the conditions of lower concentrations of cadmium and later phase of the experiment were favorable

Table 2: The Tolerant and Sensitive Algae and Protozoa for Cadmium Toxicity									
Conc. of cadmium	Tolerant	sp.	Sensitive sp.						
(mg/l)	Algae	Protozoa	Algae	Protozoa					
Control	Anacystis sp. Chlorella vulgaris Oscillatoria brevis	Spasmostoma sp. Onchomonas sp. Balantidium coli	Ulothrix zonata Scenedesmus acuminatus Euglena viridis	Acanthamoeba sp. Oikomonas termo Thylacomonas compressa					
0.5 mg/l	Scenedesmus acuminatusAnacystis sp. Chlorella vulgaris	Spasmostoma sp. Onchomonas sp. Stylonychia sp.	Microcystis sp. Lyngbya martensiana Coscinodiscus sp.	Pelomyxa palustris Acanthamoeba sp.					
1.0 mg/l	Chlorella vulgaris Anacystis sp. Oscillatoria brevis	Onchomonas sp. Balantidium coli	Diatoma hiemale Zygnema pectinatum Lyngbya martensiana	Podophrya fixa Centropyxis arcelloides					
5 mg/l	Chlorella vulgaris Anacystis sp.	Spasmostoma sp. Balantidium coli	Scenedesmus acuminatus Oscillatoria brevis Euglena viridis	Pelomyxa palustris Stylonychia sp. Acanthamoeba sp.					
10 mg/l	Anacystis sp. Chlorella vulgaris	Spasmostoma sp. Balantidium coli	Zygnema pectinatum Scenedesmus acuminatus Euglena viridis	Podophrya fixa Thylacomonas compressa Pelomyxa palustris					

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for these organisms. *Escherichia coli* were reported only till day 8 in the pond treated with lower concentrations. The study on removal of pathogens from wastewater revealed that increased pH, predation by zooplankton and the formation given by the incident of light are the reasons for reducing the number of pathogens in water (Curtis *et al.*, 1994).

CONCLUSION

The study on the effect of cadmium on the physico-chemical and biological parameters revealed that lower concentrations of cadmium influence the growth of algae and fungi. In higher concentrations, some of the species like *Anacystis* sp. (algae), *Spasmostoma* sp. (protozoa), *Pseudomonas aeruginosa* (bacteria) were showed tolerance to cadmium. Oxidation pond provides useful means of measuring variations in physico-chemical parameters of sewage and also changes in algal, protozoan, bacterial and fungal diversity in presence of cadmium. This study also confirms that oxidation pond is one of the best biological control methods for wastewater treatment.

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