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Comparison study of two different varieties of pea (Pisumsativum) crop under different quality of water

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Agricultural practices are highly dependent on irrigation while the sustainability of agriculture is directly related to the quality of irrigation water. Industrial wastewater effluents used for agricultural practices due to decrease of water supplies in agroecosystems but unpromising effects on plants physical and chemical properties have been reported. Experiment was carried out to study the effect of textile industry effluent via measuring its different physicochemical properties along with trace heavy metals and also in order to assess its quality to be used being an alternate source of irrigation. The experiment was comprised of three treatments (T1= Canal water, T2= Treated water, T3 = Untreated water). The effects of irrigation water quality on two pea (Pisumsativum) crop varieties (Mateur and Pea09) yield were also significant. T3 produced the highest yield on Pea09 variety followed by T2 and T1 which produced the lowest yield as compared to Mateur variety, respectively. As the evaluated results, it can be used to develop future scenarios for optimization of the industrial treated wastewater for agriculture.

Keywords: Irrigation, Agriculture, Wastewater effluents, Pea crop

Introduction

Pakistan is an agricultural country having arid semiarid climatic conditions [1] and facing water scarcity problem at present scenario. For intensive cropping system, canal water is not enough to achieve the maximum potential of soil and crops. Groundwater is also an important source of water for agricultural use but this water is contaminated and is unfit for irrigation [2]. Farmers are purposely using industrial and municipal wastewater for irrigation due to the scarcity of water, especially for raising vegetables and fodders [3]. Faisalabad and Lahore cities are considered to become the main industrial cities of Punjab, Pakistan. Unchecked disposal of industrial and municipal effluents has deteriorated the quality of underground water of major cities of Pakistan including Lahore and Faisalabad [4]. Combined industrial and municipal effluents exhibit high COD, BOD, TDS, toxic metals, toxic chemicals, and pathogenic microorganisms.

Treated community wastewater exemplifies a substantial latent source of domestic water for certain valuable reuse. In industrialized countries, almost 73% of the residents are assisted by the collection of wastewater and treatment services. However, only 35% of the developing countries the population is aided by the collection of wastewater (USEPA 2004). From the environmental aspect it is interesting that by using treated effluents for agriculture purposes, the agricultural field can be considered as a tertiary treatment step, while non-controlled environmental pollution is prohibited if well managed[5]. With increasing overall population, the gap between the supply and demand for water is reaching such shocking levels that in some areas of the world it is affectation a risk to human survival. Scientists around the world are working on alternates of conserving water or conjunctive use water [6, 7]. It is a favorable time, to refocus on the ways to recycle water through the reuse of wastewater, for irrigation and other purposes [8, 9, 10].

Wastewater is being irrigated 0.3 million hectares of agricultural land. The safe removal and use of wastewater enhance crop production and reduce environmental pollution [11]. The potential for irrigation to raise both agricultural productivity and the living standards of the rural poor has been recognized. This potential is even more pronounced in arid areas, such as the Near East Region, where only 30 percent of the cultivated area is irrigated but it produces about 75 percent of the total agricultural production. Where-as more than 50 percent of the food requirements are imported and the rate of increase in demand for food exceeds the rate of increase in agricultural production [12]. Wastewater can be

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used for the agriculture irrigation in towns. Flowing of wastewater in canals used to irrigate minor land area where fodder, grass or some other product that could be used in slight amounts for the diet of living organisms and may pose a serious health risk[13, 14]. Microbial community patterns substantially differed between water treatments, and bacterial population is influenced by different qualities of treated wastewater used for irrigation [15, 1]

Therefore, it is necessary to investigate the fate and transport of metals pollution in crop plants irrigated with treated and untreated industrial effluents. Further, the findings of this study would help us in achieving sustainable land use practices, to overcome the bidirectional issue of water scarcity which ultimately; affecting plants productivity.

