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Assessment on Growth Performance of Green gram (Vigna radiate (L) Wilzeck). by Using Phytotreated and Non Phytotreated Waste Water

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ABSTRACT

Assessment on growth performance of Vigna radiate L. by using Phytotreated and Non Phytotreated waste water. The plants were raised in petridish containing different concentrations of raw and treated waste water (C, 10%, 10% treated, 25%, 25% treated, 50%, 50% teated, 75%, 75% treated,100% and 100 % treated). The morophological parameters like, seed germination percentage, seedling growth, (Such as, root and shoot length; fresh and dry weight fo root and shoot), vigoue index, tolerance index were measured on 7th days after sowing. All the morophological parameters were increased at 25 treated waste water in a petridish, when compared with control. Further increases in the waste water (50%-100%) in the soil have a negative effect on these parameters.

Keywords: Seed germination; Phytotreated; Non Phytotreated; Waste water; Seedling growth. Vigna radiate L.

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1. Introduction

Waste waters discharged by the industries are one of the major causes of environmental pollution, particularly in the developing countries. Pulp and paper manufacturing is one of the oldest and largest in India with an installed capacity of about three million metric tons per annum finished product. Since on an average about 300 m3 water is consumed per ton of paper produced, the industry generates a huge volume of highly colored and toxic effluents. About 500 different chlorinated organic compounds have been identified in paper mill effluent (Savart *et al*., 2006 and Jayakumar *et al*., 2013).

The environmental pollution is one of the most severe problems nowadays. Among various water pollutants, heavy metals are of major concern because of their persistent and bio accumulative nature (change *et al*., 2009). Heavy metal contamination is aquatic and soil environments are a serious environmental problem, which threatens aquatic ecosystems, agriculture, water resources and human health (Overesch *et al*., 2007). Heavy metals are metallic chemical elements with a high atomic weight and density much greater (at least five times) than water (Anjuli *et al*., 2012). When the heavy metals absorbed by the human body, some kinds of them can react with human physiology add molecular substances such as protein and enzyme (Dejun *et al*., 2014). A high concentration of lead can be found in industrial wastewater, in domestic detergents and other laundry products and in cigarettes (Celebi and Kendir, 2002). The major source of environmental lead is metal smelting (Caussy *et al*., 2003), but agriculture, industry and urban activities are also important sources of BP pollution (Marchial *et al*., 2004).

The global diversification of textile manufacturing and export with a considerable reduction in its share in western developed countries and an increase in eastern, especially Asian developing nations has had significant implications for the Indian textile sector. Since most of the textile centers have developed as small. Scale clusters, pollution management and enforcement are not at a satisfactory level. Hence in many places the pollution load discharged into the environment has exceeded the assimilative capacity and caused severe degradation of eco-systems and ultimately affected the livelihood of the people who depend on the services provided by these eco-systems (Marwaha *et al*., 1998).

Contamination of the aquatic environment by toxic metal ions is a serious pollution problem (Glass, 2000; Khamar and Ronneau, 2000), unlike organic pollutants, chemical (or) biological processes cannot degrade toxic metal ions. To remediation the aquatic environment, the toxic metal ions should be concentrated in a form that can be extracted conveniently, possible for reuse (or) at least for proper disposal. Natural resources including plants and microorganisms are extensively explored to combat metal ion pollution.

Anthropogenic activities accelerate the rate of water pollution, which enter into ecosystems disturbing natural balance. Industrial development and explosion of population are the main dreadful factors, winding the quality of water day by day as the influx of both inorganic and organic pollutants increases in it (Kutty *et al*., 2009). The municipal Sector consumes significant volumes of water, and consequently generates considerable amount of waste water discharge (Dixit *et al*., 2011). Regular monitoring of these contaminating routes and their effective action plan has to be evolved for better control of water pollution. The health of aquatic ecosystem is depending on the biological diversity and physicochemical characteristics (Venkatesharaju *et al*., 2010). This treatment of sewage water has been designed to render the organic matter in sewage harmless and inoffensive to living being (Sangar *et al*., 2011).

