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ORIGINAL ARTICLE

Waste water treatment and metal (Pb^{2+}, Zn^{2+}) removal by microalgal based stabilization pond system

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Abstract A case study was undertaken for the treatment of domestic wastewater generated at village of Sanghol, Distt. Fatehgarh Sahib, Punjab (India), using a schematic designed algal and duckweed based stabilization pond system, which is discussed here for winter months only (November to March) as there was no growth of duckweeds and only algae dominated the whole system. A proficient increase in pH and dissolved oxygen was observed after the treatment with reduction in chemical oxygen demand and biochemical oxygen demand by 93% and 79% respectively. *Chlorella* sp. was the dominating algal species in the stabilization pond water during entire period and was studied for its Zn^{2+} and Pb^{2+} metal removal efficiency. 60–70% removal of Zn^{2+} was observed from culture medium containing 5-20 mg L^{-1} Zn^{2+}, which declined to 42% at 50 mg L^{-1}. A constant decline in cell number from 538×10^5 to 8×10^5 cells ml^{-1} was observed indicating zinc toxicity to *Chlorella*. Lead was maximally removed by 66.3% from culture medium containing 1 mg L^{-1}. The lead removal efficiency was 45 to 50 % at higher 5 to 20 mg L^{-1} of external lead concentrations. The increase in cell number indicated no signs of Pb^{2+} toxicity up to 20 mg L^{-1}. The maximum uptake (q_{max}) by live *Chlorella* biomass for both Zn^{2+} and Pb^{2+} was 34.4 and 41.8 mg/g respectively.

Keywords *Chlorella* sp. · Waste-water stabilization pond system · Chemical oxygen demand · Biochemical oxygen demand · Lead

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Introduction

Uncontrolled discharge of untreated domestic and industrial wastewater contaminates both surface and underground water by releasing huge amount of wastewater with high BOD and COD loading whereas, metal processing and electroplating industries add various heavy metals (Cd, Cr, Co, Cu, Pb, Mn, Zn, Hg and V) in it resulting in high levels of water pollution. With the limited availability and increasing pollution of the ground water, it has become imperative to make the water reusable by removing the pollutants and therefore wastewater treatment has become both an ecological as well as economical necessity.

Presently the application of conventional wastewater treatment systems in countries with low GNP is limited because of high cost and technological complexity. Worldwide, there is a continuous interest in algal-based waste stabilization pond systems that are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants [1]. However, high algal concentration of about 100 mg TSS L^{-1} may be occasionally reached in the effluent [2], causing severe clogging problems in drip irrigation system [3]. These type of sustainable technologies for wastewater treatment, which are within the economical and technological capabilities of developing countries, need to be developed further.

Cyanobacteria and microalgae play an important role in system since they supply molecular oxygen to heterotrophic partners and thus support the initial steps of degradation [4]. Nutrient removal with aid of algae compares very favorable to other conventional technologies [5–8] moreover, some cyanobacteria and algae might remove xenobiotics from the environment by sorption, transformation and degradation [9]. Treatment of both domestic and agricultural wastewater by algae has been investigated thoroughly by Oswald in 1988 [10], where in several attempts have been made to explore the efficiency of microalgae for metal removal [11–13]. It is obvious that a system consisting of several micro-organisms is preferably in bioremediation processes since it is nearly impossible to find a microorganism species that can degrade a mixture of different pollutants completely by itself [14]. The performance of oxidation pond is dependent on the biotic community present [15] and the biotic community exposed to pollutants. Among the organisms exposed to metal pollution in the lake or pond water, phytoplankton like *Chlorella vulgaris* are the most affected. Thus, it can be a potential candidate for metal bioremediation studies. This green alga has been well studied for various metabolic and stress investigations because of its small size, fast growth rate, and similarity to plants higher in terms of physiology and biochemistry [16].

