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Comparison of Crushed and Boiled Modified *Azadirachta indica (Neem)* Leaf Extracts in Industrial Wastewater Treatment

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ABSTRACT

Bioremediation of polluted water using plant materials is a major area in biotechnology. Extracts of neem leaves were used for upgrading the quality of industrial wastewater. Neem leaf extractions were done by two different methods; crushed and boiled methods. The concentration of neem leaf extract used for the preliminary study was 20 g/100 ml. Different concentrations of crushed and boiled modified neem leaf extracts (30 g/100 ml and 40 g/100 ml) were used for further experiments on different types of industrial wastewater treatment. Crude leaf extracts of *Ocimum sanctum* and *Plectranthus amboinicus* (40 g/100 ml) were also prepared to be used as control plants for the wastewater treatment. The quality of wastewater was determined by analyzing parameters; pH, turbidity, total solids and microbial load. The results revealed that crushed modified neem leaf extract was found to be more effective than boiled modified neem leaf extract especially in oil contaminated wastewater treatment. Crushed and boiled modified neem leaf extracts reduced total solids in oil contaminated wastewater. Potential of neem leaf extracts on different wastewater treatments varied with concentration of neem leaf extracts. Crude leaf extracts of the control plants (40 g/100 ml) were comparatively less ability to remediate the oil contaminated wastewater than crushed modified neem leaf extract, confirming the efficiency of neem leaf extract on wastewater treatment especially in the oil contaminated samples. However, the effect of modified leaf extracts of *Ocimum sanctum* and *Plectranthus amboinicus* has to be tested further on wastewater treatment.

KEYWORDS: Bioremediation, Neem, *Ocimum sanctum, Plectranthus amboinicus*

INTRODUCTION

Explosion of population and rapid industrialization propel to discharge of effluents and domestic sewage into natural water bodies. It causes infectious diseases and threatens the biodiversity of aquatic system.

Biological treatment holds promise in solving environmental problems in a cost effective way. Moreover, it is capable to oxidize a wide variety of organic compounds. Plant parts such as modified bark of *Artocarpus heterophyllus* for the removal of Cd^{2+} (Swami *et al.*, 2002) and pods of *Moringa oleifera* for biosorption of Pb^{2+} from aqueous solution (Adelaja *et al.*, 2011) have been used in wastewater treatment.

Neem is a multifarious ever green tree, belongs to the family Meliaceae. Its different parts were used for remediation purposes due to antimicrobial effect of neem (Biswas *et al.*, 2002). Neem has been studied on removal of metal ions in synthetic wastewater by using biosorption technology (Innocent *et al.*, 2009) and adsorption of malachite green dye from wastewater using neem sawdust (Khattri and Singh, 2009). Although a large number of studies have been conducted on neem leaf extracts, still none of the studies have been shown to use the potential of neem leaf extract to treat different types of wastewater.

Ocimum sanctum (Tulsi) and *Plectranthus amboinicus* (Indian Borage) are also vastly studied for medicinal purposes. Antimicrobial activity of Tulsi (Prakash and Gupta, 2005) and Indian Borage (Nagalakshmi *et al.*, 2012) have enormous potential to control pathogenic bacteria habited in highly polluted wastewater.

In previous studies, the crushed modified neem leaf extract used to treat different types of wastewater was found to be an effective method for reducing turbidity, microbial load, total nitrogen, ortho-phosphate and electric conductivity and also improving pH and dissolved oxygen (Srirathan *et al.*, 2011).

The objective of this study is to compare the effectiveness of crushed and boiled modified neem leaf extracts on wastewater treatment by optimizing the concentration of neem leaf extracts.

MATERIALS AND METHODS

Experimental Site

The study was carried out at Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka from January to April 2013.