Bioremediation is an emerging clean up technology for contaminated soils, groundwater, and waste water that is both lowtech and low-cost. The cleanup technology is defined as the use of green plants to remove, contain or render harmless, such environmental contaminants as heavy metals, trace elements, organic compounds and radioactive compounds in soil or water. Phytoremediation takes advantage of the unique and selective uptake capabilities of plant goat systems together with the translocation, bioaccumulation and contaminant storage/degradation abilities of the entire plant. Aquatic macrophytes systems can be effectively used to reduce pollutant levels in water bodies. This is typically much less expensive than excavation followed by disposal or incineration or other in situ treatment strategies and reduces or eliminates the need to pump and treat a common practice at the site where hydro carbon have contaminated ground water.

Aquatic plants play an important role in structural and functional aspects of aquatic ecosystems by various ways making them interesting research candidates especially for the treatment of industrial effluents and sewage water (Andra *et al*., 2010). The ultimate goal of wastewater treatment is the protection of the environment in a manner commensurate with public health and socio-economic concerns. Ecological engineering with culture of aquatic macrophytes such as water hyacinth, pistia, phragmites etc for the purpose of pollution abatement has received growing acceptance (Iram *et al*., 2012). Many researchers like chavan and Dhulap, (2012 b), Lu *et al*., (2011) and Dar *et al*., (2011) worked on phytotreatment of sewage water by *Cana indica*. Pistia and *Eichhornia* respectively. *Azollz pinnata,* a genus of floating aquatic ferns is distributed throughout tropical and temperate regions of the world (Kitah *et al*., 1993). Azolla possesses the ability to utilize atmospheric N_2 due to a symbiosis with the blue green alga Anabaena, *Azolla*, which grows in the cavities of *Azolla* leaflets. *Azolla* has been used extensively and effectively for green manure in rice fields instead of chemical fertilizer in Asia. Interest in the use of this plant as a biological fitter for the renovation of waste water has increased (Watanabe *et al*., 1992).

Green gram is one of the import pulse crop in India. It has been reported that Green gram has been cultivated in India since ancient times. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand. Green gram supplies protein requirement of vegetarian population of the country. It is a protein rich staple food. It contains about 25 percent protein, which is almost three times that of cereals. It is consumed in the form of split pulse as well as whole pulse the moong dal khichdi is recommended to the ill or aged person as it is easily digestible and considered as complete diet. Roti with moong dal and moong dal chawal is an important ingredient in the average Indian diet. It is a drought resistant crop and suitable for dry land farming and predominantly used as and intercrop with other crops.

1.1. Aim and Objectives:-

1.1.1. Aim and Objectives of the Present Investigations are:-

1) To find out the physical – chemical properties of municipal waste water.

2) To find out the seedling growth of green gram under municipal waste water.

3) To find out the fresh weight and weight of seedling of green gram under municipal waste water.

4) To find out the Vigour index and tolerance index of green gram and municipal waste water.

5) To find out the Phytotoxicity effect of green gram under municipal waste water.

6) To find out the growth performance of green gram under biologically treated municipal waste water.

2. Materials and Methods

The present work deals with the effect of waste water on seed germination and seedling growth of Green gram (*Vigna radiate* (L.) Wilzeck).

2.1. Area of the Study

The waste water collected from Cauvery River, Mayiladuthurai. The waste water has been widely used for irrigation of crop plants like sugar cane, paddy, groundnut and pulses, so the present investigation has been carried out to find the effect of waste water on germination studies of Green gram (*Vigna radiate* (L) Wilzeck).seedlings.

2.2. Materials

2.2.1. Municipal waste water:

The waste water samples were collected in plastic containers from the cauvery river, Mayiladuthurai. Waste water analysed at wardex pharmaceutical private limited, Chennai. Physio-chemical properties of the waste water were analysed adopting the procedure prescribed by Aptha (1985).

2.2.2. Seed material

The Green gram seeds were obtained from Tamil Nadu Rice Research Institute, Aduthurai. (ADT 3).

2.2.3. Analysis of Waste Water Sample

The waste water samples were collected in plastic containers from the cauvery river, Mayiladuthurai. Waste water analysed at wardex pharmaceutical private limited, chennai. The coovun river water was brought to the same factory's laboratory and TNPCB and the physio chemical properties of the waste water were analyzed adopting the procedure prescribed by Aptha (1985).

