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Biosorption of Heavy Metal Arsenic (AS) and Growth Promoting Efficacy of Marine Macroalga, *Ulva fasiciata* (L.) on *Vigna radiata*(L.) Seedlings

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ABSTRACT

The impact of heavy metal Sodium arsenate on the growth,yieldand biochemical characteristics of *Vignaradiata* (L.) was determined.The addition of high concentrations of heavymetal resulted in decrease in the growth, biochemical andyieldcharacters of *Vignaradiata*.The marine macroalga,*Ulvafasciata(L.)*as liquid fertilizer and algal biomasswas screenedfor its heavy metal biosorptionand growth promoting effect. The supplementation with *Ulvafasciata*extractincreases the percentage of growth, yield and biochemical contentof *Vignaradiata(L.)*compared with control treatedwith Sodium arsenate alone. Maximumincrease in percentage of growth and yield parameters were observed at 20%Liquid Fertilizer and 15g algal biomass of *U.fasciata* treated seedling. The results led to confirm that marine macroalgaecould be used to remove the toxicity of heavy metal inpolluted environmentfor sustainable agriculture. It is an economic, cost-effective and safe alternate to existing commercial adsorbents of heavy metal.

1. Introduction

The industrialisation coupled with the technological advances,had led to inadvertent discharge of toxic compounds which adversely affected the ecosystem. The heavy metals are among the toxic compounds which are harmful to the biological systems and do not undergo biodegradation. Metalpollutants can easily enter into the food chainif heavy metal contaminated soil is used for production of food crops resulting in reducing farm productivity (Gosavi*et al*., 2004).Arsenic is one among the heavymetal thatpollutes the environment andhas become a global environmental threat. It inhibits plant growthand yield. The World Health Organisation (WHO) and Environmental Protective Agency (EPA) have declared that inorganic arsenic is a human carcinogen. The health of children and adults exposed to high amount of arsenic are affected (Leo and Irudayaraj, 2010). The arsenic contamination also poses a major threat to the marine flora and fauna; the consumption of arsenic contaminated fish causes various ill effects.Many remediation techniques have developed to control and remove the metal pollutants from the environment. But those techniques are highly expensive and environmental disruptive (Gonzaga*etal*, 2006). The application of biotechnology in controlling andremovingmetalpollution has been paid much attention. The process ofbiosorption is a new alternative method, which utilizes certain naturals of biological origin, including bacteria, fungi, yeast and algae which possess metal sequestering property (Wang and Chen, 2009) and which have advantages of being cheap, highly efficient andenvironmentally friendly (Farooq et *al*., 2010; Srivastava and Majumder 2008; Abdel – Aty*et al*., 2013).

Marine macroalgae or seaweed are largegroup of marine benthic algaegrown abundantly in the shallow sea water,estuaries and backwater.Numerous studies revealedtheir wide range ofbeneficial effects on crop improvement and yield and elevatedresistanceto bioticand abiotic stress (Norrie and Keathley,2006). Besideseliciting agrowth promoting effecton plants,macroalgae has been proven as a potent biosorption agent(Davis*et al.,* 2000;Vijayaraghavan*etal*., 2004; Hashim*et al*., 2004).Hassan*et al*., 2004 evaluated metals like Cd, Hg and Pb uptake capacities of green marine macroalgae*viz;Cladophorafascicularis, Ulvalactuca* from contaminated solution which was attributed totheir uniformcell sizeand number of metal binding sites on their cell wall (Ramelow*et al*., 1992). These sitesinclude carboxyl groups,polysaccharides and sulfhydryl groups (Yu *et al.*, 2001). Further, biosorption approach using macroalgae would be technically feasible and economically attractive.

The normal growth and metabolism of plants requires the presence of heavy metals as they have a very important role in the biosynthesis of some enzymes and growth hormones (Nanson and McElroy, 1963). However, their excess supply hinders the plant growth and metabolism of many plant species as reported earlier by Foy*et al.* (1978). Thepresent study attempts tofind out the effect ofvarious concentrations of Sodium arsenate on the Green Gram (*Vignaradiata* (L.)). The biosorptioncapacityof different concentrationsof Liquid Fertilizerand algal biomass of*Ulva fasciata*(L.)onthe growth yield and biochemical content of Sodium arsenate treated *Vignaradiata* (L.) seedlings was also studied.

