IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 7, Issue 3 Ver. I (Apr. 2014), PP 62-72
www.iosrjournals.org

Effect of pH and Temperature on the Efficiency of Indigenous Algae to Remove Contaminants from Artificial Wastewaters

Abdus Salam[1], Zahir Shah[1], Khitab Ullah[2], Imran Khan[2], Faiz Ali Khan[3], Naseer Ahmad[4] and Kamran Khan[4]

[1]Department of Soil and Environmental Sciences, The University of Agriculture Peshawar- Pakistan
[2]Department of Soil and Environmental Sciences, The University of Agriculture Peshawar- Pakistan
[3]Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar- Pakistan
[4]Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture Peshawar- Pakistan

Abstract: *A series of Lab incubation experiments were conducted to assess the effect of different pHs (5.5., 7.0 and 8.5) and temperature ranges (25, 30 and 35 ^{0}C) on the ability of indigenous algal species for removal of contaminants and other heavy metals, from artificial contaminated water during 2012-13. For this purpose, algal samples were collected from different areas in district Buner. Artificial contaminated waters were prepared and amended to pH 5.5, 7.0 or 8.5 using dilute alkali or acid and then each set was inoculated with mix culture of Microspora, Navicula, Chaetophora, Spirogyra, Apanothece, and Hydrodictyon as these were most abundant and common algal species in Buner. After inoculation, each set was incubated at 25, 30 or 35 ^{o}C for 10 days. The results obtained on the efficiency of indigenous algae collected from Buner to remove contaminants and other heavy metals from artificial wastewaters under different pH levels (5.5, 7.0 and 8.5) and temperature ranges (25, 30 and 35 ^{0}C) showed that a mix culture of algae viz., Microspora, Navicula, Chaetophora, Spirogyra, Apanothece, and Hydrodictyon removed variable amount of heavy metals ranged from 37 % in case of Cu up to 96 % in case of Ni from wastewaters. The results revealed that change in pH between 5.5 and 8.5 and in temperature between 25 and 35^{0}C had no remarkable influence on the efficiency of algae to remove these elements from contaminated water.*

I. Introduction

Industrial wastewaters are normally very complex, heterogeneous, and poorly characterized mixtures of a large number of contaminants. The wastewaters from all industrial sources are purified in chemical, physical, and biological treatment processes. Treating wastewater biologically is efficient and cheap. Recently, biological removal processes have been attracting considerable attention for removing heavy metals from aqueous wastes. Among biological means, microorganisms having higher potential for removing heavy metals and other contaminants from waste waters. Microbial removal of heavy metals offers the advantages of low operating cost, minimizing secondary problems with metal-bearing sludges and high efficiency in detoxifying very dilute effluents.

There are several industrial estates throughout Pakistan. Each industry is releasing huge amount of wastewaters mostly untreated. These untreated wastewaters are mostly entering the water bodies and agricultural lands and causing pollution. The waste waters must be treated before use on agricultural lands.

In Pakistan, very little attention is paid to the treatment of wastewaters; particularly the concept of biological treatment of wastewater is almost non-existence. Wastewaters from industries are in most cases directly discharged into rivers and irrigation channels that are often loaded with heavy metals and other contaminants.

Industrial effluents having heavy metal contamination have been an issue of environmental and public health concern since the advent of industrialization, owing to their non-biodegradable, toxic and bio-accumulative nature (Bailey *et al*., 1999; Nomanbhay and Palanisamy, 2005). Also the exponential increase in industrial activities is due to the rapid growth in world population and urbanization, accompanied by an increase in the amount of industrial wastes being discharged into the environment. Hence, the increase in heavy metals such as cadmium, mercury, lead, copper, zinc, nickel and chromium posing significant threat to human, water and soils health.

Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. The role of algae in purification facilities to remove the nutrients continues to be studied widely (Megharaj *et al*., 1992; Oswald, 1995; Kaya and Picard, 1996; Craggs *et al*., 1995. Rocchetta *et al*. (2003) studied two strains of *Euglena gracilis* having higher resistance to the heavy metals. The study deals with a heavy metal resistant euglenoid, *Distigma proteus*, isolated from wastewater. Metal uptake ability of the *D. proteus* has also been assessed with a view to detoxify industrial wastes of heavy metals.