2.2.3.1. Colour

The colour of the waste water sample was observed usually.

2.2.3.2. Odour

Odour of the sample was categorized as objectionable or non-objectionable by smelling of the sample.

2.2.3.3. pH

The pH of the waste water sample was determined by pH meter using a glass electrode as seen as it was collected.

2.2.3.4. Dissolved solids

The dissolved solids were estimated by filtering a known volume (100 ml) of well mixed sample through a stand is glass fiber filter; the filtrate was evaporate to dryness.

2.2.3.5. Suspended solids

The suspended solids were estimated by filtering 100 ml of well mixed sample through Whatmann No. 4 filter paper, the residue obtained was dried to constant weight at 104-105 C. It is only the difference between total solids and dissolved solids.

2.2.3.6. Biological Oxygen Demand (BOD)

BOD of the waste water was determined by incubating BOP bottles containing the samples kept at 20 C for five day. The pH of the sample was diluted with distilled water, 1 ml each of phosphate buffer, magnesium, sulphate, calcium chloride and ferric chloride were added to aerated distilled water. The dissolved oxygen constant of one set was estimated immediately following the Winkler's method of dissolved oxygen; another set was incubated for 5 days in a BOD incubator and estimated for dissolved oxygen.

BOD (mg/l) =

2.2.3.7. Chemical

In a 500 ml COD flask, 10 ml of 0.1 N potassium dichromate, 1g silver sulphate and mercuric sulphate were added besides 30 ml of concentrated sulphuric acids, the mixtures were kept for 2 hours in a water bath. After cooling, the solution was made upto 140 ml by adding distilled water, 0.5 ml was added and mixed thoroughly. Then the solution was titrated against 0.1 N ferrous ammonium sulphate. For each experiment appropriate blank was used.

COD (mg/l) =

2.2.3.8. Sulphate

Ten ml of NaCl-HCl reagent solution was added with 20 ml of sample and 10 ml of barium chloride was added then made upto 50 ml. The sulphate content of the sample was estimate with spectrophotometer; a calibration curve was drowning using sulphate standards.

One ml of potassium chloride indicator was added with the known value of waste water and titrated against standard silver nitrate.

Chloride (mg/l) =

2.2.3.9. Oil and grease

One ml of sulphuric acid and 50 ml of petroleum ether and little quality of ethyl alcohol were added to 500 ml of waste water. The solution was shaken well and allowed to stand for some time in separating funnel. The lower layer of the sample was discarded. The supernatant, petroleum ether was filtered through petroleum ether soaked filter paper in a pre weighed glass beaker and evaporated. The final weight of the beaker with residue was recorded. O and G =

2.3. Germination Studies

The experiment was conducted ADT 3 varieties of Green gram (*oryza sativa* L.). Seeds under different concentrations (control, 10, 25, 50, 75 and 100%) of waste water treatment. The seeds were surface sterilized, with 0.2 per cent mercuric chloride ($HgCl_2$) solution. The seeds were thoroughly washed under tap water and then distilled water. The seeds were arranged especially in sterilized Petridish. Each Petridish were filled with soil and irrigated with uniform quantity of different concentration of the waste water in addition, a set of Petridish were irrigated with distilled water treated as control. Each treatment including control was replicated for five times. The Petridish were kept under diffused light at room temperature. The number of seeds germinated in each variety in all the treatment was counted daily upto 7th day after sowing and germination percentage was calculated. The emergence of radical was taken as criteria for germination. The seedlings from each replicate were selected for recording the morphological parameters such as length of root and shoot, fresh and dry weight of the 7th day seedling.

2.3.1. Germination percentage

Germination refers to the initial appearance of the radicle by visual observation. It was calculated by using the following formula.

Germination percentage =

2.3.2. Vigour index

Vigour index of the seeding was calculated by using the formula proposed by Abdul Baki and Anderson (1973).

Vigour index = Germination percentage length of the seedling

2.3.3. Tolerance index

Tolerance index of waste water was calculated by using the formula suggested by Turner and Marshal (1972).

