



## Increase of biomass in some aquatic plants grow under stress with using alternative sources of water and use it as contamination indicators

Georgena Wahib Rizk Gabra

Agriculture Research Center, Horticultural Research Institute, Botanical Gardens Department, Antoniadis Garden, Alexandria, Egypt.  
[georgena.rizk@yahoo.com](mailto:georgena.rizk@yahoo.com)

**Abstract:** Living organisms are distributed in ideal ecosystems in balanced proportions without prejudice to the existence of each other. The "Eutrophication" phenomena that means biomass aggravation increment of some aquatic plants occurs when increase the dietary excess by organic, inorganic nutrients and elements in waterbodies because of sewage water 'wastewater' leakage into them. These aquatic macrophytes play a major role in conservation of the aquatic ecosystem or even aesthetically for some aquarium, ponds and artificial lakes as a part of the botanical gardens, water parks, fish farms and it may spread a lot in slowly flowing canals and rivers. The purpose of this study is to estimate the rates of increase in plants biomass produced under stress of using alternative water sources. Also, using these macrophytes as contamination biomarkers and in phyto-remediation process and what's for/on it and its impact in/on its presence in fresh water bodies. The most important of these macrophytes are *Pistia stratiotes* "water lettuce" as floating plant and *Ceratophyllum demersum* as submerged one. The experiment was carried out in open field of El- Harrery village, El Montaza, at the east of Alexandria, Egypt, during two successive summer seasons 2014 & 2015 for 28, 34 days for each plant respectively. The plant samples were obtained from the National Center for Oceanography and Fisheries, El- Anfoushi, Quiet Bay, Alexandria, Egypt. The means of initial weights of plants were average at 47g and 32g wet weight for each plant respectively. The treatments media were Tap Water as a control [TW] - Primary Treated Domestic Wastewater [PTW 15%, 25%] - Ground Well Water [GWW 15%, 25%] - Mix 15%, 25% [TW+PTW+GWW] from full volume 2.5 L of plastic Jars. The treatments were increased by the same concentration when the water evaporated. The results reached to highly significant differences between treatments in vegetative growth parameters, chemical constituents [total chlorophyll, N, P, K in plants tissues] and Na, Cd and Pb in plants treated with PTW 25%, sediment determination and water quality tests [color and turbidity] before and after the same treatment. The treatment of PTW 25% proved its superiority in most vegetative growth parameters in both plants, but it caused that the fastest growing and the most degraded in water lettuce plant. Thus, it can be recommended that these plants can be released as pollution indicators and monitored in their environment and use this in phytoremediation process.

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### 1. Introduction

Living organisms are distributed in ideal ecosystems in balanced proportions without prejudice to the existence of each other. However, with the increase of dietary excess by organic, inorganic nutrients and elements then increase the biomass aggravation "eutrophication" of some aquatic plants in water-bodies (Smith et al, 1999; Ding et al, 2011). These macrophytes could affect the distribution of atmospheric oxygen in the water and then the preservation of aquatic organisms or even aesthetically for some ponds, artificial lakes as a part of the botanical garden, water parks, fish farms and slow flowing rivers. Thus, Water gardens can

contain many varieties of types of ornamental plants, from submerged to emergent to free-floating with no contact with the substrate (Mahr, 2012). So, it was important to study some of these plant distributions and their worsening growth biomass and what's for/on it and its impact in/on its presence in fresh water bodies. Heavy metal contamination in aquatic habitats has been considered as one of the most serious problems threatening water sources, aquatic organisms and human health (Cheung et al, 1997; Sternberg and Dom, 2002; Hanafiah et al, 2018). Eutrophication and heavy metal problems lead us towards a greater problem, which is the leakage of sewage water 'wastewater' into fresh waterbodies,

which is an important ecological problem that all poor and rich countries suffer from, which results in changing the bio-physiochemical properties of water and contaminating it with heavy metal (Ahmad et al., 2016 and Ali et al., 2020). Unfortunately, the advanced methods to removal of heavy metals is required high energetic costs and they have limited capacities or not sufficient to treat even very dilute solutions (Foroughi et al., 2011). Thus, Phytoremediation is one of the most eco- friendly, promising technology and low-cost methods of removing pollutants from aquatic ecosystem and sediment (Miretzky et al., 2004). This method is most effective for lowering high concentrations of hold excessive nutrients, organic and inorganic pollutants, and even more like dyes and crude oil (Volesky, 2003; Akapo et al, 2011 and Valipour et al, 2014). Plant species considered to be ideal phytoremediation agents have a high biomass, a good root system, and have easy fast-growing. These plants are classified according to their treatment of pollutants, so two or more techniques are used in order to be able to perform the function of phytoremediation. These mechanisms are phytoextraction [useful for mining purposes], phytoaccumulation [suitable for water purification], phytovolatilization [convert the pollutants to aromatic components and volatilize them into the atmosphere] and phytochelatins for soil amendments or etc (Mitsch and Jorgensen, 1989; Kushwaha et al, 2015; Fu and He, 2015 and Gabra, 2021). All of these plants can be used for medicinal, pharmaceutical and fodder purposes, so it can be used to improve the economic situation, especially in developing countries (Malathy and Stanley, 2015; Abu, 2017 and Shaltout et al., 2009). On the other hand, its unconditional release in nature, it causes many problems, such as hindering navigation, the spread of fish farm diseases, and the death of marine organisms. Also, it can clogs the river channels and causes alkalinity and salinity of the soil as one of the indirect negative effects (Gabra, 2021). So, the invasive aquatic macrophytes can escape into the surrounding environment and have a devastating effect on water quality and destroying their media and also the livelihoods of communities who depend on them (Yang et al., 2014; Thijs et al. 2017). Some countries are fighting these plants as a pest that must be eliminated, especially countries that suffer from water poverty under the pretext that these plants waste a large amount of water via evaporation. However, the environmental role of hydrophytes necessitates maintaining their presence due to their high ability to remove pollutants that cause poisoning of the water that is the source of life (Yadave et al., 2009). The high concentrations of heavy metals

