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Combined Treatment Method for the Remediation of Fe^{2+}, PO_4, SO_4 and NO_3 from River Patalganga

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ABSTRACT: The generalized use of Bermuda grass extract for medicinal purposes is common whereas the phytoremediation of soil and water for the accumulation of heavy metals is a traditional method in the field of waste water treatment. The current paper presents a combined treatment method for the remediation of Fe^{2+}, PO_4, SO_4, and NO_3. The laboratory scale work resulted in a novel technique in which the methanolic extract of Bermuda grass and the biochar obtained after the methanolic extraction process had the potential of removing NO_3> SO_4>Fe^{2+}> PO_4 from the Patalganga River water under investigation. The difference in removal of Fe^{2+}, PO_4, SO_4, and NO_3 was 5.59%, 2.34%, 10.34%and 19% more than the $KMnO_4$ method. The competence of the results attained by the Bermuda grass activated charcoal (BGACs) was more than that of commercial carbon (COM.ACs). The results were analyzed statistically by one way ANOVA and the analysis exhibited there was a significant effect of treatment at P < 0.05 level. The Levens test revealed P=0.006 and as P< 0.05 hence confirmed that there is a significant difference in the BGE treatment and the $KMnO_4$ treatment. The Post hoc test confirmed BGE treatment is more effective than KMnO4 treatment. Hence it can be concluded that the present study can substantially remove Fe^{2+}, PO_4, SO_4 and NO_3 by BGE and BGACs treatment.

Keywords: Iron; Cynodon Dactylon; $KMnO_4$ treatment; biochar; methanolic extract and Remediation.

INTRODUCTION: Cynodon Dactylon belongs to the Poaceae family of Plantae kingdom and is known by different names like Kweek grass, Chiendent Pied-de-Poule, Bahama grass, Common couch, Devil's grass, Giant Bermuda grass, Green couch, Hariali grass, Indian couch, Plain couch, Quick grass; Cynodon dactyle. It is very well-known for its pharmacological activities like antimicrobial, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, antioxidant activities. Its photochemical analysis shows the presence of flavonoids, alkaloids, glycosides, terpenoides, triterpenoids steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils and fixed oils, linoleic acid[1 & 2] along with antioxidative enzymes like Catalase, Superoxide dismutase, Peroxidase and Glutathione reductase.[3] This characteristic composition of Cynodon Dactylon makes it exceptionally significant in the treatment of polluted land and water by means of biomeneralization, phytostabilization, hyperaccumulation.[4] A. Adeli and D. E. Rowe successfully applied Cynodon Dactylon for removal of Nitrogen and Phosphorous.[5] Bermuda grass can efficiently remove nitrogen more than any other turf grass.[6] Likewise hyperaccumulation of heavy metals by Bermuda grass too is a method to treat polluted soil.[7] Use of hemicellulose from Bermuda grass extract for remediation of water pollution is another technique which is very effective.[8]

Cynodon Dactylon is effectively employed in treatment of pollution since ages back however the current paper presents an exclusive treatment method which achieves complete remediation on water pollution. Bermuda grass extract is widely used for medicinal purposes but almost no work is done using this extract for the treatment of polluted water. The current paper presents a novel method to treat any polluted source of water, here Patalganga River water was used for investigation. Remediation was accomplished by using Bermuda grass extract (BGE) and activated charcoal obtained from Bermuda grass (BGAC) and *Mangifera indica* Activated carbon. The Patalganga river water consisted of high concentrations of Fe^{2+}, PO_4, SO_4 *and* NO_3 hence the method needed for its remediation was expected to be efficient enough to completely remediate not only Fe^{2+}, PO_4, SO_4 *and*

NO_3 but also other impurities like Al, Zn, Cd, Pb, Cu etc present in the river.

MATERIALS AND METHODS:

Location: The River Patalganga lies in the Rasayani area located 18.893◦N and 73.157◦E in Raigad district of Maharashtra state. Since 1960 many industrial units have come up in this belt which extends from Khopoli to Rasayani.