2. Materials and methods

Seed weed collection and preparation of Seaweed Liquid Fertilizer (SLF)

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The macroalgae, *Ulvafasciata*(L.)was collected from Manavalakurichi coast(8'27''N Latitudeand 77'18''E Longitude)of Kanyakumari district during early morning low tide period. It was washed thoroughly, allowed to shade dry and finely powdered using mixer grinder. The powder was sieved and preserved (algal biomass) in polythene bags for metal biosorption studies anddetermination of plantgrowthpromotingefficacy.

Seaweed Liquid Fertilizer (SLF) was prepared from the dry algal powder as per procedure of Thangam and Rani (2006). The algal powder mixed with distilled water in the ratio of 1:20 (w/v) and autoclaved for 30min.The hot extracts obtained was filtered through cheese cloth and centrifuged at 10,000rpm for 15 minutes. The Supernatant was dried at 60^{0}C for 48hrs. The dried macroalgal extracts considered as 100% seaweed liquid fertilizer andstored at 4^{o}C. From this, different concentrations *viz*., 1,5,10,15,20 and 25% of SLF were prepared using double distilledwater.

Preparation of Heavy Metal Solution

The heavy metal, Arsenic(As) stock solution (100 ppm) was prepared by dissolving analytical grade sodiumarsenate in deionised water. From the stock solution, different concentrations*viz.*, 1,5,10,15,20and25ppmwerepreparedbydilutionusingdistilledwater.

Experimental Setup

The viable seeds of *Vignaradiata*(L.) were obtained from Tamil Nadu Agricultural University, Pechiparai. The seeds were subjected to viability test; those with 90% viability were selected for the experimental studies.

The planting medium was prepared by mixing garden soil, sand and cow dung in the ratio of 1:2:1. The prepared medium was taken in mud pots of size 30 x 33 cm and is filled off about two-third of its height. Ten viable seeds of *Vignaradiata*(L.) were sown to a depth of 1.5cm in each pot. The seeds were watered daily to germinate. After seven days, the seedling of *Vignaradiata* (L.) WILCZEK were treated with various concentrations of Sodium arsenate*i.e*., 1,5,10,15,20and25ppm.After seven days of treatment, various morphometric and biochemical characters were analysed. In the other set, 20ppm sodium arsenate treated seedlings were applied withvarious concentrationsof SLF and algal biomass of *Ulvafasciata(L.).* After seven days of thetreatment,morphometric, biochemicaland yield parameters were recorded.

The morphometric characters such asroot and shootlength, leaf area,number of lateral roots, freshweight, dryweight, fruitlength and number of fruits were measured. The biochemical characters were analysed by the standard methods – Chlorophyllcontent as per Wellburn and Lichtenthalar(1984), Protein content as per Lowry *et al*. (1951), amino acid content as per Jayaraman, (1981) and total soluble sugar content based on Duboise*et al*. (1951). The experiments were carried out in three replicas.

3. Result and discussion

Effect of sodium arsenate on the growth and yield of *Vignaradiata* (L.)

The addition of increased concentrations of sodium arsenate resulted in gradual decrease in growth and yield of *Vignaradiata* (L.) seedlings. Highest reduction in growth and yield was obtained at 20ppm concentration of sodium arsenate.The percentage reduction in root and shoot length of sodium arsenate (20 ppm) treated seedlingswas 63.6 and 48.5 respectively.There was a drastic reduction in the number of lateral roots (56.8%), leaf area (76.5%), fresh weight (74.2 %) and the numberand length of fruits(76.9 and 50% respectively) compared with the control treated with water alone (Table 1).

Table -1 Effect of heavy metal Sodium arsenate on the growth and yield parameters of *Vignaradiata* (L.)

