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Research Paper

IMPACT OF IRON ON THE BIODIVERSITY OF ALGAE AND PROTOZOA IN OXIDATION PONDS

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Studies were carried out on the toxicity of iron on the physico-chemical and biological parameters of oxidation ponds which are used as one of the biological treatment method to purify wastewater. The level of pH, DO and density of algae and protozoa were decreased and BOD and phosphate concentrations were increased as the concentration of iron was increased in the oxidation pond. The percentage removal of BOD on day 20 was 62.5%, 56.9%, 43.8%, 32.5% and 28.8% respectively in control, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of $FeSO_4$ treatment. The percentage removal of phosphate was 40%, 37.5%, 35% 30% and 25% respectively in control, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of $FeSO_4$ treatment. The activity of enzymes namely catalase, amylase, protease and phosphatase were reduced in ponds treated with higher concentration of iron. The assessment of iron toxicity on the predominance of algae and protozoa showed that *Scenedesmus acuminatus* and *Vorticella campanula* were recorded as most tolerant species of alga and protozoa respectively, whereas *Oscillatoria brevis* and *Podophrya fixa* were recorded as the most sensitive species of alga and protozoa respectively for ferrous sulphate treatment.

Keywords: Oxidation pond, Algae, Protozoa, Iron, Enzymes

INTRODUCTION

Iron is the fourth most abundant element in the earth's crust. Its greatest use is for structural iron and steel, but it is also used for making dyes and abrasives. It is an essential micronutrient required in trace quantities for the normal metabolism of plants and animals. It is a constituent of cytochromes and non-heme iron proteins involved in photosynthesis, nitrogen fixation and respiratory linked dehydrogenases (Noggle and Fritz, 1986). Ingestion of excessive amounts may inhibit the activity of many enzymes. The amount consumed must be very large, because only a small proportion of all iron ingested is absorbed from the gastrointestinal tract. Inhalation of iron dust can cause benign pneumoconiosis and can enhance harmful effects of sulphur dioxide and various carcinogens.

Iron plays a vital role in oxygen transport and energy production. It is an essential element in

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the daily diet. Iron in the human body is almost exclusively involved in the uptake and release of oxygen at the cellular level (Bothwell, 1995). The average adult body contains about 4 g of iron. Out of this, 3 g in active or functional form and about 1 g in storage or transport form. Most of the active iron forms integral parts of hemoglobin, myoglobin, and the enzymes cytochrome, catalase and peroxidase. The majority of this iron is found in the hemoglobin of red blood cells (erythrocytes). The hemoglobin molecule's ability to take up and release gases is dependent on the presence of iron. Myoglobin works on a similar mechanism, but it takes up and releases oxygen only in the muscle. The cytochrome enzyme system functions in energy production (Bothwell, 1995). Iron is an essential nutrient for the growth of the malaria parasite, *Plasmodium falciparum*. The iron-chelating agent desferrioxamine given as a constant intravenous infusion for 72 h enhances clearance of *Plasmodium falciparum* (Gordeuk *et al.*, 1992).

Many streams are poisoned by high levels of iron in acid mine drainage. Pyrite (iron sulphide), is often found in close association with coal deposits. Upon exposure to moisture and atmospheric oxygen, the ferrous ion is oxidized to the ferric state, a reaction which is frequently accelerated by bacteria of the *Thiobacillus-Ferrobacillus* group. The ferric ion can then react with sulphide in the presence of water to produce sulphuric acid, or react directly with water to produce a yellow, flocculent mass of ferric hydroxide. Besides being acidic, water affected this way becomes deficient in oxygen. Such poisoning of streams is reckoned to be one of the main causes of fish kill. Although particularly associated with mining, streams running through iron-laden strata may become poisoned spontaneously and severely affect the microorganisms present in the aquatic body.

