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RESEARCH ARTICLE

AN ASSESSMENT OF HEAVY METAL ACCUMULATION CAPACITY OF FIVE AQUATIC MACROPHYTES AND BIOCHEMICAL RESPONSE.

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Abstract

The phytoextraction capacity of five aquatic macrophytes (*Eichhorniacrassipes,Hydrillaverticellata Pistia stratioties, Salviniamolesta and Lagenandra toxicaria*) with respect to heavy metals like Ni, As, Zn, Cr and Fe were studied. It was done to study the plants capacity comparatively using monometallic system. Inorder to assess the biochemical response of the studied plants to heavy metal stress, metabolites like total carbohydrates, total soluble protein, tannin, total carotenoids, alkaloids, flavanoids, terpenoids, saponin and phenol were quantitatively estimated before and after treatment. With regard to Ni, As and Fe sample II showed maximum absorption and least by sample V. Zn absorption is greatest in plant sample II and least in sample I. As with Cr plant sample IV showed maximum absorption and least by sample I. The most significant biochemical change observed is the substantial increase in quantity of total carbohydrate, total soluble protein, total carotenoids, saponin and phenol content of the treated plants. Similarly there is a substantial reduction in metabolites like tannin, alkaloids, flavanoids and terpenoids after treatment. The studied macrophytes proved to the useful in the uptake of heavy metals in the monometallic system and showed great of potential for further applications in the industrial and waste water treatments.

Introduction:-

General contamination of heavy metals in the environment is a major global concern, which has provoked the emergence of phytpremediation technologies for cleaning aquatic environment. Heavy metals are released into the environment from a wide range of natural and anthropogenic sources. Aquatic systems often act as final receptacles to these metals whose concentration in interstitial water might increase several thousand folds by effluents from water (Bastian and Hammer 1993). Heavy metals are strong environmental pollutants and many of them are toxic, even at very low concentrations. Contrary to organic materials, they cannot be degraded and therefore they accumulate in water, bottom sediment and living organisms.

Heavy metals are the stable metals or metalloids whose density is greater than 5g/cm3, namely mercury, cadmium, cobalt, lead, molybdenum, nickel, copper, zinc etc(Nies 1999). Heavy metals are natural constituents of the Earth's

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crust. Human activities have drastically altered the biochemical and geochemical cycles and balance of some heavy metals. In the early seventeenth, several researchers carried out series of investigations to enhance the quality of water using natural means (2-5). Since then extensive research is being proceeded worldwide by numerous investigators to study the performance of many species of aquatic plants in removal of nutrients and metals in different aquatic water bodies (Dana Ahmed 2014) among different varieties of plant, the concept of using aquatic plant are experimentally acknowledged to mitigate a considerable amount of pollutants time, organic contaminants, metals from aquatic environment through absorption, and / or adsorption to incorporate then directly into their plant tissues. (Espinoza 2001).

Aquatic plants are well known for accumulation and concentration of a great amount of various substances. Among them metals, which they take from the environment and concentrate in the trophic chains with accumulative effect. Therefore they are useful indicators of local pollution. It is well known that all plants can accumulate heavy metals.

Biochemical Responses:-
Several heavy metals such as Fe, Cu, Mn, Mo and Ni are essential elements to plant metabolism. In higher concentrations, many heavy metals inhibit plants biochemical production and this has been extensively studied and reviewed (Feranndes and Henriques, 1991; Sarma and Sarma, 2007; Sarma et al. 2009). Photosynthetic pigments of plants belonging to different group's exhibit differential tolerance to metals (Vajpayee et. al., 2001). Heavy metal substitute chlorophylls and related porphyrins have been known in vitro for a long time (Kupper et al., 2000). Many researchers examined the effect of heavy metals on photosynthesis and observed a decrease in fluorescence (Atal et al., 1991; El-Sheekh, 1992).