stimulate the biomass production of the macrophytes but for a limited period and not a concentration that lead to physiological death (Stebbing, 1981 and Sharma et al., 2012). Then a strange phenomenon occurs, which is called as "biphasic dose response" always characterized by J or inverted-U shaped curve, also called Pistia-model (Eid et al. 2016). This phenomenon occurs naturally in the growth curve of aquatic plants. This phenomenon appears clearly in water lettuce plant, when the size of its rosettes becomes smaller and surrounding decreases until conditions improve for growth, so it regains its normal growth (Jia et al., 2013 and Farnese et al., 2014). Thus, these plants can be useful as biomarkers for toxicity. The characterization of biomarkers of toxicity is essential, since bio-indicators are, most of the time, more efficient than the physical and chemical parameters normally measured in the field (Barcelo et al., 1988).

*Pistia stratiotes* L., commonly called "water lettuce" because of its superficial resemblance to "lettuce" the green leafy vegetable. It is also occasionally called water cabbage or Nile cabbage, but is not edible. This species in the arum family (Araceae), it is the only one in the genus and likely originated in tropical or subtropical zone also, it is a strong competitor to other aquatic fresh water plants. This is an herbaceous perennial monocot with a rosette of overlapping, stem less leaves densely covered with fine hairs that prevent wetting of the actual leaf surface and trap air so the plant has increased buoyancy to float easily. The plant propagation vegetatively by 'stolons', each of which grows another rosette, with the daughter plants remaining connected to the mother plant. Under ideal conditions numerous stolons and new rosettes are formed, allowing the colony to grow quickly to cover the water surface. Although water lettuce can reproduce by seed, this is not a main means of propagation, especially when grown as an ornamental aquatic plant. It is adversely impact native flora and fauna - native submerged plants can be shaded out as the mat of leaves blocks light penetrating the water and fish may be killed from reduced oxygen in the water (Mahr, 2012 and Eid et al., 2016).

While, *Ceratophyllum demersum* L., "Hornwort" plant is a dicotyledonous, submerged aquatic angiosperm belonging to the family Ceratophyllaceae. Sometimes the plant can branch but one for each node while, the branches are brittle and easily broken and they can grow apart with lack roots, or are present as capillaries. The flowers are unisexual; both staminate and pistillate on the same plant. The fruit is an achene, oval in shape, having one oblong seed 4-6 mm long, with the spineless

lateral edges but having 1-2 basal spines (**Abu, 2017**). This plant grows in the aquarium for the beauty of vegetative growth, but in nature it can grow in slow-flowing freshwater-bodies such as lakes, canals and ponds with a high percentage of BOD. It has a high capacity for bio-sorption of iron, Mn and Cu from liquid wastes of factories (**Ali and Soltan, 1999** and **Ali et al., 1999**).

**Galal and Farahat 2015** founded that *P. stratiotes* has been shown to have great potential for the management of water quality, due to its capacity for the accumulation of heavy metals from wastewater. While, **Eid, 2017** concluded that the Pistia-model can be used as a predictive tool for assessing the potential growth of *P. stratiotes*. It might be useful for practical applications, such as the management of irrigation and drainage canals, and to derive management recommendations for *P. stratiotes* control in regions where the species is considered invasive by assessing the critical growth stages of this plant. Also, **Lu et al., 2010** investigated that water lettuce has a great potential for removing N and P, reducing water suspended solids and turbidity from storm-waters, and improving water quality. Also number of leaves, plant weights, roots lengths and its numbers decreased with the increase of days affected by many factors like water salinity and physiological limitations of the plant. **Brix, 1997** recommended that for efficient water purification, grown-up biomass of aquatic macrophytes must be removed from water bodies to keep an optimum plant density. If not harvested, the vast majority of the nutrients that have been incorporated into the plant tissue will be returned to the water by decomposition processes. **Victor et al., 2016** studied that the phytoremediation with water lettuce plant were decreased the metal contents in treated wastewaters upto 3.51-93.51% after 15 days, and reduced 58.87% of ammonium content, 50.04% of PO<sub>4</sub>, 82.45% of COD and 84.91% of BOD. **Kumar et al., 2019** showed that the highest removal of metals such as Cu%, Fe% and Hg % were observed in 5mg/l treatment. The fresh biomass of water lettuce, chlorophyll content, and kinetic growth rate and plant root size were maximized in 5 mg/l treatment. **Sidek et al., 2018** note that after 28 days, the physical observation shows that the water hyacinth was healthier than the water lettuces. Furthermore, water lettuce (*P. stratiotes*) was the most effective plant in removing Iron (96.0%) and the turbidity (50.0%) of the mining lake. In addition, **Ali et al., 2020** experimented that the successful phytoremediation to remove the pollutants from wastewater and ground water with 3 species of aquatic macrophytes such as water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and duck weed (*Lemna minor*) they