Parameters	Control (water)	Concentration of Sodium arsenate (ppm)					% decrease (compared to control)
		1	5	10	15	20	
Root length(cm)	8.25± 0.26	4.0± 0.70	3.5± 0.41	3.2±0.32	3.1 ± 0.25	3.0±0.15	63.6
Shoot length(cm)	16.5± 0.18	13.6± 0.2	12.5± 0.4	11.4±0.6	10.6 ± 0.8	8.5±0.2	48.5
Number of lateral roots	18.5± 0.3	15±0.2	14± 0.3	12±0.5	10 ± 0.4	8 ± 0.3	56.8
Leaf area(m^2)	17±0.3	14.8± 0.5	14.5± 0.8	13.1±0.4	10.4 ± 0.2	7.8 ± 0.7	54.1
Fresh weight(gm)	19.1 ± 0.54	17.6± 0.20	13.5± 0.1	12.9±0.1	7.1 ± 0.04	4.49 ± 0.03	76.5
Dry weight(gm)	6.2±0.92	6.01± 0.7	4.5± 0.5	4.2±0.4	2.05 ± 0.3	1.63 ± 0.05	74.2
Number of fruits	6.5±0.8	5.2±0.2	4.3± 0.09	4.0±0.2	2.1 ± 0.6	1.5 ± 0.01	76.9
Length of fruits(cm)	10± 0.03	8±0.9	7±0.4	6.5±0.7	6.5 ± 0.2	5 ± 0.02	50

The findings were in accordance with the study conducted by Upadhyaya*et al. (*2014) on the effect of Sodium arsenate on mung bean seedlings. The uptake of metal occurs primarily through the rootswhich inhibit the root and shoot growth. The metal uptake also reduces the fresh and dry weight of seedlings treated with heavy metal sodium arsenate (Aruduini*et al.,* 1996).Similarly, the reduction of leaf area in response to sodium arsenate treatment was also related to accumulation of arsenic in leaves. The resultscoincided with the finding of Pandey and Pathak (2006) in which some of the plants under arsenic stress showed constriction of leaves with appearance of burning spots in to leaf apex. The previous findings of Abedin*et al*.(2002) and Dhankher*et al*. (2006) also support the present findings.

Similarly, there were variations in the biochemical content of*Vignaradiate* (L.) seedlings treated with Sodium

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arsenate.The sodium arsenate (20 ppm) significantly reduced the total chlorophyll (58.8%) and chlorophylla content(40.9%) and slight reduction noted for chlorophyllb(16.3%) content.Similarly, the protein, amino acidand starch content was reduced to 32.3, 66.7and 53.4 percentage respectively compared to the control.The decrease in thecontents of chlorophyll, protein, amino acid and starch of *Vignaradiata (L.)*treated with sodium arsenate could be due to the generation of reactive oxygen species like superoxide and hydroxyl radicals and hydrogen peroxide that have potential to damage nucleic acid and amino acid involved in the biosynthetic pathway of chlorophyll synthesis (Abbas *et al*., 2018). Srivastava*et al.* (2013) also reported a similar decline of chlorophyll a, chlorophyll b andtotal chlorophyll content in *Hydrillaverticillata*at higher doses of arsenictreatment (Table2).

Table - 2 Effect of heavy metal, Sodium arsenate on the biochemical content of *Vignaradiata* (L.)

Parameters		Concentration of Sodium arsenate (ppm)					% decrease (compared to control)
	Control	1	5	10	15	20	
Chlorophyll a (µg/ g)	3.5 ± 0.041	3.32 ± 0.054	3.06 ± 0.036	2.84 ± 0.047	2.42 ±0.286	2.07 ± 0.027	40.9
Chlorophyll b (µg/g)	1.236 ± 0.024	1.207 ± 0.042	1.144 ± 0.026	1.134 ± 0.072	1.112 ± 0.05	1.035 ± 0.017	16.3
Total Chlorophyll (µg/g)	5.85 ± 0.062	5.37± 0.062	4.49 ± 0.049	4.17 ± 0.012	3.53 ± 0.021	2.41 ± 0.026	58.8
Protein (mg/g)	15.50 ± 0.85	14.10 ± 0.661	13.3 ± 0.051	12.7 ± 0.028	11 .6 ± 0.017	10.5 ± 0.06	32.3
Amino acid (mg/g)	6.0 ± 0.1	5.5 ± 0.75	4.5 ± 0.26	3.5 ± 0.17	2.3 ± 0.71	2.0 ± 0.68	66.7
Starch (mg/g)	5.8 ± 0.02	4.0 ± 0.5	3.8 ± 0.12	3.5 ± 0.06	2.9 ± 0.18	2.7 ± 0.20	53.4