Cd induced reduction of photosynthetic pigment were recorded in two species viz., M. heterophyllum and P. crispus. The highest decrease in chlorophyll a was recorded in 7.34 mg g-1 in M. heteriohyllum and 8.09 mg g-1 in P. crispus (at 64 mg L-1 and 96 h) have given as evidence for the Cd toxicity to chlorophyll.

The Cr uptake by many aquatic plants influenced in biochemical process results in alteration of pigments and amino acids. Further, Vajpayee et al. (2000) suggested that Cr VI could exchange the Mg from active site of enzyme resulting into phaeophytin and thus depleted chlorophyll contents in Cr treated plants. Chromium also inhibits chlorophyll biosynthesis by creating nutrient imbalance (Barcello et al., 1986).

Mercuric cations have a high affinity for sulphydryi (-SH). In almost all proteins contain sulphydry I groups or disulphide bridges, Hg could disturb the normal functions of proteins in binding in two sites of a protein molecule without deforming the chain, leading to protein precipitation. (Clarkson, 1972).

Mercury affects both light and dark reactions in photosynthesis and caused inhibitions of electron transport activity, oxygen evolution and quenching of chlorophyll fluorescence in photosystem II (PS II). Substitution of the central atom of chlorophyll, magnesium, by mercury in vivo is an important damage mechanism, because it prevents photosynthetic light harvesting in the affected chlorophyll molecules and results in the breakdown of photosynthesis (Krupa and Baszynski, 1995).

Study Area:-
North Paravur is located at 10.14° N 76.7° E. It has an average elevation of 10 metres (32 feet). The town is situated at north end of Ernakulam district and bordering with Thrissur district. The towns in Thrissur district like Kodungallore, Mala, Chalakudy and the towns Kalamassery, Aluva, Angamaly, Vypin island are located near to this town. The ParavurTaluk lies in the flat delta region of the Periyarriver and cut by several canals, which have resulted in the formation of many islands. The Kodungalloor Kayal (backwaters) andVarappuzha Kayal (backwaters) are in this taluk.

Materials and Methods:-
Five native aquatic macrophytes were selected as and collected from in and around N. Paravoor. The plant species selected were: *Eichhorniacrassipes (Mart) Solms, Hydrillaverticellata, Pistiastratiotes, Salviniamolesta and Lagenandratoxicaria*. The plants were thoroughly washed at the sampling site with a jet stream of tap water until the surfaces appeared to be clean. The collected plants were kept separate in five plastic tanks, filled with tap water.

After the growing period, the plants were used for Ni, As, Zn, Cr, Fe phytoremediation experiments. They were kept for acclimatization for a period of 3 days in modified Hoagland solution in laboratory condition. The heavy metal stock solutions were made of salts such as Nickel nitrate, Arsenic nitrate, Zinc chloride, Chromium Chloride and Iron sulfate. The reduction in concentration in the medium were estimated by using Atomic absorption spectrophotometer at different time intervals of 24,48, 72 96 hrs and the percentage of absorption of metals was calculated.

In order to investigate the responses of the plants through heavy metal treatments, primary and secondary metabolites like total carbohydrates, total soluble protein, tannin, total carotenoids, alkaloids, flavanoids, terpenodis, Saponin and phenol were quantitatively estimated before and after treatment Test methods were: total carbohydrates (Hodge and Hofreite, 1962) Total soluble protein (Lowry and Resebrougn) Tannin, (Makkar et.al, 2012), Total carotenoids (Zakaria et.al 1979) Alkaloids (Ajanaletal, 2012) Flavanoids (Zhisen et. al, 1999) Terpenoids (Ferguson, 1956), Saponin (Hia et. al, 1976) Phenol, (Asis 1989).

During the phytoremediation experiments, the plants were exposed to laboratory condition at a temperature of 26-31^{0}C. After the adaptation period, the plant were exposed separately with different metals of appropriate quantity in water and noted for phytoremediation experiment. In the present investigation, the control plants were left without heavy metal salt treatment. The phytoextraction capacity of each plant selected with respect to Ni, As, Zn, Cr and Fe were thus estimated.