founded that the aquatic plants were prominent metal accumulator plants for the remediation of heavy-metal polluted water.

In the same field, **Foroughi et al., 2010** experimented that the phytoremediation "biosorption" with *C. demersum* on raw municipal wastewater (RMW) and treated municipal wastewater (TMW) for 18 days. The results showed that the COD of (RMW) and (TMW) were decreased from 664 to 152.75 mg/l and 260 to 64.5 mg/l, respectively. Also, the amount of ammonium decreased from 135 to 15meq/l and 90 to 10meq/l, respectively. The amount of nitrate in RMW and TMW had a similar decreased from 60 to 30 meq/l as well as 4.48 to 0.53meq/l, respectively and the amount of phosphorus in RMW and TMW declined from 13.68 to 1.15 meq/l and 4.48 to 0.53 meq/l, respectively. So, the significant amounts of these macro elements were removed by *C. demersum*. And the EC of treated municipal wastewater was reduced from (1.34 to 0.95 dS/m) and of raw municipal wastewater (from 2.68 to 2.12 dS/m) also, due to biosorption with *C. demersum*. Meanwhile, **Patel and Kanungo, 2010** studied that the net primary productivity of *Ceratophyllum demersum* L. in domestic wastewater culture for a week interval was determinate by increase of biomass to 1.58m<sup>2</sup>/day. But an increase in value of pH, DO and Percentage oxygen saturation value while, the other parameters viz. Turbidity, Salinity, EC, TDS, Alkalinity, Free carbon dioxide, Chloride, COD, Total hardness, Calcium hardness, Ca, Mg, N and Phosphorus as Phosphate recorded a significant decrease in values due to absorption of nutrients during phytoremediation. So, the quantitative reduction of nutrients in domestic wastewater and increase in biomass productivity value had suggested *Ceratophyllum demersum* L. as an ecologically efficient aquatic plant for phytoremediation of nutrients from domestic waste water. **Al-Ubaidy and Rasheed, 2015** investigated that the ability of *C. demersum* to reduce or eliminate the harmful and toxic effect, which resulting from two concentrations of cadmium chloride (3.714, 4.952 mg/l). The results showed that cadmium accumulated rapidly in the plant and increased dramatically with time, thus the highest level of cadmium was observed at day nine. *C. demersum* showed high ability to remove cadmium and was at the 9<sup>th</sup> day of the experiment and that was (1.53, 1.089 mg /l) when exposed to 3.714, 4.952 mg /l of cadmium respectively. The current study proved strongly the ability of *C. demersum* to eradicate the cadmium in ecosystem. Also, **Ghobrial, 2000** studied that the phytoremediation of *C. demersum* on 2:5 wastewater concentration within 9 days to realizing maximum

uptake and accumulation of Cd and Cu. She founded that these contents were increased to 2.9 and 3.9 folds higher than the control plants. And Zn was increase of 2.2-75.5 times the initial content in the plant. The accumulation of Fe was greatest in plants grown on 4:5 wastewater dilutions in 7 days treatment. While, Mn ion increase slightly and K, Na ions were decrease at the beginning of treatment followed by considerable increase in K content. The growth rate was excess 4g/day in fresh weight even at the low waste dilution. Thus *C. demersum* may prove useful in the detoxification of domestic wastewater and has a capacity to accumulation heavy metals within its tissues.

So, the purpose of this study aimed to determined the increase of biomass of two types of freshwater aquatic hydrophytes such as 'Water lettuce' *Pistia stratiotes L.* as a emergent or free-floating plant and *Ceratophyllum demersum L.* as a submerged one and use their growth rate as a biomarker or pollution indicator especially, they have a proven ability to survive and tolerant high concentrations of pollutants. Meanwhile, their large biomass is an indication of the timing of its accumulation of the largest amount of pollutants, when it grows in several concentrations of primary treated wastewater and groundwater wells.