Location of Sampling Points: The Bermuda grass used for the studies was acquired from two locations that were free from any source of pollution. The first location was from the author's kitchen garden in the residential colony of Hindustan Organic Chemical Ltd. factory where the research work was carried out. The second location was kitchen garden near the author's bungalow at distance of 5Km.

Sampling: The fresh green shoots of Bermuda grass were collected from both the locations for a period of five months every day from July 2016 to Nov 2016. The leaves were cleaned thoroughly to remove any unwanted material and washed thoroughly several times with tap water and three times with distilled water. This cleaned Bermuda grass was now ready for extraction.

Production of Bermuda grass Extract (BGE): Cleaned and shredded Bermuda grass leaving behind the roots and unwanted dry leaves were transferred to the Soxhlet extractor and methanol extracts of Bermuda grass were acquired. The methanol was then distilled off and pure extracts of Bermuda grass was obtained which was used for remediation.

Production and Activation of Bermuda grass Charcoal (BGACS): The Bermuda grass remained after the extraction in part A was heated to 300°C temp in a muffle furnace to obtain Bermuda grass Charcoal. The charcoal hence obtained was subjected to activation using chemical activation method using freshly prepared $ZnCl_2$.The charcoal was boiled for an hour in the $ZnCl_2$ solution for dehydration of organic molecules. The Charcoal was then washed with distilled water for neutralization. After neutralization the water was cast off and charcoal was transferred to the muffle furnace for carbonization at 700°C temp. After one hour the BGACS was kept in the desiccator for cooling.

Preparation of Sand for filtration: The sand used for filtration was acquired from the sea shore of Goa (India). The sand was first sieved and washed several times with tap water until the sand was free from mud and all unwanted things like leaves small stones, clay etc. This washed sand was then acidified with 1M hydrochloric acid and kept overnight. The acidified sand was again washed off with distilled water in order to neutralize the sand. After neutralization the sand was rinsed three times with double distilled water and dried in sun for 8 hours. This sand was used in the filtration chamber.

Remediation Method:

BGE Treatment to Contaminated water: Patalganga River water samples from two different sampling points were collected. 200 ml of each sample was taken in clean beakers and to it three BGE of different weights was added the water sample was made alkaline with NaOH this was followed by coagulation/flocculation. This was followed by aeration using compressed air. The sample was kept overnight and for the settlement of the complex acquired and then passed through filtration chamber.

BGACS Treatment in Filtration Chamber to BGE TreatedWater: The filtration chamber was divided into two fragments the first chamber consisted of two segments the first portion was a sand unit, the second portion was of activated charcoal obtained from *Mangifera indica* this was the equalization section. The equalization chamber was followed by main purification chamber divided into four sub sections BGACS compartment followed by sand filter succeeded by commercial activated carbon (Com. ACS) again followed by sand filters shown in figure 1.

Comparative Study of BGE Treatment with two Com. ACS and Sand filtration Chambers: The BGE treatment method for the sample was same as (IA) the only change was the filtration chambers. The BGACS chamber in (IB) was replaced by Com. ACS rest of the assembly was the same as (IB).

Comparative Study with KMnO4 Treatment With two Com. ACS and Sand Chambers: 200 ml of river water sample was taken. The sample was collected from sampling point 1of the river Patalganga. The water sample was made alkaline with NaOH for the analysis. It was treated with $KMnO_4$ by varying the weights of $KMnO_4$. The weights of $KMnO_4$ were kept same as that of BGE. The procedure for treatment of $KMnO_4$ was same as that for BGE treatment. The sample was kept overnight and passed through filtration chambers which consisted of two chambers of Com. ACS each separated by sand filtration chamber same as in (II).