Result and Discussion:-

Analysis of metal accumulation by plants have practical values in outlining ore deposits of variety of metals and also in making new discovery. The hyper accumulation capacity of five aquatic macrophytes with regard to Ni, As, Zn, Cr and Fe from N. Paravur area of Ernakulam District. Heavy metals are removed from the aquatic environment by physical, biological and biochemical processes which take place in the water, biota and suspended solid. The domination of some processes depends on the composition of the system, the pH, the redox state and the pollutant nature (Miretzky et. al 2004). Dunabin and Bormer 1992 have proposed that emergent plants influence metal storage indirectly by modifying the substratum through oxygenation, buffering, pH and adding organic matter. There are two mechanisms- "Bio concentration" Uptake from ambient environment) and "biomagnifications" (up take through the food…), which are responsible for persistence and accumulation of heavy metals in water, sediment and into the tissues of the living organisms (Chaphekar 1991).

Result regarding the accumulation of heavy metals (Ni, As, Zn, Cr, and Fe) the studied macrophytes were analysed and tabulated in Figure 1-5. In the present analysis, with regard to Ni the concentration in the medium reduced from 31% to 2 % during treatment with plants sample I from 24- 95 hours of treatment whereas with plant sample II 16.5% to 6%, plant sample III 27 to 10%, with plant sample IV, 20-6.4% and finally with sample V the reduction in percentage varied from 28% to 10%. Thus with regent to Ni the best hyper accumulation is plant sample I and the least by plant sample II and V. As with Arsenic the plant sample I showed a reduction in concentration in the medium from 25% to 8% from 23 to 96 hours, with plant sample II concentration reduced from 70% to 26%, with plant sample III from 63 to 1990, plant sample IV Greatest Arsenic accumulation is thus shown by plant sample I and the least by plant sample V.

The percentage of reduction in concentration of Zinc from 24 to 96 hours ranged from 52 to 11% in plant sample I, 22 to 8% with plant sample II, 24 to 17 % with plant sample III, 21 to 9 % with plant sample IV and 37 to 11% with plant sample V. As with zinc the highest accumulator is plant sample at least is plant sample I.

Chromium concentration in the medium reduced from 83 to 27% during 24 to 96 hours in plant sample I, from 47 to 17% with plant sample II, from 9.7 to 5.3 with plant sample III, 23 to 8% with plant sample IV and from 65 % to 16% with plant sample V. Thus with regard to chromium the best accumulator species is plant sample III and least is plant sample I.

Concentration of Fe in the medium reduced from 12 to 3% with plant sample V, from 7 to 1.4% with plant sample III, from 10 to 3.2% with plant sample IV and from 9 to 1.5% with plant sample V. The best accumulator species for Fe is plant sample IV and the least plant sample I.

These results showed that aquatic macrophytes possess different accumulation ability for selected metals. The study also gained a better understanding of the importance of aquatic plants in heavy metal accumulation and detoxification mechanisms that may lead to design of new pollution control and prevention facilities.

Figure 1:-Heavy Metal Absorption By Aquatic Macrophytes.Decrease in concentration of Ni in percentage in a time interval of 24, 48, 72 and 96 hours