Cold climate is normally not favorable for phycoremediation due to the poor light availability and low temperature, however Gronlund in 2004 have successfully demonstrated the feasibility of using certain micro algae with special attributes to treat wastewater. Erik Gronlund [17] used *Coelastrum, Chlamydomonas, Chlorella, Selenastrum, Scenedesmus and Micractinium pusillum* in high rate algal ponds with artificial illumination and covered with green house plastic. Tang and his co-workers [18] compared polar cyanobacteria and *Chlorococcales* for tertiary wastewater treatment. The cyanobacteria are preferred due to their flocculation properties, active growth and nitrate and phosphate uptake at low temperature. Craggs and his co-workers [19] showed in Scotland the possibilities of treating wastewater by diluting it with seawater and using macro algae (*Phaeodactylum tricornutum*).

With this approach, present investigation was done to assess wastewater treatment potential of a stabilization pond in cold weather for treatment of domestic wastewater generated from the village of Sanghol, Distt. Fatehgarh Sahib, Punjab (India) and the most dominating alga *Chlorella* sp. of pond was studied for its metal (Pb^{2+} and Zn^{2+}) removal and metal uptake potential.

Material and methods

Sampling Procedure: The surface water sample was collected after 30 days interval from Equalization Tank, Pond 1 and Pond 2 of the stabilization pond situated at village of Sanghol Distt. Fatehgarh Sahib, Punjab (India). Ponds are natural ponds meant to polish the domestic effluent. The effluent after treatment in Pond 1 with algae and duckweeds flows into the pond 2 where treated water is used for culturing of fishes. The study was contended in winter months only (November to March) in which only algae dominate the whole system. Wastewater samples for algal diversity analysis were collected from the euphotic depth. The effluent samples for estimation of dissolved oxygen were fixed immediately after collection using manganese sulphate and alkali-iodide-azide reagents [20]. Parameters such as water temperature, conductivity, salinity and dissolved oxygen (DO) were recorded using probes from Thermo Orion Model 150 and 125 respectively.

Analytical Methods: The pH, BOD_5 and COD were measured as per the protocol given in Standard Methods [20]. Heavy metals (Cr, Pb and Zn) analysis was done after filtering wastewater by whatman filter paper no. 42 (Whatman International Ltd, Maidstone, England) and acidifying using concentrated HCl by atomic absorption spectrophotometer (GBC 932 AA, APHA, Australia). Bacterial count was estimated by standard plate count agar method on nutrient agar plates following serial dilution technique [21]. Identification of algae in pond wastewater was done following monographs on algae [22, 23].

Isolation, characterization and purification of Chlorella sp.*:* Pure culture of *Chlorella* sp. was isolated from wastewater samples and strain was identified based on their morpho-cytology and growth response in BG-11medium [24]. It was purified by series of streak plating on 1.5% agarized BG-11 medium. Axenicity was regularly checked by inoculating the culture in BG-11 medium with 0.1% yeast extract and 0.1% glucose.

Lead and Zinc removal experiments: Metal ion solutions were prepared by diluting 1 g L^{-1} of stock solutions, which were obtained by dissolving weight quantity of zinc sulphate heptahydrate (4.39 gm) and lead nitrate (1.59 gm) of analytical grade in double distilled water. Metal sorption studies were carried out to evaluate the capacity of live *Chlorella* sp. to adsorb metal ions form solution. In batch process, 50 ml of BG-11media without EDTA (Ethedium diamine tetra acetic acid, which act as metal chelating agent) [25] having different concentration of metal ions (1, 5, 10, 20 and 50 mg L^{-1}) for zinc or lead was taken in 250 ml Erlenmeyer flasks and each inoculated with 1 ml of log phase culture. The samples withdrawn from each flask after inoculation represented the initial metal concentration in the solutions (C_i). Erlenmeyer flasks were kept under shaking condition in growth room maintained at 28 ± 2°C at 3000–3500 lux, light intensity provided by cool white daylight fluorescent lamps.

At end-point of experiment after 12 days, cultures were filtered and the final metal concentration (C_f) in the filtrate was analysed using atomic absorption spectrophotometer (*GBC 932 AA; GBC Scientific Equipment Pvt. Ltd., Australia*). The salts and chemical used for stock solutions were procured from S.D. fine chemical ltd. Bombay.