RESULTS AND DISCUSSION: The weight of BGE coagulant in the two remediation methods was kept

same. The experimental condition of coagulation, flocculation, aeration, settlement timings was kept exactly the same. A control was run along with the experimental samples. All the experimental conditions were kept same except the addition of the BGE as a coagulant. It was observed that both the conventional $KMnO_4$ water treatment method and the BGE treatment method gave high quality of water purification and could effectively remove Fe^{2+}, PO_4, SO_4 and NO_3 from polluted source of water. The results obtained were analyzed statistically on General linear Model of SPSS ver. 24(2016). The results of one way ANOVA conducted to compare the effects of $KMnO_4$ and BGE treatment and the analysis showed that there was a significant effect of treatment at $P < 0.05$level for the three conditions [$F(2,9)$ $P = 6.569$, $P=0.017$]as seen in table 3.The post hoc test of multiple comparisons using Tuckey HSD test and LSD indicated that mean score of $KMnO_4$ treatment was [M=83.27, SD=20.07]. BGE was [M=92.5, SD=13.29] and for control was [M=32.2.27, SD=36.6] as seen in table 1. The Levens test (Table 2) shows P=0.006 and as P< 0.05 hence confirms that there is a significant difference in the treatments. The Post HOC (Table 4) test prove that the effect of the two treatments is different even though they lie the same homogenous subsets as seen in table 5 figure 2 shows that BGE treatment is more effective than $KMnO_4$ treatment.

When the Patalganga river water sample was coagulated and flocculated in alkaline medium with BGE it was observed that the methanolic extract of Bermuda grass formed a homogenous solution and as soon as the sample was aerated the extract started reacting within the solution. Aeration with the help of an air compressor initiated the photochemical process as BGE consists of chlorin dye which is a natural photosensitizer which can absorb light in the UV as well as the visible region[9] leading to the photocatalytic oxidation in solar light. This initial bubbling of air increased the $O_2^{\bullet-}$ dioxygen concentration in the solution hence leading to the conversion of linoleic acid in the BGE[10 & 2] into hydrogen peroxide.

Table 1: Acquired removal percentage of contaminants by different treatments.

Descriptives								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
$KMnO_4$+COMACS +SAND	4	83.2700	20.07374	10.03687	51.3282	115.2118	53.65	96.87
BGE+BGACS+SAND	4	92.5700	13.29053	6.64527	71.4218	113.7182	72.65	99.89
CONTROL	4	32.2375	36.71442	18.35721	-26.1833	90.6583	.00	67.97
Total	12	69.3592	35.95921	10.38053	46.5118	92.2066	.00	99.89

Table 2: Homogeneity of Variances.

Acquired removal percentage			
Levene Statistic	df1	df2	Sig.
9.372	2	9	.006

Table 3: ANOVA.

Acquired removal percentage					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8441.089	2	4220.544	6.569	.017
Within Groups	5782.627	9	642.514	-	-
Total	14223.715	11	-	-	-

Table 4: Post hoc Tests.

Multiple Comparisons							
Dependent Variable: Acquired removal percentage							
	(I) Remediation	**(J) Remediation**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Tukey HSD	$KMnO_4$+COMACS+ SAND	BGE+BGACS+SAND	-9.30000	17.92364	.864	-59.3429	40.7429
		CONTROL	51.03250*	17.92364	.046	.9896	101.0754
	BGE+BGACS+SAND	KMnO4+COMACS+SAND	9.30000	17.92364	.864	-40.7429	59.3429
		CONTROL	60.33250*	17.92364	.020	10.2896	110.3754
	CONTROL	KMnO4+COMACS+ SAND	-51.03250*	17.92364	.046	-101.0754	-.9896
		BGE+BGACS+SAND	-60.33250*	17.92364	.020	-110.3754	-10.2896
LSD	KMnO4+COMACS+ SAND	BGE+BGACS+SAND	-9.30000	17.92364	.616	-49.8461	31.2461
		CONTROL	51.03250*	17.92364	.019	10.4864	91.5786
	BGE+BGACS+SAND	$KMnO_4$+COMACS+SAND	9.30000	17.92364	.616	-31.2461	49.8461
		CONTROL	60.33250*	17.92364	.008	19.7864	100.8786
	CONTROL	KMnO4+COMACS+SAND	-51.03250*	17.92364	.019	-91.5786	-10.4864
		BGE+BGACS+SAND	-60.33250*	17.92364	.008	-100.8786	-19.7864
*. The mean difference is significant at the 0.05 level.							