Collection of Samples

The industrial wastewater was collected from asbestos sheet manufacturing factory (ASMF) located in the industrial zone of Makandura for the preliminary study. For

further experiments, wastewater was taken from various sources. Oil contaminated wastewater was obtained from service station (SS) located in Negombo. Industrial effluent was collected from maoya river (MR) located in Makandura. Wastewater was collected in glass containers which were rinsed three times thoroughly with the same wastewater samples before collection and stored at 4^0C.

Different Methods of Leaf Extraction

Healthy, immature neem leaves were selected and washed with sterile distilled water. Then the leaves were air dried for 2 hr. Air dried leaves were used for preparation of crushed and boiled crude neem leaf extracts. Crushed crude was extracted as described in previous studies (Warusawitharana and Vivehananthan, 2010). Air dried leaves were cut into fine pieces and added into 100 ml of boiling sterile distilled water for the preparation of boiled crude of neem leaf extract (20 g/100 ml). Then, it was mixed for 2 min. The mixed sample was filtered through autoclaved gauze. Both Tulsi and Indian Borage extracts (40 g/100 ml) were also prepared following the same procedure as for neem leaf extract. The crude of crushed and boiled neem extracts were modified to pH 10 gradually to prepare modified neem leaf extracts (Warusawitharana and Vivehananthan 2010).

Different concentrations of modified neem leaf extracts (30 g/100 ml and 40 g/100 ml) were also prepared. Then all the crude and modified extracts were autoclaved and stored at -20^0C.

Various Types of Treatments

During the preliminary study four different treatments with control were used to test the effectiveness of neem on industrial wastewater (ASMF).

Treatment **I:** Industrial wastewater (100 ml) without any neem leaf extracts.

Treatment **II:** Industrial wastewater (100 ml) was treated with 1 ml of crushed crude neem leaf extract (20 g/100 ml).

Treatment **III:** Industrial wastewater (100 ml) was treated with 1 ml of crushed modified neem leaf extract (20 g/100 ml).

Treatment **IV:** Industrial wastewater (100 ml) was treated with 1 ml of boiled modified neem leaf extract (20 g/100 ml).

The treatments were further extended with various concentrations of neem leaf extracts for different industrial wastewater such as oil contaminated wastewater, maoya industrial effluent and asbestos industrial wastewater. In parallel crushed and boiled crude extracts of both Tulsi and Indian Borage (40 g/100 ml) were also tested in oil contaminated wastewater separately. All the samples were placed on the shaker at 120 rpm for continuous agitation. The quality parameters; turbidity (Spectrophotometer 6305 JENWAY at 540 nm), pH, total solids content (Standard method) and microbial load were measured initially and weekly for all samples.

RESULTS AND DISCUSSION

Preliminary Study (1) on Industrial Wastewater from ASMF

The initial study showed that treatment I (control) (Table 1a) and treatment II (Table 1b) have less potential to treat the industrial wastewater as there was no any substantial improvement observed in all the quality parameters used in this study during the treatment while treatment III gave desirable outcomes for pH and turbidity after 3 weeks treatment, indicating the effect of crushed modified neem leaf extract on the wastewater treatment (Table 1c). However, microbial load was reduced with the addition of crushed modified neem leaf extract during the initial 2 hours treatment and it was subsequently increased with time which may be due to less concentration of neem leaf extract (20 g/100 ml) used in the preliminary study as previous studies showed better effect on wastewater treatment with 30 g/100 ml of crushed modified neem leaf extract (Srirathan *et al.*, 2011).

Treatment IV was found to be inefficient for improving the quality of wastewater however, the treatment reduced total solids during the initial 2 weeks treatment (Table 1d). Based on the results obtained from the preliminary study, concentration of neem leaf extracts was increased during further experiments.

Preliminary Study 2

The desirable effect on upgrading of wastewater quality was not exhibited even with the 30 g/100 ml concentration of crushed modified neem leaf extract as expected because which may vary with types of contaminants present in different types of industrial wastewater (Table 2a and 2b). Therefore, concentration of neem leaf extract was further increased to 40 g/100 ml to be used to treat wastewater collected from different sources.