Tolerance index =

2.3.4. Percentage of phytotoxicity

The percentage of phytotoxicity of waste water was calculated by the formula suggested by Chou *et al*. (1978).

Percentage of phytotoxicity =

2.4. Morphological Parameters

The various morphological growth parameters such as shoot length, root length, dry weight and fresh weight (shoot, root) and were recorded in 7th day seedlings.

2.4.1. Root and shoot length (cm/seedling)

Three seedlings were taken from each treatment and their shoot and root lengths were measured by using a scale and these values were recorded.

2.4.2. Fresh weight (g/seedling)

Ten seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pen balance.

2.4.3. Dry matter production

Table 1

1. **Municipal waste water collected from Cauvery river**

2. ***Azollz pinnata***

Table 2 Biologically treated municipal waste water

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Three plants were taken at random from each concentration for the study of dry matter accumulation for observation.The plants were separated into shoot-root, leaves and fruits, they air-dried in hot air oven at 80 C for 24 hours. The dry weights of plant materials were taken using a electrical single pan balance and the average dry weight of a plant was calculated.

3. Experimental Results

The present work deals with the effect of municipal waste water on seed germination and seedling growth of Green gram (*Vigna radiata* L. Wilzeck).

The municipal waste water collected from Cauvery River, Mayiladuthurai. The municipal waste water has been widely used for irrigation of crop plants like sugar cane, green gram, groundnut and pulses.

3.1. Physico- chemical properties of the municipal waste water

The physcio- chemical properties of the waste water are shown in table1. Physcio chemical properties of the waste water. Were analyzed adopting the procedure by Aptha (1985).

3.2. Germination studies

The experiment was conducted ADT 3 of green gram (vinga radiate L.). seeds under different concentration (Contral 10,25,50,75,and 100%) of waste water treatment.

3.2.1. GROWTH PERFORMANCE

3.2.1.1. Germination percentage

The germination percentage of green gram cultivar as affected by different concentration of waste water are furnished in table 2 and the germination percentage of the green gram cultivars are found to be maximum at 25 concentration of waste water for ADT 3 germination percentage of all the cultivar of green gram decreased gradually with increase of waste water concentration. The minimum Percentage of germination for ADT 3, were recorded in all cultivars of green gram in 100 percent concentration of waste water treatment.

Table 1. Physico – Chemical Properties

S. No.	General Parameters	Raw	Treated
1.	Colour	Dark Gray	Colour
2.	Odour	Foul	Odourless
3.	PH	6.48	6.2-9.0
4.	Temperature	26ºC	37ºC
5.	Total Solids	4100.00	2.170
6.	Total Dissolved Solids	1449.00	98
7.	Suspended Solids	470.00	97
8.	BOD	100	-
9.	COD	123.00	-
10.	Oil and Grease	2	-

All the parameters are expressed as mg/1, except pH, Ec and temperature.

Table 2. Effect of waste water on seed germination, vigour, tolerance index and phytotoxicity (%) green gram

Sl. No.	Treatment	7th day seedlings			
		Germination %	**Vigour index**	**Tolerance index**	**Percentage of Pytotoxicity**
1.	Control	87.44	3.812	-	-
2.	10%	91.23 (4.334)	4.333 (13.66)	1.110	93.24
3.	25%	98.46 (12.60)	5.232 (37.25)	1.158	98.08
4.	50%	63.58 (-27.28)	2.536 (-33.47)	0.923	7.649
5.	75%	56.37 (35.53)	1.811 (-52.49)	0.782	1.699
6.	100%	49.62 (-43.25)	1.201 (-68.49)	0.655	1.712

(Percent over control in given parenthesis)

Table 3. Effect of waste water (Raw + Treated) on growth (cm) of Green gram (ADT 3)