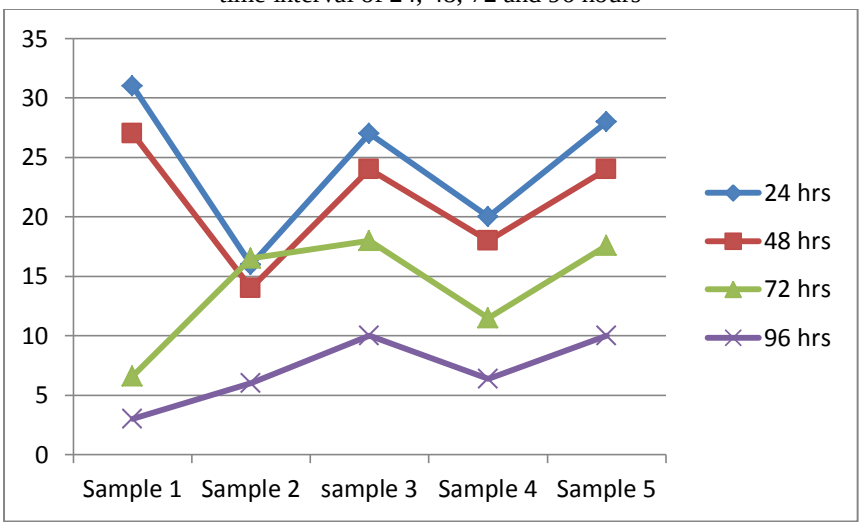


Figure 2:-Decrease in concentration of As in percentage in a time interval of 24, 48, 72 and 96 hours.

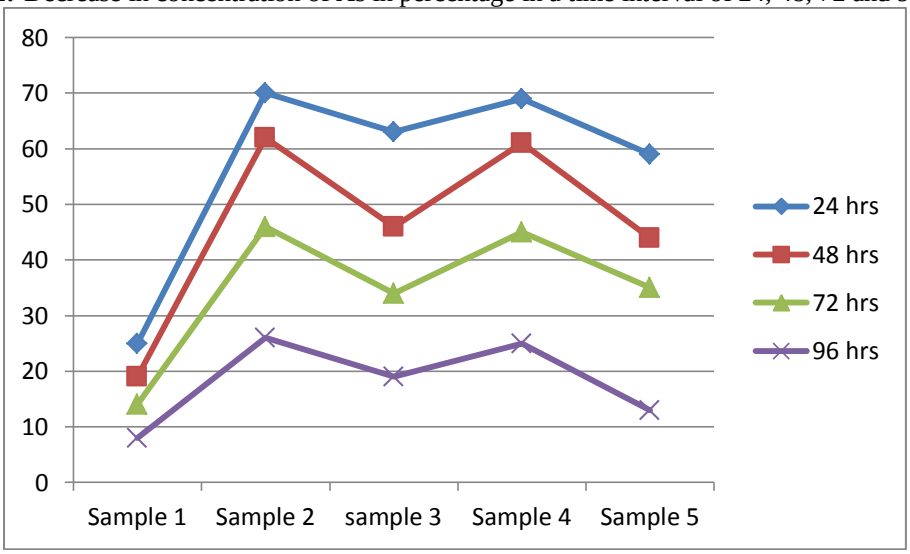


Figure 3:-Decrease in concentration of Zn in percentage in a time interval of 24, 48, 72 and 96 hours.

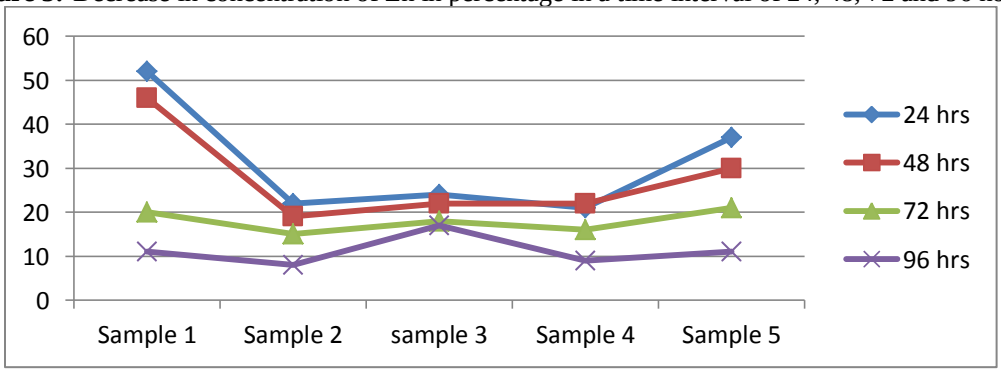


Figure 4:-Decrease in concentration of Cri in percentage in a time interval of 24, 48, 72 and 96 hours.