### Methodology of Experiment

The experiment was carried out in open field of El- Harrery village, El Montaza, at the east of Alexandria, Egypt, during two successive summer seasons 2014 and 2015. The purpose of this study is to estimate the rates of increase in biomass production of *Pistia stratiotes L.* and *Ceratophyllum demersum L.* under stress of using two types of alternative water sources such as primary treated domestic wastewater and underground well water. Also, how can using these plants in phytoremediation process and contamination biomarkers.

The plant samples of *Pistia stratiotes L.* and *Ceratophyllum demersum L.* were obtained from the National Center for Oceanography and Fisheries, El-Anfoushi, Quiet Bay, Alexandria, Egypt. The means of initial weights of plants were average at 47 g and 32 g for each plant respectively. All samples were washed with tap water to be cleaned of any soil or sediment particles attached to the plant surfaces. Thus, they were cultivated at 15 Jun lasted until 28, 34 days for each plant respectively in both seasons.

The treatments media were Tap Water as a control [TW] - Primary Treated Domestic Wastewater [PTW 15%, 25%] from " Domestic Wastewater Treatment Station, Smoha-Alex., Egypt " - Underground Well Water [GWW 15%, 25%] "from Vadinas nursery at 24 km, Alexandria-

Matrowh high way, the Northwest Coast of Egypt" – and Mix 15%, 25% [TW+PTW+GWW] from full volume 2.5 L of transparent plastic Jars. The chemical and physical analyses of three kinds of water before treatments with plants were appeared in Table (1).The treatments were increased by the same concentration when the water evaporated. All of growth criteria, and chemical constituents for each plant and sediment wet weight were determined at the major biomass stage at 21<sup>st</sup> day and 28<sup>th</sup> day from cultivated *P. stratiotes L.* and *C. demersum L.*, respectively. After this stage the treatments were continued to determine the decrease of biomass rate (negative values) and the frayed tissues was excesses in both plant. But the water tests were determined in the highly concentration treatment of wastewater [PTW 25%], before and after the majority of plants biomass stage.

### A- Vegetative growth criteria of *Pistia stratiotes L.* were:

1- leaves number, 2- Plant fresh weight (g), 3- The growth rate (g/day), 4- Shoots dry weight (g), 5-Leaf thickness (cm), 6- Leaf width (cm), 7- Rosettes diameter (cm), 8- Root length (cm), 9- Roots dry weight (g), and 10- Sediment wet weight (g), then, 11- Plant wet weight at 28<sup>th</sup> day (g), 12- The decrease of biomass rate (g/day), 13- the decrease of rosettes diameter (cm) and 14- Wet weight of frayed tissues (g).

The equations of the growth rate and the decrease of biomass rate were:

$$\text{Growth rate (g/day)} = [\text{last fresh weight} - \text{initial fresh weight}] \div \text{days number}$$

### B- Vegetative growth criteria of *Ceratophyllum demersum L.*:

1- Shoots fresh weight (g), 2- The growth rate (g/day) by the same equation, 3- Shoots dry weight (g), 4- Sediment wet weight (g), 5- Plant wet weight at 34<sup>th</sup> day (g), 6- The decrease of biomass rate (g/day). 7- Wet weight of frayed tissues (g).

### C- Chemical constituents:

1- Total chlorophyll mg/g fresh weight in the leaves was determined by direct spectrophotometer method according to (Moran, 1982). 2- N% was determined according to (Evenhuis and Deward, 1980), P% was determined according to (Trouge and Meyer, 1939) and K % was determined according to (Brown and Lilliland, 1946) in the dried plant tissues from 1/2 numbers of replicate plants. 3- Na, Cd, Pb in plant shoot of 25% PTW at 21<sup>st</sup> day, 28<sup>th</sup> and 28<sup>th</sup> day, 34<sup>th</sup> day for both plant respectively 4- water

quality tests [color and turbidity] before /after major biomass in treatment of 25% PTW.

The experimental design was CRD [complete randomized design] as 7 treatments were replicated three times each replicate contains 6 Jars. The experiment consists of 126 plants [one plant /jar], in both seasons for each plant. Also, Means of data of the different treatments were compared using Duncan's Multiple Range test according to (Snedecor and Cochran, 1974).

### 3. Results and Discussion

Increase of biomass of *Pistis stratuotus L.* and *Ceratophyllum demersum L.* grow under stress with using alternative sources of water [primary treated domestic wastewater and underground well water]

and use its criteria of biomass productivity as contamination indicators.

#### 1- The vegetative growth criteria of *Pistis stratuotus L.* 'Water lettuce':

The estimated measurements shown in Table (2, 3) for both two successive summer seasons 2014 and 2015 are [leaves number, plant fresh weight (g), the growth rate (g/day), shoots dry weight (g), leaf thickness (cm), leaf width (cm), rosettes diameter (cm), root length (cm), roots dry weight (g), and sediment wet weight (g)] as in Table (2) at the 21<sup>st</sup> day or the majority biomass stage of water lettuce. And parameters of plant wet weight at 28<sup>th</sup> day (g), the decrease of biomass rate (g/day), the decrease of rosettes diameter (cm) and wet weight of frayed tissues (g) in Table (3) at the end of experiment.