Sl. No.	Treatment	7th day seedlings					
		Root Length	**Shoot Length**	**Root fresh weight**	**Root dry weight**	**Shoot fresh weight**	**Shoot dry weight**
1.	Control	17.78	25.82	0.269	0.040	0.552	0.221
2.	10% (Raw)	19.74 (1.666)	27.76 (2.474)	0.284 (-78.67)	0.045 (-108.5)	0.569 (-47.87)	0.241 (-86.94)
3.	10%(Treated)	19.92 (1.665)	29.42 (2.468)	0.288 (-80.16)	0.056 (-136.0)	0.662 (-64.72)	0.252 (-91.92)
4.	25% (Raw)	20.60 (1.662)	32.54 (2.455)	0.289 (-80.53)	0.070 (-171.0)	0.764 (-83.20)	0.316 (-120.8)
5.	25%(Treated)	23.95 (1.678)	35.61 (2.444)	0.300 (-84.62)	0.079 (-193.5)	0.972 (-120.8)	0.339 (-131.2)
6.	50% (Raw)	16.42 (1.685)	23.47 (2.478)	0.66 (-218.4)	0.033 (-78.5)	0.534 (-41.53)	0.228 (-73.82)
7.	50%(Raw)	19.54 (1.668)	26.81 (2.478)	0.88 (-300.2)	0.033 (-78.5)	0.534 (-41.53)	0.228 (-81.06)
8.	75% (Raw)	13.92 (1.699)	18.22 (2.511)	0.72 (-240.7)	0.045 (-108.5)	0.324 (-33.49)	0.116 (-30.38)

9.	75%(Treated)	14.54 (1.696)	19.42 (2.506)	0.79 (-266.7)	0.054 (-131.0)	0.368 (-11.46)	0.192 (-64.77)
10.	100% (Raw)	11.65 (1.712)	12.56 (2.533)	0.84 (-285.3)	0.028 (-66.0)	0.243 (-11.17)	0.078 (-13.19)
11.	100% (Treated)	13.72 (1.700)	14.78 (2.524)	0.91 (-311.3)	0.035 (-83.5)	0.264 (-11.73)	0.092 (-19.52)

(Raw – un treated waste water: BT– Biologically Treated waste water) (Percent over control in given parenthesis)

Table 3

1. Effect of municipal waste water treatment on *Vigna radiata* (L.) Wilzeck

control	10%	25%	50%	75%	100%

3.2.1.2. Vigour index

The vigour index of green gram seedling grown under different concentration of waste water is presented in Table 2. The maximum vigour index of seedling 5.2320 occurs in ADT 3 at 25 percent waste water concentration. The minimum vigour index 1.201 was is ADT 3 variety at 100 percent waste water concentration.

3.2.1.3. Tolerant index

The tolerant indexes of green gram seedling grown under different concentrations of waste water are presented in Table 2. The maximum tolerant indexes of seedling (1.158) occur in ADT3 at 25 percent waste water concentration. The minimum tolerant index (8.655) was observed in ADT 3 variety at 100 percent waste water concentration.

3.2.1.4. Phytotoxicity

The phytotoxicity of green gram seedling grow under different concentration of waste water are presented in Table 2. The minimum phytotoxicity of seedling (-98.08) occurs in ADT3 at 25 percent waste water concentration. The maximum phytotoxicity (1.712) was observed in ADT 3 variety at 100 percent waste water concentration.

3.3. Morphological Parameters

3.3.1. Root Length

The maximum root length was recorded at 25 percent biological treated waste water 23.95 cm concentration and minimum root length was recorded at 100 percent biological treated waste water 13.72cm on 7th day (Table 3).

3.3.2. Shoot length

The maximum shoot length was observed at biological treated waste water (35.61cm), when compared with control, biological treated waste water treatments have shown a gradual increase in shoot length upto 25 percent concentration and then it decrease with increase in biological treated waste water (Table 3).

3.3.3. Root fresh weight

The maximum root fresh weight (0.300g plant) was observed at 25 percent biological treated waste water on 7th day and the minimum (0.083g/plant) was in 100 percent biological treated wastewater, there was a gradual increase in fresh weight of 25 percent biological treated waste water and decreased in other concentration (Table 3).

3.3.4. Root dry weight

The maximum root dry weight (0.076g/plant) was observed at 25 percent biological treated wastewater on 7th day and minimum (0.032g/ plant) was in 100 percent biological treated waste water, there was a gradual increase in dry weight of 25 percent biological treated waste water and decreased in other concentration (Table 3).