Determination of biosorption efficacy of SLF from *Ulvafasciata*

Studies on the biosorptionefficacy of heavy metal Sodium arsenate by macroalga, *Ulvafasciata(L.)* revealed that at seaweed liquid fertilizer (SLF) from *Ulvafasciata*at all the studied concentrations*viz.,*1,5,10,15, and 20 % enhanced growth in terms of rootlength (40%), number of lateral roots (61.7%),shoot length (40.5%), leaf area (33.6%), fresh weight(257.5%)dry weight(324.5%),number of fruits(172.2%) andlength of fruits (68.8%)of *Vignaradiata*(L.) compared with the control *i.e*., the seedlings treated with20 ppm sodium arsenate (Table 3).

Table 3. Cumulative effect of SLF of *U. fasciata(L.)*andheavy metal Sodium arsenate (20ppm) on the growth and yield parameters of *Vignaradiata (L.)* seedlings.

Parameters	Untreated	Concentrations of SLF (%) and 20ppm Sodium arsenate						% increase (compared to control)
		Control (20 ppm sodium arsenate)	1	5	10	15	20	
Root length(cm)	11.2 ± 0.2	7.5 ± 0.4	7.85 ± 0.10	8.1 ± 0.40	9.15± 0.55	9.75 ± 0.62	10.50 ± 0.74	40.0
Shoot length(cm)	25.5 ± 0.84	15.8 ± 0.25	15.8 ± 0.08	16.1 ± 0.14	19 ± 0.49	22.1 ± 0.55	22.2 ± 0.70	40.5
Number of lateral roots	32.5 ± 0.65	8 ± 0.3	18 ± 0.53	25.5 ± 0.42	27 ± 0.81	28.5 ± 0.34	29.1 ± 0.85	61.7
Leaf area(m^2)	17 ± 0.3	13.1 ± 0.7	13.7 ± 0.2	14.2 ± 0.5	15.8 ± 0.2	16.5 ± 0.1	17.5 ± 0.32	33.6
Fresh weight(gm)	19.1 ± 0.54	4.49 ± 0.25	7.05 ± 0.21	13.03 ± 0.17	14.3 ± 0.28	15.9 ± 0.12	16.05 ± 0.07	257.5
Dry weight(gm)	6.5 ± 0.92	1.63 ± 0.05	2.05 ± 0.12	5.1 ± 0.15	5.13 ± 0.28	6.7 ± 0.25	6.92 ± 0.71	324.5
Number of fruits	1.5 ± 0.01	1.8 ± 0.2	2 ± 0.7	3.1 ± 0.2	4.2 ± 0.1	5.1 ± 0.2	4.9 ± 0.7	172.2
Length of fruits(cm)	5 ± 0.02	8 ± 0.5	8.2 ± 0.2	9.5 ± 0.3	10.2 ± 0.5	10.5 ± 0.7	10.37 ± 0.5	68.8

The lowest root length was recorded with sodium arsenate (20 ppm) treated seedlings. The root length of the plants treated with different concentrations of liquid fertilizer of *Ulvafasciata*(L.) was comparatively more.The root length was recorded the highest (90.5% increase) compared with the control) for the seedlings treated with 20% SLF of *Ulvafasciata* (L). The studies by Revathi*et al*., (2013) on the phytotoxic effect of chromium and EDTA on growth of *Sesbaniagrandiflora* L. supported the findings. Their results indicated that the increase in concentrations of chromium, EDTA, even at very low concentration were effective in phytoextraction of chromium. The phytoremediation efficacyof macroalgae is due to the biosorption of heavy metal because of the large surface area and metal binding sites on their cell wall. They also have the functional groups like carboxyl, amino acid, polysaccharides and sulfhydrol groups which are present on the cell wall of macroalgae (Yu *et al* .1999). The present findings coincided with the findings of Selvaraj*et al*., (2010) on the application of seaweed *Gracilariacorticata* at different concentrations with 6mM nickel chloride treated Vigna*radiata (L.)* seedling.Their studies indicated the stress relieving effect of macro algae, on thenickel chloride treated seedling of*Vignaradiata (L.)*.