Table (1): The chemical and physical analysis of three kinds of water treatments before experiment [22 °C of water] as follow:

Chemical and physical analysis of three types of water	Units	Primary treated wastewater [PTW]	Underground well water [GWW]	Tap water [TW]
pH		8.3	8	7.3
Ec	dS/m	16.12	7.93	1.63
TDS	mg/l	7459.2	3148.0	137.7
DO	mg/l	20.06	9.21	0.98
SO4	ppm	89.25	38.44	6.05
NO3	mg/l	102.3	0.528	0.116
NH4	mg/l	116.7	6.67	0.232
HCO3	ppm	60.75	8.01	0.532
Fe	mg/l	5.68	2.25	3.10
Na	mg/l	175.90	58.4	18.5
K	mg/l	41.4	30.32	10.2
Ca	mg/l	59.67	25	5.95
Mg	mg/l	63	14.4	1.56
Mn	mg/l	0.421	0.187	0.121
Zn	mg/l	2.604	0.466	0.29
Cd	meq/l	6.43	3.53	0.507
Pb	meq/l	1.109	0.601	0.439
Total P	mg/l	21.2	8.14	1.72
Turbidity	NTU	25.5	12.52	5.8
Color	Pt/co	54 gray	18 yellowish	12 bright

All of these parameters were agreed together on the superiority of the most concentrated treatment of pollutants was (25%PTW), which gave the highly significant values for all biomass measurements in both seasons followed by 15%PWT in some cases such a leaves number, plant fresh weight, the growth rate and shoots dry weight in both seasons and sediment wet weight 2<sup>nd</sup> season. While there were no

significant differences between the treatments of 25% PTW and 25% Mix in the parameters of leaves number in 1<sup>st</sup> season and leaf thickness and root length in second season. This is a greater indicator to the plant efficiency to assimilate the nutrient mineral elements in its tissues and convert them from an overly toxic polluted state to organic biomass. Also, the significant increase in the sediment weight

resulting from the treatment of 25%PTW explains that the root may have excreted some substances or adsorbed particles suspended in the water and turned them to sediment, which indicates the extent of the ability of plant to reduce the turbidity of the aqueous media. All of these results indicate that the treatments that contain a higher concentration of pollutants as a result of the addition of sewage water 'primary treated wastewater' led to an increase in the organic mass of *Pistis stratuotus L.* plant during the first 21<sup>st</sup> days of the experiment. Where these results are consistent with the findings of **Lu et al., 2010; Jia et al., 2013 and Farnese et al., 2014; Victor et al., 2016; Eid et al., 2016; Eid, 2017 and Kumar et al., 2019.**

On the other hand, the growth criteria in Table (3) indicated to the limited decrease of plant's organic mass with a long time which shown in parameters of [plant wet weight at 28<sup>th</sup> day, the decrease of biomass rate, the decrease of rosettes diameter and wet weight of frayed tissues]. The significant values of decrease of plant wet weight in parallel with the decrease of biomass rate of plants and the faster with time at the day of 28<sup>th</sup> were

resulting from the treatment of 25% PTW followed by 15% PTW in some cases, rather it indicates that the more concentrated treatments of pollutants after a short time, became the least abundant of nutrients and lack of availability of more these elements as a result of the high rate of accumulation in plant tissues. So the least of metal was led to the rapid destruction of plant tissues and the excess of the frayed tissues despite this, the plants were still alive but had lost layers of lower leaves close to the surface of the water.

Also, the results show that the rate of decrease of biomass [negative values] was faster than the rate of positive growth rate, which is an important indicator that confirms that there is a maximum growth limit at a certain point in time which the accumulation of pollutant elements is the maximum in the plant. At this time if the plants in not removed from aquatic system, its tissues will degrade and the elements will be liberated, leaving again to pollute the aquatic ecosystem and this is known as Pistia-model according to **Eid et al. 2016** or called as "biphasic dose response" according to **Jia et al., 2013; Farnese et al., 2014 and Kumar et al., 2019.**

Table (2): The vegetative growth criteria of *Pistis stratuotus L.* 'Water lettuce' grows under stress under stress with using alternative sources of water[ primary treated domestic wastewater and underground well water] during two summer seasons 2014 and 2015.