3.3.5. Shoot fresh weight

The maximum shoot fresh weight (0.972g/plant) was observed at 25 percent biological treated waste water on 7th day and the minimum (0.264g/ plant) was in 100 percent biological treated waste water, there was a gradual increase in fresh weight of 25 percent biological treated waste water and decreased in other concentrations (Table 3).

3.3.6. Shoot dry weight

The maximum shoot dry weight (0.339 g/ plant) was observed at 25 percent biological treated waste water on 7th day and the minimum. (0.092g/plant) was in 100 percent biological treated waste water there was a gradual increase in dry weight of 25 percent biological treated waste water and decreased in other concentrations (Table 3).

2. Effect of raw and biological treated municipal waste water treatment on *Vigna radiata* (L.) Wilzeck

Control 10% R 10% BT 25% R 25% BT 50% R 50% BT 75% R 75% BT 100%R 100%BT

4. Discussion

Municipal waste water treatment systems are most concerned with the reduction of suspended solids, organic matter, pathogens, phosphates, and ammonium and organic nitrogen. Other kinds of waste water trea5tent wetlands may be concerned with these same contaminants in addition to other organic compounds, residual explosives, or metals. Some system designs anticipate exactly what kids of contaminants the wetlands will receive, and at what levels, while others face variable and unpredictable wastewater flows. Some wetland systems treat specific substances, such as airplane deicer fluid. Other systems, such as a storm water runoff system; many receive a mixture of contaminants, with levels of incoming water varying widely with season and year.

The polluted river water is mostly used for irrigation of crop plant. Studies are being conducted to assess the impact of industrial waste water on crop plants. The polluted water irrigation causes the sail pollution and also affects the production of field (Sims *et al*., 2000; Ametakumar *et al*., 2003 Feng *et al*., 2005; Abdelhafidth Dhown *et al*., 2006; Jayakumar *et al*., 2014).

Metal uptake by plants depends on the bioavailability of the metal in the water phase, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. Furthermore, when metals have been bounded to the soil, the pH, redox potential, salinity and organic matter content will affect the tendency of the metal to exist in ionic and plant-available form (Fritioff and Greger 2003). The success of phytoremediation may be limited by factors such as growing time, climate root depth, soil chemistry, salinity stress and level of contamination (Salido *et al*., 2003).

4.1. Germination Studies

Germination studies were conducted in laboratory to find out the effect of waste water on seed germination, seedling growth, fresh and dry weight of green gram seedlings. The seeds of green gram (*Vigna radiata* L.) were allowed to germinate in Petridis. They were treated with different concentration (control, 10, 25, 50, 75 and 100%) of waste water. The variety of green gram seeds were treatment of waste water germination percentage, seedling growth fresh weight ad dry weight were taken into concentration for experiment of the basis of data obtained from germination studies.

4.2. Germination Percentage

The preset investigation has been carried to find out the effect of municipal waste water treatment on seeds of green gram (*Vigna radiate* L.) ADT3 was, chosen for experimentation. The germination percentage of green gram seeds was increased at lower concentration of the waste water treatment and decrease with the increase in the waste water concentration.

The highest percentage of germination was noticed at waste water concentration. The same trend was observed in *Sesamum indicum* plant (Das *et al*., 2000), sewage sludge on seed germination Chinese cabbage plant (Wong *et al*., 2001). Chinese cabbage (Wang and Ketuai, 1990), coal washers effluent on seed germination *Vigna radiate* of (Pandey *et al*., 2002). Chemical industry effluent on seed germination of *Vigna radiata* and *Vino mungo* (Chidambarampillai *et al*., 1996). Ordinance factory effluent on pea (Srivastava *et al*., 1995),

4.2.1. Vigour Index

The vigour index values were found to be maximum in percent of waste water and then it decreased with increase of waste water concentration. Similarly, the vigour index value increased with the decreases of reported by Halmes, 1992).Waste water on vigour index of spinach *Spinacea oleraceae* (khedkar*et al*., 2005).

4.2.2. Tolerance Index

The tolerant index values were found to be maximum in 25 percent of waste water and then it decreased with increased of waste water concentration. Similarly, the vigour index value increased with the decreases of green gram.