Effect of Neem Extracts on Oil Contaminated Wastewater and Industrial Effluent (Maoya)

The pH of the wastewater samples in all types of treatments used in this study reached

more or less the neutral pH. However, there were not much differences in pH of the different types of treatments where aeration itself has a considerable effect on wastewater treatment (Table 3a, 3b, 4a and 4b).

Interestingly, turbidity and microbial load were reduced with addition of crushed modified neem leaf extract in oil contaminated wastewater during 3 weeks treatment (Table 3a) while there was not much beneficial effect in the maoya industrial effluent throughout the treatment (Table 4a). Since oil contaminated wastewater contains emulsified oils, suspended solids and heavy metals (Kanluen and Amer, 2000) and also dye, turbidity reduction during the treatment of 3 weeks in oil contaminated sample may be due to absorption of dyes (Sharma *et al.*, 2005), heavy metals and removal of suspended solids. The decline of microbial population may also be resulted by the antimicrobial effect of neem. Since control showed high turbidity and microbial load in oil contaminated wastewater after 3 weeks treatment (Table 3a), confirmed the effectiveness of crushed modified neem leaf extract on oil contaminated wastewater. Further, there was no reduction in microbes with boiled modified neem leaf extract (Table 3b).

Although, positive effect was observed on reduction of total solids in the oil contaminated wastewater with both crushed and boiled modified neem leaf extracts (Table 3a and 3b) the crushed modified neem leaf extract has shown the great potential to reduce total solids at the end of 3 weeks treatment. In addition, maoya industrial effluent showed reduction in the total solids with addition of crushed modified neem leaf extract after five weeks treatment (Table 4a), indicating modified neem leaf extract has a great effect on reducing total solids in all the samples.

Table 1a. Analysis of different parameters for control (ASMF)

Parameters	Original	Aerated						
	Initial	1W	2W	3W	4W	5W	6W	7W
pH	6.19	6.97	7.22	7.26	6.89	6.90	6.55	6.79
T	0.309	0.123	0.139	0.033	0.102	0.181	0.162	0.169
TS (g/l)	3.2	0.46	0.41	0.095	0.165	0.42	0.22	0.435
ML/ml	7800	UC	11300	11700	20000	13900	5700	13200

W- Week, TS – Total Solids, ML – Microbial Load, T – Turbidity, UC >34400 colonies/ml

Table 1b. Analysis of different parameters for crushed crude neem treatment

Parameters	Treated with crushed crude neem leaf extract (20 g/100 ml)							
	2H	1W	2W	3W	4W	5W	6W	7W
pH	6.93	7.12	7.12	7.00	6.72	6.73	6.45	6.62
T	0.284	0.136	0.164	0.088	0.088	0.177	0.560	0.352
TS	3.6	0.43	0.445	0.045	0.26	0.455	0.635	0.84
ML	1650	UC	UC	UC	UC	UC	UC	UC

H- Hour

Table 1c. Analysis of different parameters for crushed modified neem treatment

Parameters	Treated with crushed modified neem leaf extract (20 g/100 ml)							
	2H	1W	2W	3W	4W	5W	6W	7W
pH	7.28	7.29	7.23	7.06	7.32	6.50	6.90	7.26
T	0.222	0.071	0.163	0.003	0.068	0.204	0.283	0.369
TS	3.2	5	0.71	0.235	0.305	0.55	0.875	0.975
ML	6440	UC	24400	17500	18000	UC	UC	UC

Table 1d. Analysis of different parameters for boiled modified neem treatment

Parameters	Treated with boiled modified neem leaf extract (20 g/100 ml)							
	2H	1W	2W	3W	4W	5W	6W	7W
pH	7.12	7.20	7.35	7.25	7.15	7.03	6.95	7.18
T	0.296	0.173	0.190	0.085	0.123	0.205	0.318	0.332
TS	4	0.425	0.065	0.24	0.275	0.48	0.86	1.08
ML	9220	UC	UC	12900	UC	UC	UC	UC