Treatment	Leaves number	plants fresh weight (g)	The growth rate (g/day)	Shoots dry weight (g)	Leaf thickness (cm)	Leaf width (cm)	Rosettes diameter (cm)	Root length (cm)	Roots dry weight (g)	Sedimentw. weight (g)
Summer season during 2014										
TW	6.00 e	87.67f	1.99d	1.32cd	0.40c	4.2d	11.6c	6.67d	0.187f	0.25f
15% PTW	9.33a	117.33ab	2.81ab	1.98a	0.633b	5.0b	13.3b	8.51c	0.292c	0.89b
25% PTW	9.53a	121.5a	3.03a	2.03a	0.767a	6.3a	14.8a	10.33a	0.348a	1.08a
15% GWW	6.67de	100.0de	2.61b	1.16de	0.307c	3.57e	11.5c	4.57f	0.216e	0.42e
25%GWW	7.33cd	94.0e	2.24cd	1.12e	0.367c	3.0f	10.5d	4.33f	0.279c	0.70c
15% Mix	8.00bc	105.83cd	2.51bc	1.49c	0.551b	4.3cd	13.0b	5.67e	0.242d	0.35ef
25% Mix	8.67ab	112.0bc	2.76b	1.76b	0.600b	4.87bc	12.6b	9.53b	0.313b	0.55d
Summer season during 2015										
TW	6.167f	95.33d	2.54d	1.29d	0.383d	4.503c	12.93c	5.73de	0.159e	0.27e
15% PTW	9.67ab	116.67ab	3.27ab	1.95ab	0.66b	5.16b	14.37b	9.16b	0.23cd	0.94a
25% PTW	9.87a	124.0a	3.55a	2.0a	0.775a	5.392a	15.07a	10.83a	0.371a	1.017a
15% GWW	7.17e	91.67d	2.395de	1.18d	0.52c	3.15f	12.2d	6.2cd	0.212d	0.59c
25% GWW	7.83d	82.33e	2.065e	1.15d	0.407d	4.04d	11.1e	5.03e	0.26c	0.73b
15% Mix	8.47c	106.17c	2.895c	1.53c	0.617b	3.75e	13.5c	6.83c	0.323b	0.38d
25% Mix	9.13b	108.67bc	2.98bc	1.72bc	0.70ab	4.45c	13.8bc	9.93ab	0.309b	0.45d

Table (3): The vegetative growth criteria and chemical analyses of *Pistis stratuotus L.* 'Water lettuce' grows under stress with using alternative sources of water [primary treated domestic wastewater and underground well water] during two summer seasons 2014 and 2015.

Treatment	plants wet weight at 28 <sup>th</sup> day (g)	Decrease of biomass rate (g/day)	Decrease of rosettes diameter (cm)	W. weight of frayed tissues	Total chlorophyll (mg/g)	N%	P%	K%
Summer season during 2014								
TW	67.75d	-2.92cd	7.27bc	55.33d	1.020 bcd	2.15	0.333	1.25
15% PTW	94.35a	-3.45b	8.61a	76.67b	1.147 b	3.21	0.325	1.83
25% PTW	81.68bc	-5.78a	7.83ab	89.33a	1.82 a	3.32	0.416	1.98
15% GWW	87.95ab	-2.11f	6.5cd	48.0de	0.957 cde	2.895	0.358	1.73
25% GWW	77.75cd	-2.36ef	4.7e	42.33e	0.843 e	3.12	0.352	1.68
15% Mix	87.76abc	-2.71de	6.2d	64.0c	0.903 de	3.065	0.339	1.59
25% Mix	90.24ab	-3.18bc	5.77d	67.5c	1.064 bc	3.06	0.411	1.64
Summer season during 2015								
TW	79.75c	-2.26f	7.07c	59.213d	1.069cd	2.28	0.312	1.39
15% PTW	91.605a	-3.67b	7.95b	74.243b	1.155bc	3.17	0.421	1.96
25% PTW	89.75ab	-4.98a	8.94a	88.656a	1.894a	3.19	0.424	2.01
15% GWW	68.33d	-3.36b	5.04e	45.98e	1.013de	2.87	0.337	1.75
25% GWW	59.925e	-3.23cd	6.73c	43.547e	0.828f	2.93	0.328	1.78
15% Mix	86.08b	-2.85de	6.55c	64.767c	0.921ef	2.91	0.337	1.62
25% Mix	90.19a	-2.63ef	5.85d	68.523c	1.179b	3.05	0.354	1.82

## 2- Vegetative growth criteria of *Ceratophyllum demersum L.*:

It is clear from data in Table (4) that using alternative sources of two types of water such as primary treated domestic wastewater and underground well water and mix of them had a significant effect on all criteria of vegetative growth like shoots fresh weight (g), the growth rate (g/day), shoots dry weight (g), sediment wet weight (g). And the parameters of Plant wet weight at 34<sup>th</sup> day (g), the decrease of biomass rate (g/day) and wet weight of frayed tissues (g) at the end of experiment. The results were appeared that all plants treated with 25%PTW were the highest significant in the estimated values in most cases. While there were no the significant different values between the treatments of 25%PTW, 15%PTW and 25% Mix in

parameter of shoots fresh weight (g) and the growth rate in both seasons. However the control treatment gave the highly values in the shoots dry weight in both seasons and was the lowest in the sediment formation and frayed tissues. Also, the parameter of plant wet weight at 34<sup>th</sup> day with the other one of decrease of biomass rate note to the same direction results of *Pistis stratuotus L.*, where the rate of reduce in the biomass of the plant was faster than the rate of growth. However, the values of wet weight of frayed tissues resulted from *C. demersum L.* draw attention to the fact that it is less than what is found in *P. stratuotus L.*

All these results are in harmony with those of **Ghobrial, 2000; Patel and Kanungo, 2010; Foroughi et al., 2010 and Al-Ubaidy and Rasheed, 2015.**

Table (4): The vegetative growth criteria and chemical analyses of *Ceratophyllum demersum* L. "Hornwort" grows under stress with using alternative sources of water [primary treated domestic wastewater and underground well water] during two summer seasons 2014 and 2015.