The metal uptake (q) [26] by the algae and bioremoval efficiency (R) [27] of the algae were calculated by the following formulae.

$$q = \frac{(C_i - C_f)V}{M} \quad (1)$$

$$R(\%) = \frac{(C_i - C_f)}{C_i} \times 100 \quad (2)$$

where q = Metal uptake (mg/g); M = dry mass of algae (g); V = Volume of culture media (L); R = Bioremoval efficiency (%); C_i = Initial Conc. of metal in aqueous solution (mg L^{-1}); C_f = final Conc. of metal in aqueous solution (mg L^{-1}).

Algal Cell Count: Unicellular culture of *Chlorella* sp. was counted using improved Neubauer haemocytometer which is an etched glass chamber with raised sides that will hold a quartz coverslip exactly 0.1 mm above the chamber floor. The counting chamber is etched in a total surface area of 9mm^2. To determine the average number of unicellular algal cells per ml, algal (unicellular) culture was stained with a drop of Lugol's reagent (An aqueous solution of 5 g iodine and 10 g potassium iodide in 100 ml water which stain starch granules to make cells darker and also heavier for easier to visualize) and appropriately diluted. The coverslip was placed on counting chamber carefully before one drop of cell suspension was put on one side of the central groove on haemocytometer with 1 ml pipette which fills the gap between coverslip and the chamber. The whole chamber has 9 squares where the corner squares have 4 × 4 subdivisions. The square is 1mm^2 and the chamber depth is 0.1 mm; therefore the volume of each square is 0.1mm^3 (0.0001ml = 0.1µl). The average number of cells counted was per square (total cell counted / number of squares used).

The total number of cells was calculated by the following formula,

$$N \times DF \times 10^4 = \text{cells ml}^{-1} \quad (3)$$

where N is the average number of cells per square (0.1 mm^3 = 0.1µl), DF is the dilution factor for the culture.

Result and discussion

Characterization of waste water and microalgae from algal stabilization pond: A case study was undertaken for treatment of domestic wastewater generated from the village of Sanghol, Distt. Fatehgarh Sahib, Punjab (India), using a schematic designed algal and duckweed based stabilization pond system which was contended to winter months (November to March) in which midday water temperature varied from 17.7 to 24.4°C so no growth of duckweeds was observed and only algae dominated. The wastewater generated by the village community was applied directly to the pond 1 after passing through an equalization tank where the settling of sludge takes place. Pond 1 of the system was used to treat the wastewater where in algae utilize inorganic nutrient for their growth by lowering chemical oxygen demand and nascent oxygen produced during photosynthesis augments complete oxidation of organic fractions resulting in lowering of biochemical oxygen demand. Treated water was transferred to pond 2 after filtration of algae which was further used for fish culturing and irrigation.

Monthly analysis of wastewater samples for temperature, pH, conductivity, salinity, dissolved oxygen, COD and BOD_5 collected from pond 1 (inlet) and pond 2 (outlet) showed the treatment potential of this system in cold weather (Table 1). The results showed the significant increase in pH and DO values by the treatment where both measured at the same time for inlet and outlet wastewater. Taking the average of five months, the pH of wastewater increased from 8.34 to 9.56 with showing an increase of more than one unit. Similarly a considerable increase in dissolved oxygen was observed from an average of 2.83 mg $L^{-1}$of inlet water to 10.99 mg $L^{-1}$of outlet water (Table 1). In a similar study conducted on microalgal based ponds Msuya and his co-workers observed an increase in DO from 5.3 to 8.7 after treatment [28]. COD and BOD_5 values were higher in the inlet water than the treated wastewater with percent reduction in COD varied from minimum 41% to maximum 93%. Similarly BOD_5 was reduced with a minimum 53.91% to maximum of 79.12% during 5 month observation period showing clear reduction in the inorganic and organic material (Table 1). The pollutant removal efficiency of algae and duckweed-based pond systems has been shown to vary widely and it depends on retention time, water depth, initial nutrient concentration, microflora and harvesting regimes [29]. The culturable bacterial cell count in terms of colony forming units per milliliter of wastewater was also drastically reduced in wastewater after treatment than count in untreated wastewater and it was comparatively higher in both treated and untreated wastewater during December and January which could be correlated with high BOD_5 values (Table 1), however bioactivity of a complex waste is probably related to interactions among components with no substance having dominant effect [30].