The literature shows that algae have great potential to remove contaminants from wastewaters. However, little information is available about the efficiency of algae under different pH and temperature environments. This study was therefore planned to assess the efficiency of selected algal species in removing heavy metals and others contaminants from wastewaters under different pH and temperature regimes.

II. MATERIALS AND METHODS

Collection and identification of Algal samples

Algal samples were collected from natural waters from different areas in district Buner and identified with the help of guidelines as described in Prescott (1951), Tiffany and Britton (1952).

Testing of algal species for removal of heavy metals from water under different pH and temperature regimes

Desired numbers of fresh algal samples were tested for the removal of heavy metals, ammonium and phosphorus from wastewater at three pH levels (viz, 5.5, 7.0, 8.5) and three temperature regimes (viz 25, 30, 35^0C) during lab incubation experiments**.** Algae was removed after incubation, dried and digested. Algal digest and the water from which algae was removed were analyzed for heavy metals.

Table 1. Concentration of heavy metals and other elements in artificial contaminated water

Type	Element	X (mg L^{-1})	xx (mg L^{-1})
1	Pb Cd Ni Cr	5.00 0.01 0.02 0.10	10.0 1.0 3.0 2.0
2	Zn Cu Fe Mn	2.00 0.20 5.00 0.20	5.00 2.00 10.00 2.00
3	NH_4-N PO_4-P	50.00 25.00	50.00 25.00

x: maximum recommended conc. in irrigation H_2O. xx: Concentration in artificial contaminated water

Measurement of heavy metals in wastewaters

The samples were filtered through Whatman No. 42 and read for heavy metals including Pb, Cd Ni, Cr, Zn, Cu, Fe, Mn on Atomic Absorption Spectrophotometer. (Shimadzu, Model AA-6300).

Determination of Ammonium and Phosphorus in wastewaters

For determination of NH_4-N and P, the artificial wastewaters samples were filtered through Whatman No. 42 and read on Spectrophotometer. (Shimadzu, UV-1700) at 540 and 880 nm respectively after colour development.

Determination of algal efficiency in removing contaminants from wastewaters

The contaminants, other heavy metals, ammonium and phosphorus levels were measured in wastewaters before and after algal inoculation. The disappearance of metals, ammonium and phosphorus in wastewaters with algal inoculation was considered as removed by algae.

Statistical Analysis

Descriptive statistics was used for calculation of mean, standard deviations and coefficient of variations (Bhatti, 2006).

III. Results And Discussion

Composition of Algal Species in Natural Wastewaters

The results showed that *Microspora, Navicula, Chaetophora, Spirogyra, Apanothece, Hydrodictyon,*were the most abundant and common algal species , while *Fragilaria, Nitzschia, Oedogonium, Oscillatoria, Amphora, Cynarcus, Gomphonema, Hermidium, Synedra, Ulothrix and Zygnema* were least common algal speices in natural wastewaters in district Buner.

The results concerning the removal of Pb from artificial contaminated water by algae at different pH's and temperatures are presented in Table 2. The results showed that substantial amount of Pb was removed by algae from contaminated water at all pHs and temperature ranges. At pH 5.5, the removal ranged from 64 % at 35 ^{0}C to 73 % at 25 ^{0}C showing an increasing trend with decreasing temperature. At pH 7.0, the removal ranged from 42 % at 25 ^{0}C to 74 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 8.5, the removal of Pb ranged from 72 % at 35 ^{0}C to 86 % at 25 ^{0}C showing increasing trend with decreasing temperature. However, the average % removal of Pb from contaminated water by algae was not significantly affected by pH levels or temperature ranges. However, Jalali *et al*. (2001) reported that 98 % of Pb was removed by algae from aqueous solution and that metal biosorption increased with increasing pH. Rehman *et al*. (2006) reported that algae can be used for the uptake of heavy metals, metal detoxification and environmental clean-up operations. Imani *et al.* (2011) studied the bioremediation capability of *Dunaliella* algae for Pb, Cd and Hg and found that Hg had deadly effects on this organism as compared to those of Cd and Pb.