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In the present study, the stress imposed by Sodium arsenate treatment to the seedlings of *Vignaradiata*(L.) led to reductionin the quantity and yield. But the yield was enhanced when the seedlings were treated with 20% SLF of *Ulvafasciata*(L.). It was similar to the findings of Sridhar and Rengasamy(2002). They suggested that the phytoremediation activity of macroalgal extracts was due to the presence of macro and micro-elements as well as plant growth regulators likecytokinin in it.

The treatment with seaweed liquid fertilizer of *Ulvafasciata* (L.) also helped in enhancing the content of Total chlorophyll, Chlorophyll a, Chlorophyll b and Carotenoids (Table 4). The findings are indicative of the fact that of SLF even at the lowest concentration controlled the mobility of heavy metal sodium arsenate as was evident from the growth, yield and increased biochemical contents when compared with the control. The observationswere in par with that made byAzmatand Askari(2015)in mercury (Hg) stressedplants and Selvaraj*et al*(2010) in Nickel chloride treated plants of *Vignaradiata* (L.) These results clearly indicated that additionof Liquidfertilizer frommacroalgae, *U.fasciata* reduced the toxic effect of sodium arsenate and promoted the growth and biochemical content of *Vignaradiata* (L.).

Table-4. Cumulative effect of SLF of*U. fasciata(L.)* and heavy metal Sodium arsenate (20ppm) on the biochemical content of *Vignaradiata(L.)*seedlings

Parameters	Control (20ppm Sodium arsenate)	Concentrations of SLF(%) and 20ppm Sodium arsenate (As)					% increase (compared to control)
		1	5	10	15	20	
Chlorophyll a (µg/ gm)	2.07±0.07	3.44±0.041	3.18±0.050	3.13±0.030	2.91±0.028	2.92±0.026	111.0
Chlorophyll b (µg/gm)	1.035±0.17	1.23±0.050	1.45±0.065	2.99±0.024	2.18±0.954	2.11±0.047	195.0
Total Chlorophyll (µg/gm)	2.41±0.026	2.45±0.048	3.17±0.050	4.11±0.054	5.66±0.061	4.01±0.039	131.02
Protein(mg/gm)	10.5±0.06	21.1± 0.443	23.4± 0.581	20.5± 0.482	18.3± 0.391	15.2± 0.221	122.9
Amino acid (mg/gm)	2.0±0.68	5.2± 0.52	6.1± 0.89	6.5±0.75	6.4±0.67	5.5±0.63	225.0
Starch (mg/gm)	2.7±0.61	6.4±0.30	7.2± 0.52	8.9 ±0.78	8.8±0.91	7.4±0.61	174.1

The treatment with algalbiomass of Ulva*fasciata(*L.) to the sodium arsenate (20ppm) treated seedlings also showed a similar trend. A drastic increase in the growth and yield was noted with the algal biomass treated seedlings than control. It is because of the presence of some growth promoting substances such as IAA,IBA,Gibberellins,Cytokinins,micronutrients and macronutrients in algal biomass which affect the cellular metabolism in heavy metal treated seedlingleading to enhanced the growth and crop yield (Durand *et al* 2003, Ordog*et al*.,2004).