Oxidation pond is a simple scientifically designed pond with 2-6 feet depth, where BOD reduction of sewage takes place by supporting algal-bacterial growth cycle (Hosetti *et al*., 1985; Tharavathi and Hosetti, 2003). These are considered as one of the secondary treatment of sewage and are commonly used in warm climates to purify wastewater. Many literatures are available on the impact of heavy metals like Zn, Pb, Cu and Cr on oxidation pond but very little work was done on Fe. Hence the present work is focused on the impact of iron on the physico-chemical and biological parameters and also on the biodiversity of algae and protozoa grown in oxidation pond.

MATERIALS AND METHODS

The experiment was designed according to the procedure of Patil (1986). The heavy metal salt, ferrous sulphate ($FeSO_4.7H_2O$) manufactured by New India Chemical Enterprises, Cochin was selected for the present 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 iron treatment was taken as control. The second, third, fourth and fifth set of plastic containers were treated with 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of ferrous sulphate respectively which were selected from range finding test. To all the oxidation ponds, algae and protozoan communities collected from natural oxidation pond were introduced in equal quantities and all the ponds were kept in open sunlight. The experiments were carried out for 20 days. Observations are made by collecting 150 ml of

sample from each oxidation pond on every fifth day and were analyzed for pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and phosphate (PO_4) by following the standard methods prescribed in APHA (1995). Catalase activity was determined according to the method of Luck (1974). Amylase and phosphatase activities were determined according to the procedure of Sadasivam and Manikam (1992). Protease activity was determined according to the procedure of Jayaraman (1985). Algal counts were made by Lacky's drop method using lugol's iodine solution and protozoa were enumerated by using a plankton counting device called Sedgewick-Rafter Cell (S-R cell) (Trivedy *et al.*, 1998). The data was subjected to statistical analysis.

RESULTS

The data of the physico-chemical and biological characteristics of the sewage samples both in the control and different concentrations of iron treated oxidation ponds on day 10 and 20 are shown in Table 1. The list of tolerant and sensitive algae and protozoa for iron toxicity is shown in Table 2. The correlation coefficient values of physico-chemical and biological characteristics of sewage in oxidation pond on day 20 are presented in Table 3. The percentage reduction of BOD and PO_4 at different concentrations of $FeSO_4$ was shown in Figure 1. The percentage mortality of algae and protozoa on day 20 are shown in the Figure 2.

DISCUSSION

Physico-Chemical Parameters

Contamination of water with heavy metals and other toxicants may appreciably alter the physico-chemical characteristics. Many times transformed products of the toxicants may prove more toxic to the aquatic life (Madhyastha *et al.*, 1996). In the present study also there was a drastic change in physico-chemical and biological properties of sewage in the four concentrations

Table 1: Physico-chemical and Biological Parameters in Control and Different Concentrations of $FeSO_4$ Treated Stabilization Pond Samples on Day 10 and 20 (Av ± SD)

Days	10					20				
Concentration of $FeSO_4$	Control	25 mg/l	50 mg/l	75 mg/l	100 mg/l	Control	25 mg/l	50 mg/l	75 mg/l	100 mg/l
pH	7.4 ±0.16	7.2 ±0.16	7.1 ±0.16	7.0 ±0.12	7.0 ±0.12	7.4 ±0.12	7.2 ±0.12	7.0 ±0.12	6.9 ±0.08	6.9 ±0.08
DO (mg/l)	11.2 ±0.08	10.6 ±0.16	9.6 ±0.16	9.2 ±0.16	8.4 ±0.16	8.8 ±0.08	8.0 ±0.24	6.8 ±0.24	5.6 ±0.16	4.8 ±0.16
BOD (mg/l)	252 ±0.24	270 ±0.24	270 ±0.49	288 ±0.49	294 ±0.49	120 ±0.16	138 ±0.33	180 ±0.33	216 ±0.49	228 ±0.49
Phosphate (mg/l)	1.5 ±0.08	1.7 ±0.08	1.78 ±0.16	1.8 ±0.16	1.81 ±0.16	1.2 ±0.08	1.25 ±0.16	1.3 ±0.16	1.4 ±0.12	1.5 ±0.12
Catalase[a]	10 ±0.16	9.17 ±0.57	9.17 ±0.57	8.33 ±0.57	7.5 ±0.24	9.17 =0.08	9.17 ±0.08	8.33 =0.08	6.67 ±0.08	5.83 ±0.08
Amylase[b]	30±0.33	25±0.16	20±0.16	18±0.57	18±0.57	25.0±0.16	23.0±0.16	19.0±0.24	12.0±0.24	10.0±0.24
Protease[c]	10.0±0.16	9.6±0.16	9.0±0.16	9.0±0.08	9.0±0.08	9.6±0.08	9.4±0.16	9.2±0.16	8.9±0.16	8.0±0.16
Phosphatase[d]	3.8±0.08	3.0±0.16	3.0±0.16	2.6±0.16	2.5±0.16	3.0±0.08	3.0±0.08	2.8±0.08	2.0±0.08	1.8±0.08
Algae/ml	200 ±2.45	180 ±2.45	180 ±1.63	156 ±1.63	150 ±1.63	140 ±1.63	140 ±1.63	140 ±2.45	130 ±2.45	100 ±2.45
Protozoa/ml	100 ±2.45	98 ±1.63	96 ±0.82	94 ±0.82	92 ±0.82	100 ±3.27	90 ±3.27	80 ±1.63	80 ±1.63	70 ±1.63