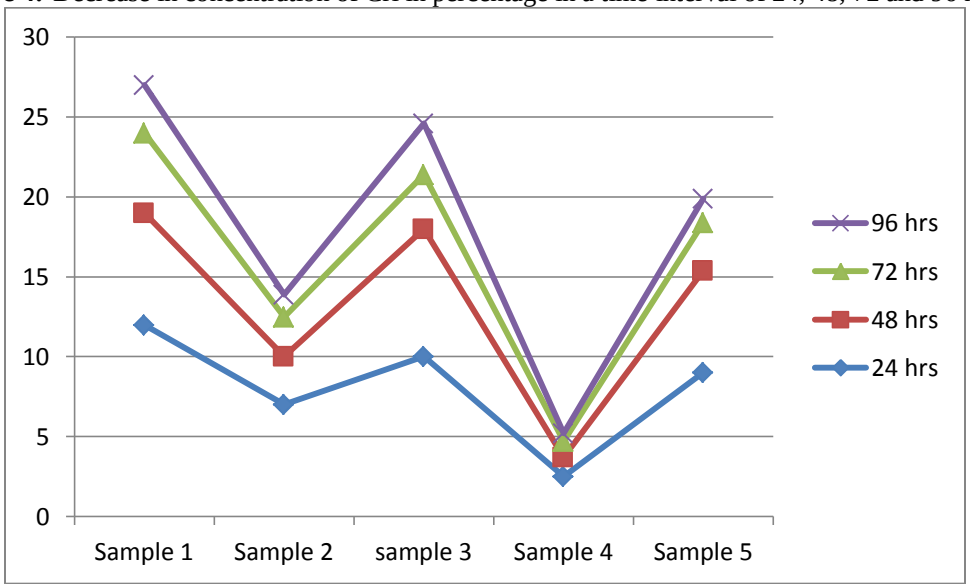
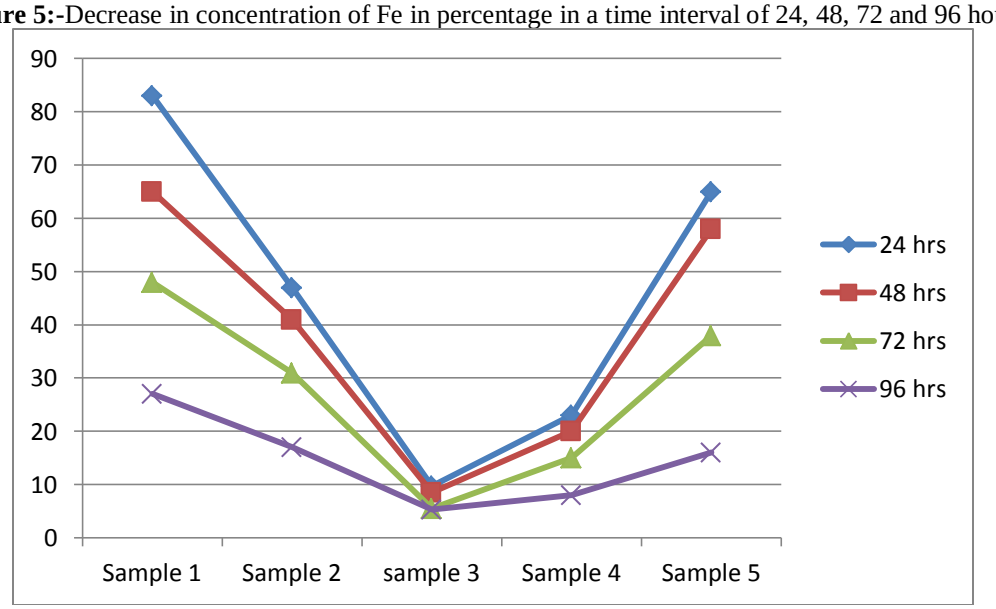


Figure 5:-Decrease in concentration of Fe in percentage in a time interval of 24, 48, 72 and 96 hours.