Algal species composition of the pond indicated that *Chlorella* sp. was (present during the whole sampling

Table 1 Characterization of wastewater sample (Inlet and outlet) collected from Algal Stabilization Pond Sanghol village, Dist. Fatehgarh Sahib, Punjab, India during November, 2004 to March, 2005

Parameters analysed		Month				
		Nov	Dec	Jan	Feb	Mar
Temp °C	Inlet	17.7	17.5	12.7	22.5	24.2
	Outlet	19.2	19.2	12.0	22.9	24.5
pH	Inlet	8.11	8.17	8.23	8.57	8.62
	Outlet	9.12	10.72	9.22	9.26	9.36
Cond (mS cm^{-1})	Inlet	3.53	4.00	4.49	1.94	4.67
	Outlet	1.78	1.88	1.97	1.75	1.89
Salinity (‰)	Inlet	1.8	2.1	2.3	1.0	2.5
	Outlet	0.9	1.0	1.0	0.9	1.0
DO (mg/L^{-1})	Inlet	2.23	3.47	4.04	3.24	1.20
	Outlet	10.08	11.60	12.00	11.50	9.80
COD (mg L^{-1})	Inlet	-	183.2	278.4	272.0	373.3
	Outlet	-	12.8	51.2	128.0	213.3
BOD_5 (mg L^{-1})	Inlet	165.4	152.1	365.8	-	320.2
	Outlet	46.7	70.1	76.3	-	104.6
Plate count (cfu $\times 10^3$ ml^{-1})	Inlet	85.5	65.0	370.0	110.0	87.0
	Outlet	5.0	31.6	73.0	86.0	11.0
Zn (mg L^{-1})	Inlet	0.25	<0.02	<0.02	<0.02	0.04
	Outlet	0.07	<0.02	<0.02	<0.02	<0.02
Pb (mg L^{-1})	Inlet	0.14	<0.02	<0.02	<0.02	0.18
	Outlet	0.11	<0.02	<0.02	<0.02	0.05

- Data not available

Table 2 Metal uptake potential (*q*) of *Chlorella sp.* at variable concentrations of heavy metals in culture medium after 12 days of incubation

Metal (mg L^{-1})	Metal Uptake *q* (mg g^{-1})	
	Pb^{2+}	Zn^{2+}
1	3.08	0.07
5	14.14	6.01
10	17.66	8.44
20	34.36	19.8
50	-	41.75

period as) one of the most dominating species followed by *Chlamydomonas*, *Lyngbya* and *Diatoms*. High algal diversity results in efficient nutrient removal from primary settled wastewater [31]. Cold climate is normally not favorable for bioremediation of wastewater in pond system due to poor light availability and low temperature, however Gronlund in 2004 successfully demonstrated the feasibility of using certain micro algae with special attributes to treat wastewater [17]. The performance of stabilization pond is dependent on the biotic community present in treatment pond, which is continuously exposed to pollutants. It has been established that the toxic chemical stress causes large changes in community structure [32]. In aquatic ecosystem new population can rapidly grow to replace the pollution damaged population. The relationship between the pollutant and the algal community in these systems is not well understood, where sometimes the changes in nutrient and pollution levels do not alter the algal species composition of and sometimes it does [33]. The response of the same species to similar enrichment condition differs, thus it is suggested that the parameters other than nutrients and pollution are more important in determining the species composition.