Table 5: Homogeneous Subsets.

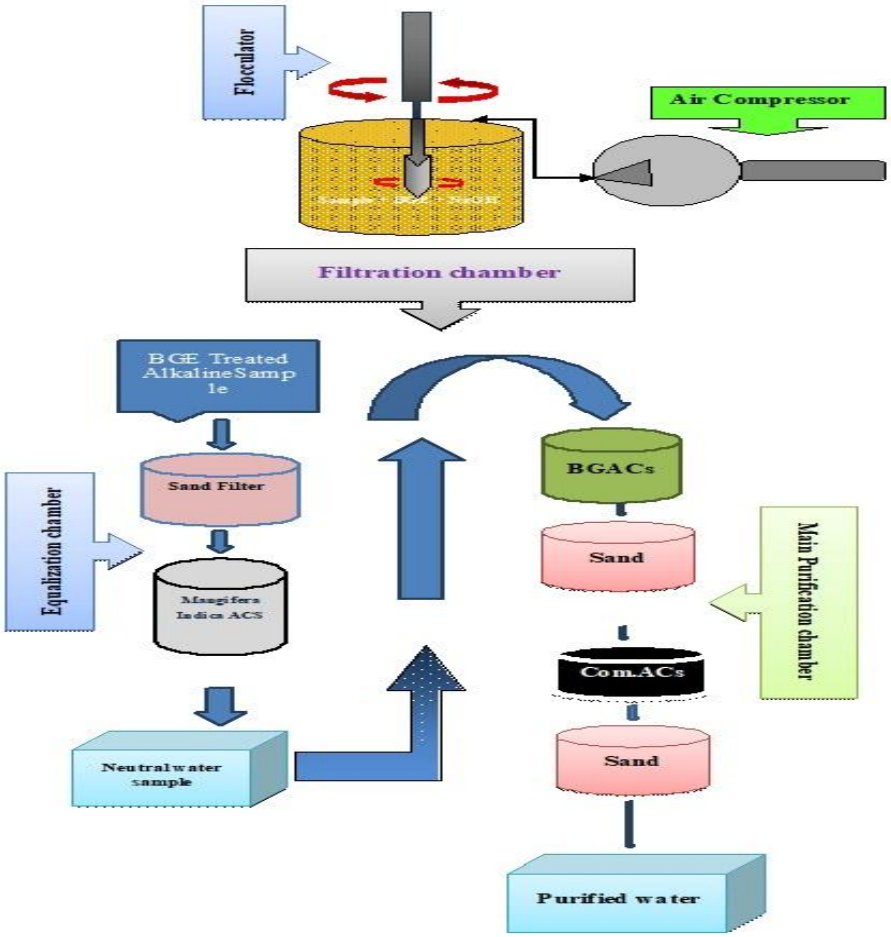
Acquired removal percentage				
	Remediation	N	Subset for alpha = 0.05	
			1	2
TukeyHSD[a]	CONTROL	4	32.2375	-
	$KMnO_4$+COMACS+SAND	4	-	83.2700
	BGE+BGACS+SAND	4	-	92.5700
	Sig.		1.000	.864
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 4.000.				