Table 2. Effect of pH and temperature on the removal of Pb from artificial wastewaters by mix species of indigenous algae

pH	Temperature (^{o}C)	Pb concentration in water (µg/50 ml)		Pb removal by algae	
		- algae	+ algae	(µg/50 ml)	%
5.5	25	402	110	292	73
	30	410	145	265	65
	35	407	147	260	64
7.0	25	403	232	171	42
	30	400	129	271	68
	35	406	107	298	74
8.5	25	402	55	347	86
	30	406	107	298	74
	35	409	113	296	72

Effect of pH (average across pHs): pH

5.5	406	134	272	67
7.0	403	156	247	61
8.5	406	92	314	77

Effect of Temperature (average across temperature ranges):
Temperature (^{0}C)

25	402	132	270	67
30	405	127	278	69
35	407	122	285	70

Table 3 shows the removal of Ni from artificial contaminated water by algae at different pH's and temperature levels. The results showed that at all pHs and temperature ranges, algae removed a remarkable amount of Ni from contaminated water. At pH 5.5, the removal ranged from 69 % at 25 ^{o}C to 71 % at 35 ^{o}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 63 % at 25 ^{o}C to 71 % at 35 ^{o}C again showing an increasing trend with increasing temperature. At pH 8.5, the removal of Ni ranged from 33 % at 35 ^{o}C to 81 % at 25 ^{o}C showing increasing trend with decreasing temperature. However, no significant differences were observed on average removal of Ni from contaminated water by algae between pH levels and temperature ranges. Nirupama (2003) reported that maximum biosorption of Ni and Cu ions by *C. vulgaris,* and found at a pH range of 6.5–7. 5, and that 35 ^{0}C was found to be the best temperature for maximum adsorption, whereas intracellular uptake was highest at 25 ^{0}C. Kızılkaya *et al*. (2012) found that biosorption of Ni from aqueous systems on living microalgae cultures *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris* under laboratories conditions. The study revealed that the metal uptake capacity of each living green algae was fast and thus living microalga cultures could be used to absorb Ni effectively from aqueous solutions.

Table 3. Effect of pH & temperature on removal of Ni from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Ni concentration in water (µg/50 ml)		Ni removal by algae	
		- algae	+ algae	(µg/50 ml)	%
5.5	25	118	36	82	69
	30	123	14	109	89
	35	110	31	79	71
7.0	25	120	44	76	63
	30	119	56	62	52
	35	115	33	82	71
8.5	25	121	23	98	81
	30	122	9	113	92
	35	121	81	40	33

Effect of pH (average across pHs):
pH

5.5	117	27	90	76
7.0	118	44	73	62
8.5	122	38	84	69

Effect of temperature (average across temperature ranges):
Temperature (^{0}C):

25	120	34	85	71
30	121	27	95	80
35	116	49	67	58

Table 4 represents the results obtained on the Cd removal from artificial contaminated water by algae at different pH's and temperatures. The results showed that algae removed large amount of Cd from contaminated water at all pH's and temperature ranges. At pH 5.5, the removal ranged from 63 % at 35 ^{o}C to 90 % at 25 ^{o}C showing an increasing trend with decreasing temperature. At pH 7.0, the removal ranged from 65 % at 25 ^{o}C to 66 % at 35 ^{o}C showing a increasing trend with increasing temperature. At pH 8.5, the removal of Cd ranged from 65 % at 25 ^{o}C to 96 % at 35 ^{o}C showing increasing trend with increasing temperature. However, no significant differences in average removal of Cd from contaminated water by algae were observed between pH levels and temperature ranges. Terry *et al*. (2001) examined the individual removals of Cd and Cu from contaminated water through biosorption using *Scenedesmus abundans*, a common green algae, and to compare metal removal using living species to that using nonliving ones. It was shown that while both living and nonliving *S. abundans* removed Cd and Cu from water, but living algae significantly outperformed nonliving algae. Rehman *et al*. (2006) also reported that algae can be used for heavy metals uptake, metal detoxification and environmental clean-up operations. Imani *et al.* (2011) studied the bioremediation capability of *Dunaliella* algae for Cd, Pb and Hg and found that Hg had deadly effects on this organism as compared to those of Cd and Pb.