4.2.3. Phytotoxicity

The phytotoxicity values were found to be maximum in percent concentration of waste water and then it decreased with increase of waste water concentration. Similarly the phytotoxicity value with the decrease of wastewater on phytotoxicity of spinach spinaceaoleracea (Khedkar *et al*., 2005).

4.3. Morphological Parameters

The morphological parameters such as root and shoot length, fresh weight of root and shoot, dry weight of root and shoot the variety.

4.3.1. Root Length and Shoot Length

The seedling growth of green gram plants were increased upto 25 percent of treated waste water concentration. Industries effluent on seedling growth of terrestrial and aquatic plant species (Crow *et al*., 2002). Acid rain in seedling growth of phaseolusaureus var.RMG62 (Devpurashikha, 2003). Industry waste water on seedling growth of crop plant (Mariappan *et al*. 2002). Coal washery effluent on seedling growth of *Vigna radiates* (Pandey *et al*., 2002). Chemical industry effluent on *Vigna radiata* and *Vigna mungo* (Chindambarampillai *et al.*, 1996).Ordinance factory effluent on seedling growth of crop plant (Srivastava, *et al*., 1995).

Wastewater on crop plant (Khedkar *et al*., 2005). Wastewater on seedling growth of different crop plants (Ramakrishnan *et al*., 2001; Subramani *et al*., 1995; Rajesh *et al*., 2013), dissolved solids in water on seedling growth of crop plant (Gautam *et al*., 1992; Hariom and Nepal sigh 1994).Sewage sludge water on seedling growth of agriculture crop plant (Wang and Su,1997; Halmes, 1992).

4.3.2. Seedling Weight

The seedling weight of Green gram plants were increased upto 25 percent of treated waste water concentration. The same trend in sewage sludge water on seedling growth of agriculture crop plant (Wang and Su, 1997; Holmes, 1992). Industrial effluent on groundnut (Sundaramoorthy and Kunjithapatham, 2000), Green gram (Radha krishnan *et al*., 2005).

Dry matter production in various parts the variety was increased upto 25 percent raw and treated waste water. Similar findings were observed in earlier reports (Villar *et al*., 2001 and Feath, 2000).

Many aquatic plants (*Eichhornia, Lemna, spirodela, Nasturtium, Ipomea, Azollaect.*) add algae have been used for reclamation of wastewaters (Hunter *et al*., 2001, Hu *et al*., 2008, Kutty *et al*., 2009). The phytoremediation potential of Azolla has been extensively reviewed recently (Sood *et al*., 2022). Another important aspect is mechanism involved in uptake of these nutrients. An up regulation of the synthesis of a carrier system is believed to contribute to the plants observed enhancement of P absorption. The kinetics of p uptake by whole plant is complex, requiring a dual mechanism; a high-affinity system operating in the low micromolar range and a low-affinity system operating at higher concentrations (Reghothama 2000). The plant phosphate transporter (PT) genes for pi transport has been classified into four families, pht1 to pht4. The varied sub cellular localization of the PT genes from the four families (Pht1, plasma membrane; Pht2, chloroplast; Pht3, mitochondria; Pht4, Golgi apparatus) suggest their diverse biological function for plant growth and development (Rausch and Bucher, 2002). Among all the known PTs, members belonging to the Pht1 family, which are presumed as high-affinity PTs, and most extensively studied. They have been indentified in several plant species including Arabidopsis, *Oryza sativa*, *Hordeuvulgare, Glycine max, Lycopersicon esculentum* (Mudge *et al*., 2002, Suchumann *et al*., 2004, Jia *et al*., 2011). However, no report is available on pi transporter genes involved in nutrient removal in Azolla-Anabaena symbiotic system.

According to stepniewska *et al*., (2005). Azolla can bioaccumulate heavy metals and also remove organic substances from wastewater. It has been reported that Azolla has a high capacity to accumulate toxic elements such as mercury, cadmium, chromium, copper, nickel and zinc (Rai, 2008) and can be used to remove contaminants from wastewater (Arora and Saxena, 2005; Rakhshaea *et al*., 2006). This fern can also remove nutrients (Forni *et al*., 2001) and organic substances like sulphonmides (Forni *et al*., 2002). The bioaccumulation potential of Azolla spp. For various heavy metals has been compared with other aquatic macrophytes by many workers (Rai and Tripathi, 2009; Rai, 2010).