This hydrogen peroxide acts as an active oxidizing agent and on coming in contact with the innate Fe^{3+} ions in the Patalganga River water converts the solution into Fenton's reaction mixture. The reaction produces catalase that thereby catalyzes the redox reaction and decomposes H_2O_2 into water and oxygen. This hydrogen peroxide is a by-product of various cellular reactions (linoleic acid oxidation). The enzyme catalase in the BGE utilizes the Fe^{3+} ions mentioned earlier in the oxidation-reduction cycle this incorporation of iron thus improves the catalytic activity as the catalase is stronger than iron in the solution. They in turn absorb photons and generate $O^{\bullet-}$ and $OH^{\bullet}$ radicals.[11, 12, 13, 14 & 15] In the second phase once the bubbling of air was stopped the particles of extract appeared glowing and within no time it was the process of complexation was initiated because it leads to depletion of dioxygen concentration consequently increasing the free radical concentration in the solution leading to the formation of different adducts of phosphates, sulphates and nitrates. The formation of these adducts is a result of the antioxidants like phenols and flavonoids present in BGE. These antioxidants possess property to form metal complexes by chelation and scavenge the free radicals as scavengers. The ability of the methanolic extract of the aerial parts of Bermuda grass to scavenge free radicals is 93.33% as confirmed by Dr. Ali Esmail.[1, 11 & 16] BGE is rich in antioxidants which increase the photochemical activity due to the presence of cytokinins.[2] Hence the sample was allowed to stand overnight so that the reaction was completed. The resting time not only completed the reaction but also helped the absolute settlement of the complex formed by chelation. The solution thus

obtained was pale yellow colored clear liquid which was free from any suspended impurities and the pH of the solution was alkaline.

In the third phase of treatment the BGE treated sample was passed through the filtration chamber. The most significant thing about filtration chamber was that the BGE treated sample was passed through it without the addition of any acid for neutralization. The first sand unit and *Mangifera indica* ACs unit acted not only as equalization compartments but also phenolic removing station as BGE is rich in phenolics.[17] This equalization chamber treated the sample to free it from all the coordination complex matter and neutralize it. The neutral sample free from phenolics was finally passed through the main purification chamber where the completely purified water sample was acquired. Here the BGACs played the key role in the removal of iron, phosphates, sulphates and nitrates. In the main filtration chamber the first compartment was the BGACs chamber which played a vital role in purifying the sample completely it was observed that when the BGACs chamber was replaced with the two commercial activated carbon chambers the results acquired were very low as compared to the results obtained from BGACs filtration chamber. The comparative results of the changes in the main filtration chambers can be seen in figure 4 which clearly differentiates the results acquired by changing the filtration chambers. The BGACs filtration unit gave maximum removal of contaminants. Whereas in first filtration chamber (as seen in figure 4) the sample was initially treated with BGE but the filtration chamber did not have BGACs instead it had two Com. ACs units. The results were better than $KMnO_4$ treatment method but lower than BGE and BGACs treatment method. Hence it can obviously be said that BGE along with BGACs makes the best solution for remediation of polluted water. The iron removal by BGE was 5.59% more than $KMnO_4$, phosphates removal by BGE was 2.34% more than $KMnO_4$ and sulphates removal by BGE was 10.34% more than $KMnO_4$ whereas the BGE could remove almost 19% nitrates more than $KMnO_4$ distinctly showing that nitrate removal by BGE treatment is the highest. The control sample did not receive any treatment so the results obtained were negligible.

Figure 1: Graphical Abstract of Remediation process.



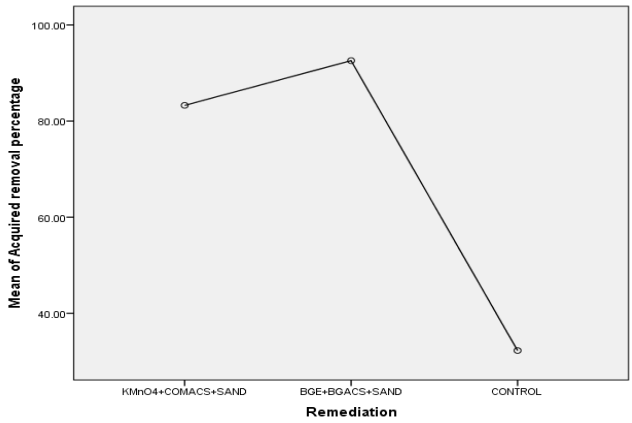


Figure 2: Comparison of means of % removal of Fe^{2+}, PO_4, SO_4 & NO_3 between BGE treatment, $KMnO_4$ treatment and Control sample.