Table 4. Effect of pH & temperature on removal of Cd from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Cd concentration in water (µg/50 ml)		Cd removal by algae	
		- algae	+ algae	(ug/50 ml)	%
5.5	25	43	4	39	90
	30	43	17	26	59
	35	44	16	27	63
7.0	25	43	15	28	65
	30	43	16	27	63
	35	43	15	29	66
8.5	25	43	15	28	65
	30	44	7	36	83
	35	43	1	42	96

Effect of pH (average across pHs):
pH

5.5	43	13	31	71
7.0	43	15	28	65
8.5	43	8	35	81

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	43	11	32	73
30	43	14	30	68
35	43	11	33	75

The results pertaining the removal of Cr from artificial contaminated water by algae at different pH's and temperatures are presented in Table 5. The results showed that significant amount of Cr was removed from contaminated water by algae at all pH's and temperature ranges. At pH 5.5, the removal ranged from 54 % at 25 ^{0}C to 58 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 42 % at 25 ^{0}C to 57 % at 35 ^{0}C again showing an increasing trend with increasing temperature. At pH 8.5, the removal of Cr ranged from 15 % at 25 ^{0}C to 68 % at 35 ^{0}C showing an increasing trend with decreasing temperature. However, no significant differences were observed in average removal of Cr from contaminated water by algae between pH levels or temperature ranges. Rehman *et al*. (2006) reported that algae can be used for the uptake of heavy metals, detoxification of metal and environmental clean-up operations. Sikaily *et al*. (2007) investigated the batch removal of toxic hexavalent Cr ions from aqueous solution, saline water and wastewater using marine dried green alga *Ulva lactuca*, and found that the maximum efficiencies of Cr removal was 92 %. Onyancha *et al.* (2008) studied the influence of pH on two algae namely, *Spirogyra condensate* and *Rhizoclonium hieroglyphicum* to remove Cr from tannery effluent. The effect of pH on Cr concentration showed that *S. condensata* to show maximum uptake of about 14 mg Cr g-^{1} of algae at optimum pH of 5.0 whereas *R. hieroglyphicum* had 11.81 mg of Cr g^{-1} of algae at pH of 4.0.

Table 5. Effect of pH & temperature on removal of Cr from artificial wastewater by mix species of indigenous algae

pH	**Temperature (^{0}C)**	**Cr concentration in water (µg/50 ml)**		**Cr removal by algae**	
		- algae	**+ algae**	(ug/50 ml)	%
5.5	25	67	31	36	54
	30	72	17	55	76
	35	69	29	40	58
7.0	25	70	40	30	42
	30	71	23	48	68
	35	70	30	40	57
8.5	25	71	23	48	68
	30	70	7	63	89
	35	70	60	10	15

Effect of pH (average across pHs):
pH

5.5	69	26	44	63
7.0	71	31	39	56
8.5	71	30	40	57

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	69	31	13	55
30	71	16	55	78
35	70	40	30	43

The results concerning the removal of Zn from artificial contaminated water by algae at different pH's and temperatures are presented in Table 6. The results showed that at all pH levels and temperature ranges large amount of Zn was removed by algae from contaminated water. At pH 5.5, the removal ranged from 52 % at 35 ^{0}C to 70 % at 25 ^{0}C showing an increasing trend with decreasing temperature. At pH 7.0, the removal ranged from 60 % at 35 ^{0}C to 65 % at 25 ^{0}C showing a increasing trend with decreasing temperature. At pH 8.5, the removal of Zn ranged from 52 % at 25 ^{0}C to 77 % at 35 ^{0}C showing increasing trend with increasing temperature. However, on average no significant differences in % removal of Zn from contaminated water by algae was observed between pH levels and temperature ranges. These results are in agreement with Chojnacka *et al*. (2003) who conducted experiments on the ability of microalga *Spirulina sp.* for trace elements removal from the conditioned (large volume, low contaminants concentration) industrial effluent and refinery and found that the removal of trace elements is the problem still not solved due to very low concentration of contaminants (on the level of µg kg^{-1}).

Table 6. Effect of pH & temperature on removal of Zn from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Zn concentration in water (µg/50 ml)		Zn removal by algae	
		- algae	+ algae	(ug/50 ml)	%
5.5	25	201	59	142	70
	30	210	95	115	55
	35	206	99	107	52
7.0	25	205	71	133	65
	30	204	87	117	57
	35	203	81	122	60
8.5	25	203	97	106	52
	30	210	44	166	79
	35	210	47	163	77

Effect of pH (average across pHs):
pH

5.5	206	84	121	59
7.0	204	80	124	61
8.5	208	63	145	70

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	203	76	127	63
30	208	75	133	64
35	207	76	131	63