Table 2a. Crushed modified neem leaf extract (30 g/100 ml) Vs Industrial effluent (MR)

Parameters	Original	Aerated				Treated with crushed modified neem leaf extract				
	Initial	1W	2W	3W	4W	2H	1W	2W	3W	4W
pH	7.11	7.00	6.97	6.89	7.05	7.27	7.24	6.69	7.54	7.23
T	0.033	0.103	0.100	0.077	0.115	0.168	0.153	0.151	0.117	0.142
TS	0.21	0.235	0.155	0.105	0.1	0.435	0.43	0.285	0.335	0.21
ML	3920	1300	200	3200	960	2000	29680	4240	23360	24240

Table 2b. Crushed modified neem leaf extract (30 g/100 ml) Vs Industrial wastewater (ASMF)

Parameters	Original	Aerated				Treated with crushed modified neem leaf extract				
	Initial	1W	2W	3W	4W	2H	1W	2W	3W	4W
pH	8.17	7.45	7.78	7.41	8.03	7.77	7.59	7.79	8.03	7.79
T	0.223	0.131	0.067	0.041	0.09	0.570	0.261	0.229	0.201	0.184
TS	0.631	0.505	0.44	0.545	0.375	0.825	0.735	0.59	0.765	0.53
ML	UC	UC	UC	UC	UC	UC	UC	UC	UC	UC

Table 3a. Comparison of crushed modified neem leaf extract (40 g/100 ml) with control in Oil wastewater (SS)

Parameters	Original	Aerated					Treated with crushed modified neem leaf extract					
	Initial	2W	3W	4W	5W	6W	2H	2W	3W	4W	5W	6W
pH	6.82	7.51	7.47	7.24	7.25	7.47	7.30	7.59	7.49	7.68	7.64	7.44
T	High	High	1.413	0.716	0.372	0.416	High	1.306	1.219	1.258	1.300	1.355
TS	1.74	4.54	3.01	3.49	2.47	1.83	1.92	1.81	1.48	1.43	1.54	1.63
ML	UC	UC	20300	2600	26400	8000	UC	UC	9200	24400	UC	UC

Table 3b. Effect of boiled modified neem leaf extract (40 g/100 ml) in Oil wastewater (SS)

Parameters	Treated with boiled modified neem leaf extract						
	2H	2W	3W	4W	5W	6W	7W
pH	6.74	7.91	7.62	7.36	7.61	7.70	7.23
T	High	1.859	1.865	1.662	1.109	1.136	0.775
TS (g/l)	2.4	1.67	1.67	1.547	1.67	1.43	1.31
ML/ml	UC	UC	UC	UC	UC	UC	UC

Table 4a. Comparison of crushed modified neem leaf extract (40 g/100 ml) with control in Industrial effluent (MR)

Parameters	Original	Aerated					Treated with crushed modified neem leaf extract					
	Initial	2W	3W	4W	5W	6W	2H	2W	3W	4W	5W	6W
pH	7.37	7.71	7.65	7.19	7.22	7.44	7.61	7.77	7.33	7.53	7.20	6.77
T	0.025	0.057	0.059	0.02	0.059	0.014	0.112	0.156	0.069	0.157	0.134	0.118
TS	0.3	0.14	0.04	0.1	0.2	0.1	0.73	0.59	0.26	0.6	0.12	0.49
ML	2320	Nil	200	2900	3200	400	9800	UC	UC	10000	UC	UC

Table 4b. Effect of boiled modified neem leaf extract (40 g/100 ml) in Industrial effluent (MR)