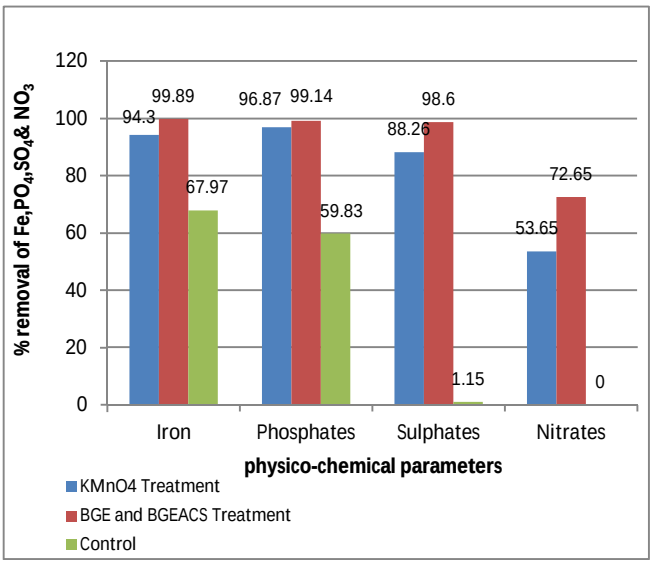


Figure 3: Comparison of % removal of Fe^{2+}, PO_4, SO_4 & NO_3 between BGE treatment, $KMnO_4$ treatment and Control sample.

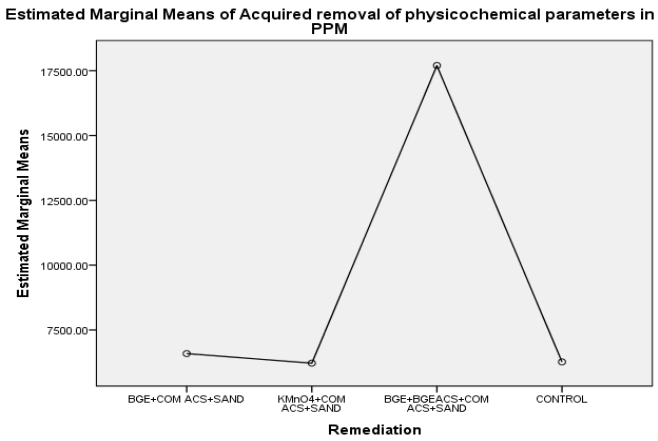


Figure 4: Comparison of removal of Fe^{2+}, PO_4, SO_4 & NO_3 in PPM between different filtration chambers.

CONCLUSION: The use phytoplankton's for the treatment of polluted river basins and polluted soil are no longer new and the use of Cynodon Dactylon for the same is common. It is known that Cynodon Dactylon is renowned for its medicinal values the use extract of Bermuda grass for medicinal purposes is conventional but its use as measure to remediate water pollution is novel. The BGE and BGACS treatment method is a unique combined treatment method which can efficiently remove iron, phosphates, sulphates and nitrates from polluted water. The results obtained after comparing it with conventional $KMnO_4$ treatment method gives that there was a significant effect of treatment at $P < 0.05$ proving that BGE treatment more effective than $KMnO_4$ treatment method. The BGE treatment method is the only method which can remove high values of iron, phosphates, sulphates and nitrates. Moreover it can also remove other impurities like Zn, Cd, Pb, and Cu which have not been mentioned in this paper

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