Table 7 shows the results obtained on the removal of Cu from artificial contaminated water by algae at different pH's and temperatures. The results showed that algae removed considerable amount of Cu from contaminated water at all pH's and temperature ranges. At pH 5.5, the removal ranged from 37 % at 25 ^{0}C to 50 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 38 % at 25 ^{0}C to 39 % at 35 ^{0}C showing a increasing trend with increasing temperature. At pH 8.5, the removal of Cu ranged from 38 % at 25 ^{0}C to 70 % at 35 ^{0}C showing increasing trend with increasing temperature. However, on average the effect of pH levels and temperature ranges on % removal of Cu from contaminated water by algae was not significant. Terry *et al*. (2001) studied the individual removals of Cu and Cd from contaminated water through biosorption using *Scenedesmus abundans*, common green algae, and to compare metal removal using living species to that using nonliving ones. It was shown that while both living and nonliving *S. abundans* removed Cd and Cu from water, but living algae significantly outperformed nonliving algae. Nirupama. (2003) studied the mechanism of Cu and Ni ions biosorption by *C. vulgaris*, distinguishing adsorption from intracellular accumulation under various conditions. It was found that maximum uptake was reported at a pH range of 6.5-7.5, and that 35 ^{0}C was found suitable temperature for maximum adsorption, whereas intracellular uptake was highest at 25 ^{0}C that Cu was significantly removed from biological treatment of contaminated water by green algae.

Table 7. Effect of pH & temperature on removal of Cu from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Cu concentration in water (µg/50 ml)		Cu removal by algae	
		- algae	+ algae	(ug/50 ml)	%
5.5	25	72	45	27	37
	30	81	49	32	39
	35	80	40	40	50
7.0	25	76	47	29	38
	30	80	45	35	44
	35	80	49	31	39
8.5	25	77	48	29	38
	30	79	50	29	37
	35	81	24	57	70

Effect of pH (average across pHs):
pH

5.5	78	45	33	42
7.0	79	47	32	40
8.5	79	41	38	48

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	75	47	28	38
30	80	48	32	40
35	80	38	43	53

The results pertaining to removal of Fe from artificial contaminated water by algae at different pH's and temperatures are presented in Table 8. The results showed that remarkable amount of Fe was removed from contaminated water by algae at all pH's and temperature ranges. At pH 5.5, the removal ranged from 68 % at 25 ^{0}C to 79 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 75 % at 25 ^{0}C to 76 % at 35 ^{0}C showing a increasing trend with increasing temperature. At pH 8.5, the removal of Fe was 87 % at both 25 ^{0}C and 35 ^{0}C showing no effect of temperature. However, no significant differences in average removal of Fe from contaminated water by algae were observed between pH levels or temperature ranges. Chojnacka *et al.* (2003) studied microalga *Spirulina sp.* ability for the removal of trace elements from the industrial effluent and refinery and found that the removal of trace elements is the problem still not solved due to very low concentration of contaminants (on the level of μg kg ^{1}).

Table 8. Effect of pH & temperature on removal of Fe from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Fe concentration in water (µg/50 ml)		Fe removal by algae	
		- algae	**+ algae**	(ug/50 ml)	%
5.5	25	439	140	299	68
	30	441	102	339	77
	35	437	90	347	79
7.0	25	435	107	328	75
	30	434	114	320	74
	35	435	104	331	76
8.5	25	434	55	379	87
	30	436	54	381	87
	35	435	55	379	87

Effect of pH (average across pHs):
pH

5.5	439	111	328	75
7.0	435	108	327	75
8.5	435	55	380	87

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	436	101	335	77
30	437	90	347	79
35	436	83	353	81

Table 9 represents results obtained on the removal of Mn from artificial contaminated water by algae at different pH's and temperatures. The results showed that at given pH levels and temperature ranges algae removed considerable amount of Mn from contaminated water. At pH 5.5, the removal ranged from 42 % at 25 ^{0}C to 52 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 45 % at 25 ^{0}C to 48 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 8.5, the removal of Mn ranged from 62 % at 25 ^{0}C to 65 % at 35 ^{0}C showing an increasing trend with increasing temperature. However, on average no significant differences in % removal of Mn from contaminated water by algae were observed between pH levels or temperature ranges. Kızılkaya *et al*. (2012) studied the biosorption of Mn from aqueous systems on living microalgae cultures, *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris* under laboratories conditions, and it was revealed that the capacity of metal uptake of each living green algae was rather fast and thus living microalga cultures could be used to absorb Mn affectively from aqueous solutions.