Further, in the present study the experimental plants were subjected to phytochemical screening before and after phytoremedation in order to determine the physiological response of the plants towards metal hyper accumulation. During phytochemical screening the presence of various compounds like total carbohydrates, total soluble protein, tannin, total carotenoids, alkaloids, flavanoids, terpenoids, saponin and phenol were performed by standard methods. The result were presented in table I and II.

The results of the phytoremediation study and the consequent physiological response is expressed in the quantitative variation of the secondary metabolites. Total carbohydrates increased from 13 to 21.38 mg/gm in plant sample I, 25.2- 22.06 mg/gm in plant sample II, 19.5 to 25.07 in plant sample III, 12.5 to 20.94 in plant sample IV and 13.2 to 19.56 in plant sample V.

Total soluble proteins varied from 2.37 to 5.49 mg/gm in sample I, 2.86 to 6.62 mg/g in sample II, 2.12 to 4.91 in sample III, 1.95 to 4.52 sample IV and 1.86 to 5.31 mg/g in plant sample V.

With regard to tannin in all plant samples there is a uniform pattern of reduction in quantity is observed. In sample I 44 to 38.52 ug/g, sample II 37-32.39 ug/g, sample III 39 – 34.14 ug/g, sample IV 28 – 24.51 ug/g at in plant sample V 22-19.26 ug/gm.

As with regard to Carotenoids there is a substantive increase in quantity is observed In sample I 140- 192.56 ug/gm, sample II 100-187.54 ug/g, sample III 150- 191.31 ug/gm, sample IV 90-148.79, sample V 90 – 158.79 ug/gm.

Just like tannin , the 2ndary metabolite alkaloid also exhibited a substantive reduction in quantity after treatment with heavy metals. Sample I 29- 25.39 ug/gm, sample II 32-28.01 ug/gm, sample III 27- 23.64 ug/gm, sample IV 30- 26.26 ug/gm and sample V 35- 30.64 ug/gm.

Flavanoids exhibited a quantitative reduction from 49- 42.89 ug/gm in sample I, 48 – 42.02 ug/gm in sample II, 31 – 37.14 in sample III increase in sample IV it shows reduction in quantity from 33 to 28.89ug/gm and again with sample V 48 to 42.02 ug/gm.

The metabolite terpenoid showed a slight increase from 14-15.76 ug/gm in sample whereas a reduction in sample II from 7-6.13 ug/gm, in sample III 11-9.63 ug/gm, in sample IV 11-8.63 ug/gm and in sample V from 9-7.88 ug/gm.

As regard to the metabolite saponin there is again a substantial increase in quantity from 33-45.3ug/gm in plant. Sample I, 33 to 45.08ug/gm in plant sample II, 31 to 42. 64 ug/gm in plant sample III, 34 to 46.76ug/gm in plant sample IV and 38 to 52.27 in plant sample V.

Finally the metabolite Phenol also showed a substantial increase in quantity after treatment. In plant sample I Phenol increased in quantity from 5.2- 14.95 mg/gm, sample II 4.5 to 12.94 mg.gm, sample III 5.5 to 15.81 mg/gm, sample IV 5-7 to 16.39 mg/gm and in sample V 6.2- 17.83 mg/gm respectively.

Conclusion:-

The present study was carried out on five species of aquatic macrophytes, with the aim to determine the capacity for accumulation of four metals (Ni, As, Zn, Cr and Fe), which is important for bioindication, bio remediation and biomonitoring of aquatic ecosystems. In order to determine stress responses in plants under heavy metal treatment, biochemical parameters such as total carbohydrates, total soluble protein tannin, total carotenoids, flavanoids, terpenoids, saponin and phenol were quantitatively estimated before and after heavy metal treatment. The most significant biochemical change observed is the substantial increase in quantity of total carbohydrates, total soluble proteins, total carotenoids, saponin and phenol content of the treated plants. Similarly there is substantial reduction the metabolites like tannin, alkaloids, flavanoids and terpernoids after treatment indicating that responses of plants to heavy metal. The reduction in metabolite level can be considered as an effect of the metal ion toxicity and the increase revealed the highest heavy metal tolerance.