Treatments	Shoots fresh weight (g)	The growth rate (g/day)	Shoots dry weight (g)	Sediment w. weight (g)	plants wet weight at 34 <sup>th</sup> day (g)	Decrease of biomass rate g/day	Wet weight of frayed tissues	Total chlorophyll (mg/g)	N%	P%	K%
Summer season during 2014											
TW	95.82 c	1.877 b	1.29 a	0.621 g	82.96bc	-2.17e	25.04d	5.517 c	1.81	0.586	1.60
15% PTW	100.83 ab	2.025 ab	1.05 de	1.723 b	86.39a	-2.35de	26.72bc	5.944 a	2.64	0.701	2.03
25% PTW	103.65 a	2.107 a	1.031 e	1.968 a	83.92abc	-3.38a	32.85a	6.043 a	3.05	0.744	2.12
15% GWW	88.62 de	1.665 c	1.215 ab	0.787 f	72.17d	-2.65cd	27.63bc	5.087 d	1.92	0.594	1.62
25% GWW	86.94 e	1.620 c	1.25 ab	1.014 e	70.39d	-2.73c	26.09cd	5.099 d	2.16	0.641	1.79
15% Mix	97.07 bc	1.914 b	1.12 cd	1.163 d	85.48ab	-2.095e	28.08b	5.604 c	1.98	0.609	1.68
25% Mix	99.26 abc	1.978 ab	1.18 b	1.306 c	81.37c	-3.085b	31.44a	5.752 b	2.49	0.638	1.95
Summer season during 2015											
TW	93.66 d	1.643 d	1.32 a	0.652 d	74.39d	-3.24c	22.35f	5.59 cd	2.04	0.614	1.70
15% PTW	104.53 ab	2.073 ab	1.075 d	1.751 a	83.75bc	-3.835b	30.46c	5.97 ab	2.86	0.756	2.14
25% PTW	106.82 a	2.158 a	1.081 d	1.954 a	80.76c	-4.28a	39.57a	6.141 a	3.21	0.784	2.19
15% GWW	96.49 d	1.753 d	1.265 ab	0.755 d	73.52d	-3.71b	28.53cd	5.124 e	2.49	0.652	1.75
25% GWW	100.95 bc	1.926 c	1.275 ab	1.023 c	92.89a	-1.37e	26.36de	5.092 e	2.58	0.698	1.94
15% Mix	100.67 c	1.94 bc	1.165 c	1.064 bc	83.08c	-2.955cd	25.85e	5.55 d	2.76	0.633	1.88
25% Mix	102.38 bc	2.013 abc	1.23 bc	1.268 b	86.64b	-2.635d	36.15b	5.78 bc	2.94	0.706	2.06

### 3- Chemical constituents:

Data of the effect of using alternative sources of water [primary treated domestic wastewater and underground well water and mix of them] on chemical constituents of *Ceratophyllum demersum* L. "Hornwort" and *Pistis stratuotus* L. 'Water lettuce' are presented in Table (3, 4 and 5). The results cleared that there were significant differences between all treatments. The highest chlorophyll value was achieved from the treatment of 25% PTW in *P. stratuotus* L. and 25% PTW and 15% PTW in *C. demersum* L. plant in both seasons respectively, but the highest assimilation of total chlorophyll was increasing in *C. demersum* L. shoots than *P. stratuotus* L.

While, the highest values of N% in *Pistis stratuotus* L. shoots were resulted from 25% PTW and 25% PTW and 15% PTW in both seasons respectively, meanwhile the highest values of P% were due to 25%PTW and 25% Mix in 1<sup>st</sup> season and 25% PTW and 15% PTW in 2<sup>nd</sup> season. In the same trend the plants were treated by 25%PTW were the highest in accumulation of K%.

Also, the superiority treatment in N% in *C. demersum* L. shoots was 25% PTW during two seasons. While, the highest values of P% and K% resulted from the treatments of 25%PTW and 15% PTW in 1<sup>st</sup> season and 25% PTW, 15% PTW and 25% Mix in 2<sup>nd</sup> season.

However, it was still the plants growing in the 25%PTW treatment that achieved the highest values of nutrient assimilation. The observed results noted that *C. demersum* L. was more concentrated in total

chlorophyll and P% analysis than *P. stratuotus* L. plant. While the analyses of N% and K% were noted that there were similar accumulations among *P. stratuotus* L. and *C. demersum* L. in them tissues.