Table 9. Effect of pH & temperature on removal of Mn from artificial wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	Mn concentration in water (µg/50 ml)		Mn removal by algae	
		- algae	+ algae	(ug/50 ml)	%
5.5	25	76	44	32	42
	30	75	37	37	50
	35	77	37	40	52
7.0	25	76	42	34	45
	30	71	23	48	68
	35	77	40	37	48
8.5	25	79	30	49	62
	30	79	29	50	63
	35	77	27	50	65

Effect of pH (average across pHs):
pH

5.5	76	39	37	48
7.0	75	35	40	53
8.5	78	28	50	63

Effect of temperature (average across temperature ranges):
Temperature (^{0}C)

25	77	39	38	50
30	75	30	45	60
35	77	35	42	55

Table 10 shows the results obtained on the removal of NH_4-N from artificially contaminated water by algae at different pH and temperatures. The results showed that considerable amount of NH_4-N was removed from contaminated water by algae at all pH and temperature ranges. At pH 5.5, the removal ranged from 61 % at 25 ^{0}C to 80 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 68 % at 25 ^{0}C to 80 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 8.5, the removal of NH_4-N ranged from 72 % at 35 ^{0}C to 81 % at 25 ^{0}C showing an increasing trend with decreasing temperature. However, the average effect of pH levels and temperature on % removal of NH_4-N from contaminated water by algae was statically non-significant. Zhang *et al*. (2012) tested the efficiency of *Chlorella* sp. isolated from municipal wastewater to remove inorganic nutrients from household secondary sewage in parallel-plate bioreactor after starvation. Their results showed that complete removal of NH_4-N was achieved within 4 h of treatment. The removal efficiency of NH_4-N efficiency was 81% after 1.25 h and 98.81% after 4 h. Thus, it is concluded that *Chlorella* sp. has great capabilities in removal of nutrients.

Table 10. Effect of pH & temperature on removal of NH_4-N from artificially wastewater by mix species of indigenous algae

pH	Temperature (^{0}C)	NH_4-N contents in water (µg/50 ml)		NH_4-N removal by algae	
		- algae	+ algae	(ug/50 ml)	%
5.5	25	10852	4215	6637	61
	30	10820	3425	7395	68
	35	10730	2158	8572	80
7.0	25	10902	3505	7397	68
	30	10745	2882	7862	73
	35	10745	2169	8576	80
8.5	25	10750	2057	8692	81
	30	10475	2254	8220	78
	35	10785	3045	7740	72

Effect of pH (average across pH):
pH levels

5.5	10801	3266	7535	70
7.0	10797	2852	7945	74
8.5	10670	2452	8218	77

Effect of temperature (average across temperature ranges):

Temperature (^{0}C)				
25	10835	3259	7576	70
30	10680	2854	7826	73
35	10753	2457	8296	77

The results concerning the P removal from artificially contaminated water by algae at different pH and temperatures are presented in Table 11. The results showed that algae removed substantial amount of P from contaminated water at given pH and temperature ranges. At pH 5.5, the removal ranged from 63 % at 25 ^{0}C to 67 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 7.0, the removal ranged from 60 % at 25 ^{0}C to 64 % at 35 ^{0}C showing an increasing trend with increasing temperature. At pH 8.5, the removal of P ranged from 61 % at 25 ^{0}C to 72 % at 35 ^{0}C showing an increasing trend with increasing temperature. However, the average % removal of P from contaminated water by algae was not significantly affected by given pH levels or temperature levels. Choi and Tian (2012) tested the efficiency removal of nutrients by *Chlorella vulgaris (C. vulgaris)* and found that increasing *C. vulgaris* content from 1 to 10 g L^{-1} caused an increase of removal rate of PO_4-P from 45 to 49 %. Similarly, Zhang *et al* (2012) evaluvated *Chlorella* sp. isolated from municipal wastewater to remove inorganic nutrients from household derived effluents in parallel-plate bioreactor after starvation. The results showed that complete removal of PO_4^3 was achieved within 4 h of treatment and the PO_4^3P removal efficiency after 1.25 h was 77% and after 4 h the removal efficiency was 100 %.