Metal removal by microalgae from algal stabilization pond: Algae are known for their capacity to accumulate heavy metals from wastewater since heavy metals such as Zn, Cu, Fe, Mn, Co and Mo are required as essential nutrients [34]. To determine the removal and accumulation potential of *Chlorella* sp. which dominated the stabilization pond system during 5 months period, was isolate as pure culture and studied for its Pb^{2+} and Zn^{2+} removal over a concentrations range of 1–50 mg L^{-1} maintained in culture medium under flask conditions at average pH (9 ± 0.2) of pond water. *Chlorella* sp. showed high removal efficiency for Zn^{2+} followed by Pb^{2+} where 60–70% removal of Zn^{2+}

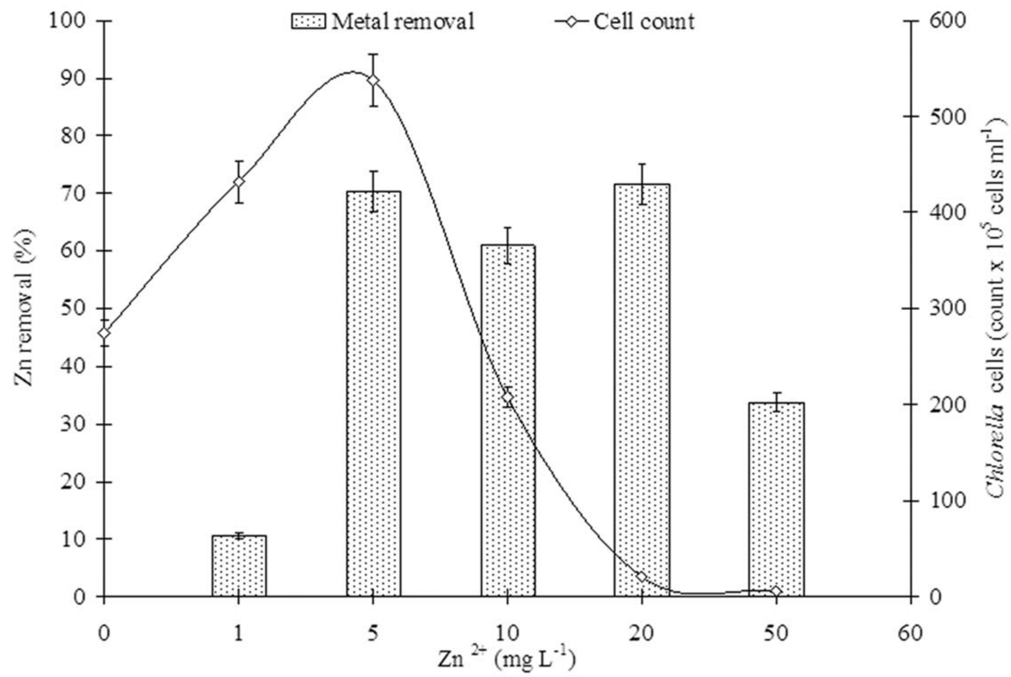

Fig. 1 Effect of Zn^{2+} concentration on metal removal and cell count of *Chlorella* sp.