Similar findings were obtained by **Brix, 1997; Lu et al., 2010; Patel and Kanungo, 2010; Galal & Farahat 2015; Al-Ubaidy and Rasheed, 2015; Eid, 2017; Sidek et al 2018 and Ali et al., 2020.**

Moreover, because the treatment of 25%PTW achieved the highest results in both plants, so it is appear in Table (5) the analyses of Na, Cd and Pb in each plant tissues at 21<sup>st</sup> day and 28<sup>th</sup> day for *Pistis stratuotus* L. and 28<sup>th</sup> day and 34<sup>th</sup> day for *Ceratophyllum demersum* L. during two seasons and water quality tests [color and turbidity] of the same treatment at before (1<sup>st</sup> day), during (at 21<sup>st</sup>, 28<sup>th</sup> day of the majority biomass for each plants) and after the experiment [at the day of 28<sup>th</sup> and 34<sup>th</sup>] for each plants respectively.

The results noted that the day of 21<sup>st</sup> and 28<sup>th</sup> for each plant respectively were achieved the highest values of bioaccumulations or phytoremediation for Na, Cd and Pb after that at 28<sup>th</sup> and 34<sup>th</sup> day for *P. stratuotus* and *C. demersum* respectively the accumulation of metals were started to decies in both plants tissue, which confirms the return of their release in water again. Also, the results of the analyses showed that *C. demersum* plant was better than *P. stratuotus* in assimilation of these metals. Meanwhile, the high values of water quality tests were decreased with the majority of biomass plant and their efficiency of metals accumulation at 21<sup>st</sup> and 28<sup>th</sup> day for both plants. But after increased of



tissues deterioration, the water purity decreased again. Also, the efficiency of *C. demersum* plant was the best than *P. stratuotus* for water purification.

These results are in harmony with those of **Ghobrial, 2000; Patel and Kanungo, 2010; Farnese et al., 2014; Ahmad et al., 2016; Sidek et al., 2018 and Ali et al., 2020.**

Table (5): the metal concentration in dry weight of *Pistis stratuotus* L. 'Water lettuce' and *Ceratophyllum demersum* L. "Hornwort" and water quality tests in the treatment of 25%PTW during two summer seasons 2014 and 2015.

Metal concentration in plant D.W.	Unit	<i>Pistis stratuotus</i> L.			<i>C. demersum</i> L.		
The plants of 25% PTW treatment		Summer season during 2014					
			21 <sup>st</sup> day	28 <sup>th</sup> day		28 <sup>st</sup> day	34 <sup>th</sup> day
		Na	4.41	2.16		6.08	2.20
		Cd	0.35	0.09		0.52	0.13
Pb	0.51	0.02		0.75	0.22		
<b>water quality test</b>		1 <sup>st</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	1 <sup>st</sup> day	28 <sup>st</sup> day	34 <sup>th</sup> day
Water turbidity	NTU	20	7	13	20	6	11
Water color	Pt/co	29	10	15	29	8	14
		Gray	Cloudy	Green yellowish	Gray	Light cloudy	Light greenish
The plants of 25% PTW treatment		Summer season during 2015					
			21 <sup>st</sup> day	28 <sup>th</sup> day		28 <sup>st</sup> day	34 <sup>th</sup> day
		Na	4.84	2.11		7.12	3.02
		Cd	0.37	0.10		0.60	0.12
Pb	0.56	0.03		0.76	0.24		
<b>Determination of water quality</b>		1 <sup>st</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	1 <sup>st</sup> day	28 <sup>st</sup> day	34 <sup>th</sup> day
Water turbidity	NTU	21	9	12	21	8	10
Water color	Pt/co	28	11	14	28	9	15
		Light gray	Cloudy	Green yellowish	Light gray	Light cloudy	Greenish

### Recommendation:

- 1- The experiment has shown that *Pistis stratuotus* L. 'Water lettuce' and *Ceratophyllum demersum* L. "Hornwort" has a high ability to phytoremediation of aquatic ecosystem from harmful pollutant elements. Hence, it can be used for aesthetic purpose in aquariums or an economic purpose in fish farms, as they purify the pond water. However, they must be removed at the maximum growth stage without delay. Otherwise, pollutants will return to be released from the worn out tissues to be released again into the water, changing its biophysicochemical properties and harms other aquatic organisms, increasing sediment and disturbing the waters smoothness, causing an infection of the lake or the pond.
- 2- The efficiency of phytoaccumulation of elements in *Ceratophyllum demersum* L. "Hornwort" is higher than that of *Pistis stratuotus* L. 'Water lettuce' plant. While, the frayed tissue in the 'Water lettuce' was higher than "Hornwort" plant.
- 3- The water quality test indicated that the plants adsorbed the turbidity or secreted substances that turned them into sediment. So it can be recommended to study this phenomenon in the future. However, the water color turned green at the end of the experiment, possibly due to the frayed parts of the plant.

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