Table 11. Effect of pH & temperature on removal of P from artificially wastewater by mix species of indigenous algae

pH	**Temperature (^{0}C)**	**P concentration in water (µg/50 ml)**		**P removal by algae**	
		- algae	**+ algae**	(ug/50 ml)	%
5.5	25	5524	2058	3466	63
	30	5164	2063	3102	60
	35	5428	1797	3632	67
7.0	25	5309	1893	3416	64
	30	5499	1988	3511	64
	35	5206	2058	3148	60
8.5	25	5236	2032	3204	61
	30	5247	1866	3381	64
	35	5349	1509	3839	72

Effect of pH (average across pH):

pH				
5.5	5372	1973	3399	63
7.0	5338	1980	3358	63
8.5	5278	1802	3475	66

Effect of temperature (average across temperature ranges):

Temperature (^{0}C)				
25	5356	1994	3362	63
30	5304	1972	3331	63
35	5328	1788	3540	66

IV. Summary

The aim of this study was to assess the removal of heavy metals, ammonium N and phosphate P from artificial wastewaters through indigenous algae. Also to find out suitable environmental conditions such as pH and temperature where these algae can efficiently remove various contaminants from contaminated waters. Eleven algal samples were collected for this purpose from natural waters in district Buner. Three types of artificial contaminated water were prepared and inoculated with desired type of algae. Afterwards, a series of laboratory incubation experiments were conducted to assess the efficiency of different indigenous algae to remove contaminants, other heavy metals, ammonium-N and phosphorus from contaminated waters under different pH levels and temperature ranges. The results showed that *Microspora, Navicula, Chaetophora, Spirogyra, Apanothece,* and *Hydrodictyon* were the prominent algal species in water samples.

The results obtained on the efficiency of indigenous algae collected from Buner to remove contaminants, other heavy metals, NH_4-N and PO_4-P from artificial wastewaters under different pH levels (5.5, 7.0 and 8.5) and temperature ranges (25, 30 and 35 ^{0}C) showed that a mix culture of algae viz., *Microspora, Navicula, Chaetophora, Spirogyra, Apanothece,* and *Hydrodictyon* removed variable amount of heavy metals, ammonium and phosphorus from artificial wastewaters. The results showed that algae removed 74 % Pb from artificial wastewaters during 10 days of incubation. The results showed that on average 76 % of Ni was removed from artificial contaminated water by algae. The results showed that about 81 % of Cd was removed from artificial wastewaters by algae. The results showed that on average 78 % of Cr was removed from artificial wastewaters by algae. The results showed that algae removed 70 % of Zn from artificial wastewaters. The results showed that on average algae removed 53 % of Cu from artificial wastewaters. The results showed that about 75 % of Fe was removed from artificial wastewaters by algae. The results showed that on average algae removed 63 % Mn from artificial wastewaters. The results showed that about 77 % of NH_4 was removed from artificial wastewaters by algae. The results showed that algae removed 66 % P from artificial wastewaters.

It is clear from the above results that mixed culture of indigenous algae removed substantial amount of contaminants, other heavy metals, ammonium and phosphorous from contaminated water at all given pH and temperature levels. Further more, the results revealed that change in pH between 5.5 and 8.5 and in temperature between 25 and 35 ^{0}C had no remarkable influence on the efficiency of algae to remove these elements from contaminated water.

V. Conclusion and Recommendations

This study has shown that *Microspora foceosa, Microspora tumidula, Navicula exigua, Chaetophora elegans, Chaetophora attenuate, Spirogyra aequinoctialis, Spirogyra crassa, Spirogyra subsalsa, Spirogyra aequinoctialis, Apanothece nidulans, and Hydrodictyon reticulatum*, were dominant algal species in natural wastewaters of Buner. Mix culture of indigenous algal species including *Microspora, Navicula, Chaetophora, Spirogyra, Apanothece, and Hydrodictyon* has large potential to remove contaminants and other heavy metals from artificial wastewaters. Change in pH between 5.5 and 8.5 and in temperature 25 and 35^{0}C had no remarkable influence on the efficiency of algae to remove these elements from contaminated water.

The following recommendations were formulated from the present study,

Further research is needed to test the above as well as other common algal species alone and in combinations for removal of heavy metals and other contaminants from artificially contaminated/ wastewaters. Efforts are also needed to increase the efficiency of such algal species for removal of heavy metals and other contaminants from artificially contaminated wastewaters.

Acknowledgement

This study was part of a research project funded by Higher Education Commission (HEC), Islamabad.