Aquatic macrophytes can accumulate significant quantity of heavy metals in their tissues ($10\text{-}10^6$) times greater concentration than in the water (Snezana *et al*., 2005). According to Nuzhat *et al*., (2015) has revealed the role of free floating macrophyte in pytoremediation technology has an excellent performance in removing the hazardous and was able to remove huge amount of heavy metals in 7 days of the experimentation period.

Azolla pinnata is a small aquatic fern belonging to phylum-pteridophyta, Class polypodiopsida, Ordersalviniales, Family Azollaceae with a monotypic genus (sood and Ahluwalia 2009). This fern represents the only example of pteridophyteharbouringsymbiotic association with diazotrophic, nitrogen-fixing cyanobacteria and bacteria residing in leaf cavities (Sood *et al*., 2008 a; b; sood and Ahluwalia 2009). This there partner association remains together during vegetative and reproductive phase of life history, there by excluding the need of re-inoculation, hence proving its potential over and above other bio fertilizers (Carrapico 2010). The free-floating habitat, fast growth rate, ability to grow in N-deficit sites, known potential to tolerate cuide range of pollutants and wider acceptance as bio fertilizer or green manure for agronomocally important crops reflects its potential to be a More promising candidate in future for phytoremediation of municipal wastewater. Therefore, this project aims at utilizing Azolla for sequestration of nutrients (mainly nitrogen and phosphorus), application of biomass generated as bio fertilizer, mining and gene expression analysis of pi- transporter genes involved in p uptake.

5. Summary and Conclusion

The present work deals with the effect of waste water on seed germination and seedling growth of five varieties of Green gram (*Vigna radiate* (L) Wilzeck).

The Green gram seeds were obtained from Tail Nadu, Rice Research Institute, Aduthurai. (ADT 3).

The waste water collected from Cauvery River, Mayiladuthurai. The waste water has been widely used for irrigation of crop plants like sugar cane, green gram, ground nut and pulses.

The growing season, macrophytes accumulate nutrients form water. When the macrophytes die, the decomposition process begins. The release of nutrients raises the nutrient concentration in the water and the oxygen consumption lowers the dissolved oxygen level in the water and in the sediment. Decomposition of aquatic macrophytes may therefore substantially regulate the recycling of nutrients in shallow freshwater ecosystems and thus influence the net carbon storage of the ecosystem for a long time.

5.1. Germination Studies

The morphological parameters like germination percentage, seedling growth, fresh weight and dry weight of seedling were taken into consideration on the basis of data obtained for germination studies.

The various concentration of the waste water (10, 25, 50, 75 and 100%) were prepared and used for germination studies. The increase in shoot length of seedling at lower concentration (25 percent) over control was noticed and then the shoot length decreased with increase of waste water concentration all the growth parameters such as shoot length root length, dry weight and fresh weight (root, shoot and leaf) of the seedling etc. are decreasing with the increase of waste water concentration.

5.2. Morphological Parameters

The experiment was conducted ADT 3 varieties of green gram (*Vigna radiata*) during the year of 2016. The various concentration of waste water were treated (10 raw, 10 treated, 25 raw, 25 treated, 50 raw, 50 treated, 75 raw, 75 treated and 100 raw, 100 treated).

The observation on the plant samples of various concentration and control were taken at 7^{th} days after souring. The plants were used for the measurement like shoot length, root length; fresh weight and dry weight (root and shoot). Growth parameters were increase upto 25 percent biological treated waste water. Then it decreased with increasing amount of waste water concentration.

It has been observed that the plants irrigated with lower concentration 25 percent of biological treated waste water exhibited a better growth than that of control plants. They further indicated that the aquatic macrophytes play a major role in removing the maximum pollutants from wastewater and the use of plant system is highly sustainable, cost effective and most acceptable. The almost all free floating aquatic plants have proved to be the best patent for treatment of municipal wastewater.

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