Parameters	Treated with boiled modified neem leaf extract						
	2H	2W	3W	4W	5W	6W	7W
pH	7.29	7.87	7.50	6.90	7.21	7.12	7.16
T	0.118	0.214	0.198	0.112	0.196	0.157	0.131
TS (g/l)	0.83	0.613	0.58	0.247	0.62	0.23	0.37
ML/ml	3800	19100	UC	14400	22400	UC	UC

Table 5a. Effect of control plants on pH and turbidity of Oil wastewater (SS)

Parameters	pH						Turbidity					
Samples	Initial	1D	1W	2W	3W	4W	Initial	1D	1W	2W	3W	4W
Aerated oil	6.69	7.99	7.30	7.63	7.29	7.02	1.402	0.704	0.562	0.486	0.580	0.420
Oil + TB	7.77	7.72	7.35	7.47	7.42	7.44	1.603	1.289	0. 922	0.902	0.958	1.084
Oil + TC	6.92	7.73	7.53	7.47	7.60	7.36	1.570	0.713	0.479	0.358	0.394	0.546
Oil + KB	6.76	7.95	7.54	7.34	7.49	7.11	1.446	0.796	0.631	0.562	0.502	0.475
Oil+ KC	7.09	7.66	7.71	7.58	7.67	7.19	1.392	0.585	0.416	0.556	0.441	0.420

TB- Tulsi Boiled, TC- Tulsi Crushed, KB- Kapparavalliya Boiled, KC- Kapparavalliya Crushed, W – Week, D – Day, UC > 34400 colonies/ml

Table 5b. Effect of control plants on total solids and microbial load of Oil wastewater (SS)

Parameters	Total solids (g/l)						Microbial load (ML/ml)					
Samples	Initial	1D	1W	2W	3W	4W	Initial	1D	1W	2W	3W	4W
Aerated oil	1.68	0.35	0.77	0.73	0.82	0.62	7920	9200	1600	2160	1040	2960
Oil + TB	1.46	0.92	0.9	1.29	1.09	1.22	6000	UC	16720	UC	13280	15520
Oil + TC	1 .39	1.41	1.39	1.36	1.86	1.43	6080	UC	32000	10800	20880	UC
Oil + KB	1	0.28	1.12	0.84	0.66	0.6	8640	UC	9360	7200	1840	4640
Oil+ KC	1.1	0.9	0.87	1.01	1.13	0.79	12240	UC	30080	19440	14000	18400

Effect of Control Plants on Oil contaminated Wastewater

The initial pH (6.69) of oil contaminated wastewater reached approximately the neutral pH during the treatment of one month in all the types of treatments including the control (Table 5a), indicating aeration itself has an extensive effect on improving pH during

wastewater treatment. Turbidity was reduced in oil contaminated wastewater with addition of crude of crushed Tulsi and Indian Borage during the treatment of 2 and 3 weeks respectively while it was increased with crude of boiled Tulsi extract in the wastewater (Table 5a). Total solids were higher than control during the treatment of crude leaf extracts of crushed and boiled Tulsi and crushed Indian Borage in the oil sample (Table 5b). Microbial load was reduced with addition of crude of crushed and boiled Tulsi in the wastewater at the early hours of treatment, and increased drastically with time throughout the treatment. It may be due to presence of heavy metals (Jena and Gupta, 2012) and other food sources in the leaf extracts for the existing microbes. Consequently, the crude leaf extracts of control plants showed less potential to give beneficial outputs for all the quality parameters measured in this study to determine the wastewater quality.

CONCLUSIONS

Addition of crushed modified neem leaf extract was found to be more effective on wastewater treatment than boiled modified neem extract for most of wastewater samples. Further, high concentration of neem extract remediated the wastewater (oil contaminated wastewater) efficiently. Total solids were drastically reduced by addition of crushed modified neem leaf extract especially in oil contaminated sample. Antimicrobial activity of neem leaf extract was highly influenced by types of wastewater and source of collection. However, modified leaf extracts of control plants have to be tested on wastewater treatment.

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