From the present observations, it is concluded that the aquatic macrophytes were found to be the potential source for accumulation of heavy metals from water. Therefore, such studies should become an integral part of the sustainable development of the ecosystems' and pollution assessment program. There is urgent need to study more of those specific macrophytes which are "responsible" for cleaning the water body from toxic heavy metals.

Table I:-Phytochemical analysis of 5 macrophytes before treatment

Sl No	Sample Name	Parameters	Result	Test Method	Unit (as fresh weight)
1	1	*Total carbohydrates*	13.0	*Hodge &Hofreite, 1962*	*mg/g*
	2		25.2		
	3		19.5		
	4		12.5		
	5		13.2		
2	1	*Total soluble protein*	2.37	*Lowry and Rosebrough, 1951*	*mg/g*
	2		2.86		
	3		2.12		
	4		1.95		

	5		1.86		
3	1	*Tannin*	44	*Makkar et al., 1993*	*µg/g*
	2		37		
	3		39		
	4		28		
	5		22		
4	1	*Total carotenoids*	140	*Zakaria et al., 1979*	*µg/g*
	2		100		
	3		150		
	4		90		
	5		90		
5	1	*Alkaloids*	29	*Harbone, 2000*	*µg/g*
	2		32		
	3		27		
	4		30		
	5		35		
6	1	*Flavanoids*	49	*Zhisen et al., 1999*	*µg/g*
	2		48		
	3		31		
	4		33		
	5		48		
7	1	*Terpenoids*	14	*Ferguson, 1956*	*µg/g*
	2		7		
	3		11		
	4		11		
	5		9		
8	1	*Saponin*	33	*Hiai et al., 1976*	*µg/g*
	2		33		
	3		31		
	4		34		
	5		38		
9	1	*Phenol*	5.2	*Asis, 1989*	*mg/g expressed as pyrogallic acid*
	2		4.5		
	3		5.5		
	4		5.7		
	5		6.2		

Eichorniacrassipes 2.Lagenandratoxicaria 3.Hydrillaverticellata 4.Pistiastratiotes 5.Salviniamolesta .

Table II:-Phytochemical analysis of 5 macrophytes after treatment

Sl No	Sample Name	Parameters	Result	UNIT (as Fresh Weight)	Test Method
1	1	*Total carbohydrates*	21.38	*mg/g*	*Hodge &Hofreite, 1962*
	2		22.06		
	3		25.07		
	4		20.94		
	5		19.56		
2	1	*Total soluble protein*	5.49	*mg/g*	*Lowry and Rosebrough, 1951*
	2		6.62		
	3		4.91		
	4		4.52		
	5		5.31		
3	1	*Tannin*	38.52	*µg/g*	*Makkar et al., 1993*
	2		32.39		
	3		34.14		

	4		24.51		
	5		19.26		
4	1	*Total carotenoids*	192.56	*µg/g*	*Zakaria et al., 1979*
	2		187.54		
	3		191.31		
	4		148.79		
	5		158.79		
5	1	*Alkaloids*	25.39	*µg/g*	*Harbone, 2000*
	2		28.01		
	3		23.64		
	4		26.62		
	5		30.64		
6	1	*Flavanoids*	42.89	*µg/g*	*Zhisen et al., 1999*
	2		42.02		
	3		37.14		
	4		28.89		
	5		42.02		
7	1	*Terpenoids*	15.76	*µg/g*	*Ferguson, 1956*
	2		6.13		
	3		9.63		
	4		8.63		
	5		7.88		
8	1	*Saponin*	45.39	*µg/g*	*Hiai et al., 1976*
	2		45.08		
	3		42.64		
	4		46.76		
	5		52.27		
9	1	*Phenol*	14.95	*mg/g*	*Asis, 1989*
	2		12.94		
	3		15.81		
	4		16.39		
	5		17.83		

Eichorniacrassipes 2.Lagenandratoxicaria 3.Hydrillaverticellata 4.Pistiastratiotes 5.Salviniamolesta .

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