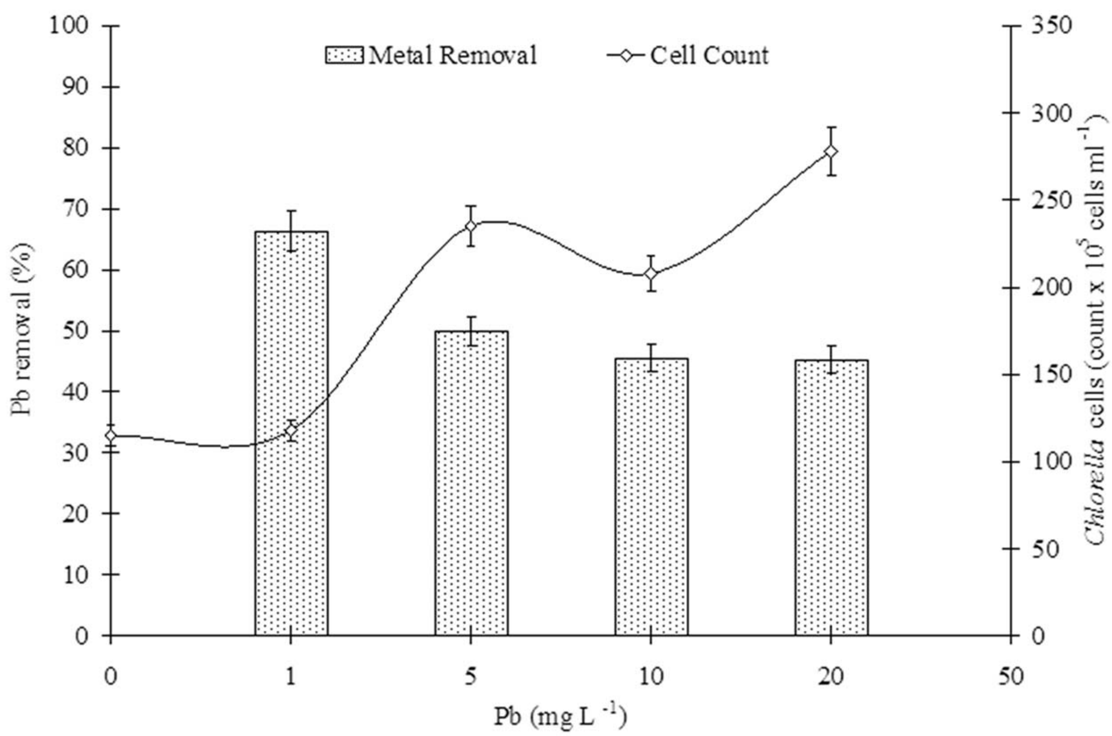

Fig. 2 Effect of Pb^{2+} concentration on metal removal and cell count of *Chlorella* sp.

was observed from culture medium concentration from 5–20 mg L^{-1} Zn^{2+} which declined to 42% at 50 mg L^{-1} (Fig. 1) A constant decline in cell number from 538 × 10^5 to 8 × 10^5 cells ml^{-1} was observed indicating zinc toxicity to *Chlorella* (Fig. 1). Interestingly, such decrease of cell count was not affecting the Zn^{2+} removal potential till 20 mg L^{-1}, supporting the contribution of dead biomass in metal removal. This growth inhibition in microalgae is related to the amount of heavy metal ions bound to the algal cell surface, and also, to the amount of intracellular heavy metal ions [35]. However, for zinc, the growth inhibition may be related to extracellular zinc concentration [36]. Lead was maximally removed to 66.3% from culture medium containing 1 mg L^{-1}. 45 to 50% removal efficiency was maintained for 5 to 20 mg L^{-1} of external lead concentration. The increase in cell number indicated no signs of Pb^{2+} toxicity up to 20 mg L^{-1} (Fig. 2). In the present study the percentage Pb^{2+} removal was higher as compared to 32.15% and 46.01% by two *Chlorella* strains WB and SB respectively isolated from Laguna de Bay, Philippines [37].

The specific metal uptake (q) for both Zn^{2+} and Pb^{2+} increased concomitantly with increase in metal concentration in medium (Table 2) with maximum metal uptake (q_{max}) of 34.36 and 41.75 mg g^{-1} for Zn^{2+} and Pb^{2+} respectively as calculated by mass balance for live *Chlorella* biomass. The *Chlorella* sp. under study showed better Pb^{2+} and Zn^{2+}, uptake potential than reported by Sandau and his co-workers [38] who observed 17.2 and 6.60 mg g^{-1} of q_{max} for Pb^{2+} and Zn^{2+}, respectively by C*hlorella vulgaris.* In the algal stabilization ponds there was 72% removal of Zn^{2+} and 73% removal of Pb^{2+} especially in the both months of November and March (Table 1). The results demonstrated a good treatment potential of algae stabilization pond system by *Chlorella* sp. alongwith (the heavy and toxic) metal remediation.

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