Note: [a] – µg moles of H_2O_2 decomposed min^{-1} 100 ml^{-1} of sample; [b] – µg of maltose liberated min^{-1} 100 ml^{-1} of sample; [c] – µg of tryptophan liberated min^{-1} 100 ml^{-1} of sample; d- ¼ g of paranitrophenol liberated min^{-1} 100 ml^{-1} of sample.

Table 2: List of Tolerant and Sensitive Algae and Protozoa for $FeSO_4$ Toxicity

Tolerant Species		Sensitive Species	
Algae	**Protozoa**	**Algae**	**Protozoa**
Scenedesmus acuminatus	*Vorticella campanula*	*Oscillatoria brevis*	*Podophrya fixa*
Chlorella vulgaris	*Oikomonas termo*	*Anacystis sp.*	*Colpoda cucullus*
Lyngbya martensiana	*Balantidium coli*	*Nitzschia palea*	*Paramecium caudatum*
Navicula lanceolata	*Pelomyxa palustris*	*Ulothrix zonata*	*Stylonychia pastuluta*
Anabaena oscillarioides		*Pinnularia viridis*	*Acanthamoeba castellanii*
Phacus suecicus		*Euglena viridis*	

Table 3: Correlation Coefficient Values of Physico-Chemical and Biological Characteristics of Sewage in Oxidation Pond on day 20

Parameters	pH	DO	BOD	Phosp.	Catal.	Amyl.	Prote.	Phosph.	Algae	Proto.
pH	1.000									
DO	+0.950	1.000								
BOD	+0.929	+0.935	1.000							
Phosphate	+0.018	+0.279	+0.356	1.000						
Catalase	+0.850	+0.968	+0.984	+0.573	1.000					
Amylase	+0.920	+0.991	+0.998	+0.354	+0.987	1.000				
Protease	+0.777	+0.920	+0.912	+0.422	+0.937	+0.907	1.000			
Phosphatase	+0.824	+0.948	+0.969	+0.492	+0.994	+0.980	+0.907	1.000		
Algae	+0.599	+0.805	+0.808	+0.553	+0.874	-0.808	+0.968	+0.852	1.000	
Protozoa	+0.950	+0.957	+0.926	+0.101	+0.870	+0.909	+0.897	+0.822	+0.760	1.000

Note: + indicates positive correlation between two parameters; All values are significant at 5% level.

of iron treated oxidation ponds. The pH values of the sewage was 7.0±0.08 in the first day of the experiment increased to 7.4 ±0.08, 7.3±0.08, 7.2±0.08, 7.2±0.08 and 7.2±0.08 respectively in control, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of $FeSO_4$ treated oxidation ponds on day 5. The increase in the pH values in these ponds may be due to the increased microbial activity, which is also due to the degradation of organic content of the sewage into ammoniacal nitrogen and phosphates by the bacteria and fungi. These nutrients are absorbed by the proliferating algae, which aerate the effluent of oxidation pond. (Tharavathy and Hosettti, 1998).