Literature Cited

[1]. Bailey, S.E., T.J. Olin, R.M. Bricka, and D.D. Adrain. 1999. A Review of Potentially Low-cost Sorbents for Heavy Metals. Water. Resour. 33(11): 2469-2479.
[2]. Bhatti, A.U. 2006. Statistical Procedure for Analysis of Agriculture Research Experiments. Department of Soil and Environmental Sciences, NWFP Agricultural University Peshawar, pp 293.
[3]. Nomanbhay, S.M., and K. Palanisamy. 2005. Removal of Heavy Metal from Industrial wastewater Using Chitosan-coated Oil Palm Charcoal. Electronic J. Biotech. 8 (1): 0717-3458.
[4]. Megharaj, M., H.W. Pearson, and K. Venkateswarlu. 1992. Removal of nitrogen and phosphorus by immobilized cells of *Chlorella vulgaris* and *Scenedesmus bijugatus* isolated from soil. Enzyme Microb. Technol. 14: 656-658.
[5]. Oswald, W.J. 1995. Ponds in the twenty-first century. Water Sci. Technol. 31: 1-8.
[6]. Kaya, V.M., and G. Picard. 1996. Stability of chitosan gel as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. Bioresour. Technol. 56: 147-155.
[7]. Craggs, R.J., V.J. Smith, and P.J. McAuley. 1995. Wastewater nutrient removal by marine microalgae cultured under ambient conditions in mini-ponds. Water Sci. Technol. 31: 151-160.
[8]. Rehman, A., and A.R. Shakoori. 2001. Heavy metal resistant *Chlorella spp*., isolated from tannery effluents, and their role in remediation of hexavalent chromium in industrial wastewater. Bull. Environ. Contam. Toxicol. 66: 542–547.
[9]. Rocchetta, I., L.B. Ruiz, G. Magaz, and V.T.D. Conforti. 2003. Effects of hexavalent chromium in two strains of *Euglena gracilis*. Bull. Environ. Contam. Toxicol. 70: 1045–1051.
[10]. Imani, S., S.R. Zarchi, M. Hashemi, H. Borna, A. Javid, A.M. Zand, and H.B. Abarghouei. 2011. Hg, Cd and Pb heavy metal bioremediation by *Dunaliella* alga. J. Med. Plant. Res. 5: 2775-2780.
[11]. Jalali, R., H. Ghafourian, Y. Asef, S.J. Davarpanah, and S. Sepehr. 2001. Removal and recovery of lead using nonliving biomass of marine algae. J. Hazard. Mater. 92: 253–262.
[12]. Kızılkaya, B., G. Turker, R. Akgul, and F. Dogan. 2012. Comparative study of biosorption of heavy metals using living green algae Scenedesmus quadricauda and Neochloris pseudoalveolaris: Equilibrium and Kinetics. J. Dispersion Sci. Technol. 33: 410 – 419.
[13]. Mallick, N. 2003 Biotechnological potential of Chlorella vulgaris for accumulation of Cu and Ni from single and binary metal solutions.World .J. Microbio. Biotechnol 9, 196–201.
[14]. Onyancha.D., W. Mavura , J.C. Ngila, P. Ongoma, and J. Chacha. 2008. Studies of chromium removal from tannery wastewaters by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*. J. Hazard. Mater. 158: 605–614.
[15]. Terry, A.P., and W. Stone. 2001. Biosorption of cadmium and copper contaminated water
[16]. by Scenedesmus abundans. Chemosphere 47: 249–255.
[17]. Chojnacka, K., A. Chojnacki, and H. Gorecka. 2003. Trace element removal by *Spirulina sp.* from copper smelter and refinery effluents. Hydrometallurgy 73: 147–153.
[18]. Sikaily, A.E., A. E. Nemr, A. Khaled, and O. Abdelwehab. 2007. Removal of toxic chromium from wastewater using green alga *Ulva lactuca* and its activated carbon. J. Hazard. Mater. 148: 216–228.
[19]. Bhatti, A.U. 2006. Statistical procedures for analysis of agriculture research experiments, Department of Soil and Environmental Sciences, NWFP Agricultural University Peshawar, Page 293.
[20]. Prescott, G.W. 1951. Algae of the Western Great Lakes Area, exclusive of Desmids and Diatoms. Science Bloomified Hills, Michigan State College, USA, pp 932.
[21]. Tiffany, L.H. and M.E. Briton. 1952. The algae of Illinois. Chicago University Press, Chicago, USA, pp 380.