Table-5. Cumulative effect of algal biomass of *U. fasciata*(L.)andSodium arsenate(20 ppm) on the growth and the yield parameters of *Vignaradiata (L.)*seedlings

Parameters	control (20ppm Sodium arsenate)	Quantity of algal biomass (gm) and 20ppm Sodium arsenate					Maximum increased in % over control
		1	**5**	**10**	**15**	**20**	
Root length(cm)	4.5±0.12	8.7±0.05	9.35±0.32	10.95±0.64	12.4±0.75	13.9±0.80	108.8
Shoot length(cm)	8.5±0.12	10.4±0.10	15.2±0.17	18.75±0.35	22.65±0.67	24.2±0.73	166.4
Number of lateral roots	8.1±0.21	11.5±0.07	18±0.42	21±0.59	27.5±0.71	33.5±0.85	116.04
Leaf area(m^2)	13.1±0.7	13.7±0.9	14.5±0.12	15.9±0.32	16.2±0.48	17.5±0.55	31.29
Fresh weight(gm)	4.5±0.12	5.23±0.24	7.4±0.15	12.96±0.28	21.06±0.17	12.9±0.81	212.4
Dry weight(gm)	2.8±0.31	3±0.25	4.52±0.17	5.61±0.15	7.4±0.22	4.23±0.21	164.2
Number of fruits	1.5±0.2	2.5±0.3	3.1±0.2	3.5±0.3	4.1±0.1	3.2±0.5	173.3
Length of fruits(cm)	5±0.21	6.4±0.2	7.7±0.1	8.8±0.2	9.8±0.3	9.1±0.2	96.0

Algal biomass of *Ulvafasciata (L.)* at different quantities treated with sodium arsenate (20ppm) on *Vignaradiata* seedlings improved the pigment and biochemical characters *viz.,* protein, sugar, amino acid and starch in *Vignaradiata*(L.).It was similar to the findings of Sevugaperumal*et al*., (2012). According to their studies on, the bioadsorption of algal biomass of *Padinacommersonni* on the *Vignaradiate (L.)* pretreated with 6mM concentration of aluminium chloride, increased the level of chlorophyll, protein, sugar, amino acid and starch compared with the control *i.e*., treated with heavy metal alone. Again these results were coincided with the findings of Jayakumar and Ramasubramanian (2009). Their results also confirmed that the addition of dry algal biomass reduce the toxic effect of sodium arsenate and promote the growth and biochemical content of *Vignaradiata*(L) Wilcekseedlings.

Table -6. Cumulative effect of algal biomass of *U. fasciata* andSodiumarsenate (20ppm) on the biochemical content of *Vignaradiata (L.)*seedlings

Parameters	Control (20ppm Sodium arsenate)	Weight of Algal biomass (gm) and 20 ppm Sodium arsenate					Maximum increase in % over control
		1	5	10	15	20	
Chlorophyll a (µg/ gm)	2.07± 0.027	3.01± 0.024	3.2± 0.062	3.91± 0.067	4.15± 0.072	3.34± 0.059	100.5
Chlorophyll b (µg/gm)	1.035± 0.017	1.24± 0.082	1.18± 0.016	1.26± 0.018	1.35± 0.019	1.18± 0.015	30.4
Total Chlorophyll (µg/gm)	2.41± 0.026	3.14± 0.027	3.27± 0.038	4.79± 0.036	5.62± 0.048	5.2± 0.042	133.1
Protein(mg/gm)	10.5± 0.06	12± 0.373	12.6± 0.328	13.7± 0.278	15± 0.451	14.5± 0.331	47.6
Amino acid (mg/gm)	2.0± 0.68	3.1± 0.69	4.5± 0.72	5.6±0.75	6.4± 0.79	6.1± 0.72	155.0
Starch (mg/gm)	2.7± 0.20	5.4± 0.19	6.0± 0.25	7.2±0.24	9.2± 0.81	8.1± 0.80	151.8

The above results clearly showed that the applicationof algalbiomass of*Ulvafasciata*(L.) on the heavy metal Sodium arsenate treated seedlings reduce the toxic effect of arsenic and enhance the growth and productivity of Vigna*radiata*(L.).

The results of present study indicate that the application of SLF and algal biomass of *Ulvafasciata*(L.) reduce the toxic effect of heavy metal Arsenic and promote the growth and yield of *Vignaradiate* (L.) WILCZEK.Thus, macroalgae or seaweed are used as cost effective,eco-friendly safe alternative to existing commercial adsorbent for heavy metal stresses plants and polluted environment.

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