DO and BOD values showed opposite trend. DO level decrease with the increase in iron treatment throughout the experimental period. This indicates that the algal species could not sustain the toxic effects of the metal and reduced photosynthesis in the ponds treated with higher concentrations of $FeSO_4$. The BOD level

Figure 1: The Percentage Reduction of BOD and Phosphate at Different Concentration of Ferrous Sulphate

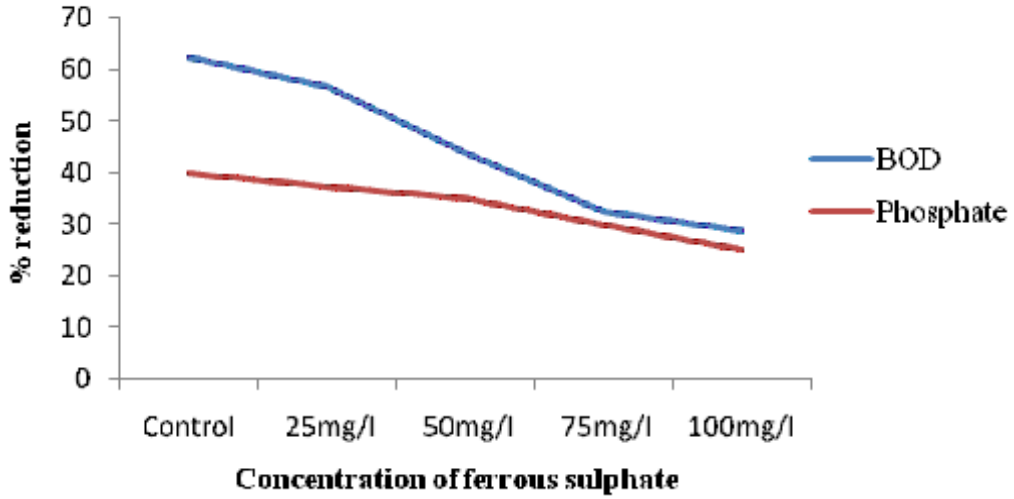
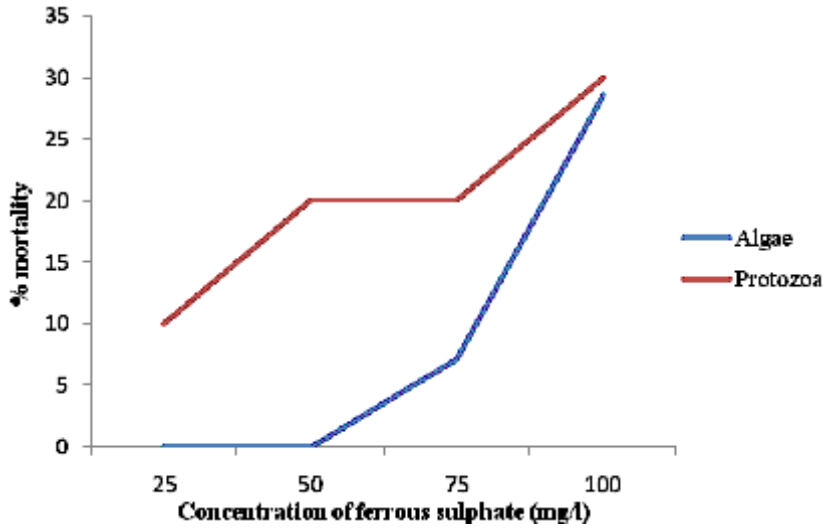


Figure 2: The Percentage Mortality of Algae and Protozoa at Different Concentration of Ferrous Sulphate



increased with the increase in iron treatment. The percentage removal of BOD on day 20 was 62.5%, 56.9% 43.8%, 32.5% and 28.8% respectively in control, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of $FeSO_4$ treatment. According to these values, the effective dose of ferrous sulphate was recorded as 39.06 mg/l of $FeSO_4$. The phosphates are important nutrients required for phytoplankton growth in oxidation ponds. It was 2.0±0.08 mg/l in sewage at 0 h and reduced gradually in the control and 25 mg/l $FeSO_4$ treatment due to the increased growth of phytoplankton in these ponds. The percentage removal of phosphate was 40%, 37.5%, 35%, 30% and 25% in control, 25 mg/l, 50 mg/l, 75 mg/l and 100 mg/l of $FeSO_4$ treatment.

Biological Parameters

All the enzyme activities are pH dependent and any change in the pH values in the effluent of oxidation pond will affect the effluent quality. The enzyme activities, namely catalase, amylase, protease and phosphatase recorded respectively 5.83±0.08 units, 10.0±0.16 units, 8±0.24 units and 1.8±0.08 units in the raw sewage. However, all the enzymes showed increased activity in the experimental ponds and there was a decline in their activity in the ponds treated with higher concentration of iron. This may be due to the formation of enzyme-metal complexes. Catalase is a hydrolytic enzyme used for the evaluation of effluents (Hosetti and Patil, 1988; Gaddad and Hosetti, 198; Hosetti and Frost, 1998 a,b) and river water quality (Hosetti and Birasal, 1989). Aerobic cells, during oxidative respiration, produce toxic secondary metabolites like alcohol, hydrogen peroxide and thiols. The aerobic cells also produce certain hydrolytic enzymes which breakdown and detoxify all the secondary by-products produce during oxidative respiration. The hydrogen peroxide is broken down into water and oxygen, by the action of the catalase produced by the cell itself. This particular phenomenon of detoxification of hydrogen peroxide by the action of catalase is used in the evaluation of water and waste water quality in the present study which was also used by the earlier workers (Gaddad *et al.*, 1982; Hosetti and Frost, 1994).

The growth inhibitory effect on algae and protozoa was maximum at 100 mg/l of $FeSO_4$ treatment. The percentage mortality of algae and protozoa on day 20 was 28.6% and 30% respectively in this pond. Municipal wastes and industrial effluents contribute number of heavy metals to the aquatic environment. Survival of aquatic organisms exposed to heavy metals depends upon their tolerance capacity (Konar, 1975). The assessment of iron toxicity on the predominance of algae and protozoa showed that *Scenedesmus acuminatus* and *Vorticella campanula* were recorded as most tolerant species of alga and protozoa, respectively, whereas *Oscillatoria brevis* and *Podophrya fixa* were recorded as the most sensitive species of alga and protozoa, respectively for ferrous sulphate treatment. The diversity of species in oxidation ponds depends on the photosynthetic activity of algae. Toxic substances like metals in the sewage may inhibit the activity of algae and disturb the normal operation of the oxidation ponds (Mara and Pearson, 1986).

The correlation matrix showed that all four enzymes exhibited significant positive correlation with all physico-chemical and biological parameters. Similar results were also reported by Tharavathy *et al.* (2003) during the study of the impact of copper on the biodiversity of algae and protozoa grown in oxidation ponds. This showed that any changes in the value of physico-chemical and biological parameter reflect the activity of enzymes namely catalase, amylase, protease and phosphatase.

CONCLUSION

Iron plays an important role in changing the physico-chemical and biological parameters in sewage of oxidation ponds. The assessment of iron toxicity on the predominance of algae and protozoa showed that *Scenedesmus acuminatus* and *Vorticella campanula* were recorded as most tolerant species of alga and protozoa respectively, whereas *Oscillatoria brevis* and *Podophrya fixa* were recorded as the most sensitive species of alga and protozoa respectively for ferrous sulphate treatment. Based on the % mortality of

algae and protozoa grown in oxidation pond, the LC_{50} value for algae is 175 mg/l and for protozoa it is 167 mg/l of $FeSO_4$. Even though iron is very important for the biological function, in excess quantity they are injurious to algae and protozoa.

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