2. Materials and Methods

2.1. Experimental design

The experimental research work was carried out in the open atmospheric natural conditions into the botanical garden of Government College University Faisalabad. Seeds of the two selected cultivars of pea (*Peas09* and *Mateur*) were collected from the Vegetables Research Institute located in Ayub Agricultural Research Institute, Faisalabad for this experimental research work. Soil for the experimental study was arranged from the nursery. The soil was homogenized, air-dried under room temperature and passed through 2 mm mesh and then filled into the 18 pots having diameter of 20cm, respectively. After that five seeds per pot were sown and seed germination started in one week after sowing. The pots were shifted to an experimental area for effluents treatment Fig.1.

Figure 1 Field Experiment at growing stage of pea crop

The complete layout of the experiment is given in Table 1.

Table 1: Layout of Experiment

Treatment 1		Treatment 2		Treatment 3	
V1R1	V2R3	V1R2	V2R1	V1R3	V2R1
V1R2	V2R1	V1R3	V2R2	V1R1	V2R3
V1R3	V2R2	V1R1	V2R3	V1R2	V2R2

V1= Peas 09, V2 = Mateur, R(1-3) = Replications, T1 = Canal water, T2 = Treated water, T3 = Untreated water

The experiment conducted under the open air conditions was a completely randomized design (CRD) with three treatments and three replicates for each of the selected genotypes of a pea.

2.2. Applied Treatments

During the first week of experiment, three treatments with three different qualities of water were conducted (canal, treated and untreated wastewater) started after the sufficient vegetative growth (leaf, stem and roots) of selected cultivars of pea crop. Each pot in the experimental design receives 250 ml of water accordingly to three treatments, designed as Treatment 1 (canal), Treatment 2 (treated wastewater) and Treatment 3 (untreated wastewater), respectively. The treatment of canal, treated and untreated wastewater applied to experiment regularly as per crop requirement and accordingly to prevailing weather patterns. Wastewater quality parameter of treated and untreated samples is shown in Table 2.

Table 2: Studied parameters of treated and untreated wastewater samples

Sr. No.	Parameter	Units	Canal water	Treated water	Untreated water	NEQS
	Flow	m^3/h		90	90	
1	Temperature	^0C	29	31	39	40
2	pH	---	7.72	7.06	6.68	6-9
3	Chemical Oxygen Demand (COD)	mg/l	72.08	51	5140	150
4	Biological Oxygen Demand (BOD)	mg/l	16.04	20	2092	80
5	Total Dissolved Solids (TDS)	mg/l	1723.5	3466	4220	3500
6	Total Suspended Solids (TSS)	mg/l	73	56	198	200
7	Chromium	mg/l	0.05	0.03	0.05	1.0
8	Copper	mg/l	0.20	0.02	0.04	1.0

2.3. Experimental Observations

During the experiment days to *germinate, plant height, flower development, chlorophyll concentration, stomatal conductance and photosynthesis rate* were measured on a regular interval as follow.

2.3.1. Plant height

Initial growth measurements (plant height and leaf number) were taken before the start of treatment. Numbers of leaves for selected cultivar *Peas 09* and *Mateur* of the experimental crop were calculated. Measurements for plant height were carried out on weekly basis at day 14, 22, 30, 37, 44, 51, 58, 65, 72, 79, 86 and 93 of the experiment, respectively.

2.3.2. Chlorophyll concentration

The leaf *chlorophyll concentrations* were measured during the experiment. Fresh leaf samples (0.1 g) were taken and directly immersed into 3 ml acetone (80%) and incubated for 24 hours at 4°C. After incubation, the optical density was determined at 480 nm, 645 nm, and 663 nm wavelength by using a UV-visible spectrophotometer (V1.7, WPA, Cambridge). *Chlorophyll a*, *chlorophyll b*, and *total chlorophyll* were calculated by using the formula of(Arnon 1949). *Carotenoid* content of leaves was evaluated by using the formula [17]. The number of pigments was represented in mg/g of fresh weight.

Chlorophyll *a* = 12. 7 (A 663) - [2. 69 (A 645) (V / W)]

Chlorophyll *b* = 22. 9 (A 645) - [4. 68 (A 663) (V / W)]

Total Chlorophyll (a + b) = 20. 2 (A 645) + [8.02 (A 663) (V / W)]

Carotenoids = 4. 16 (A 480) - [0. 89 (A 663) (V / W)]

Where, A = Absorbance at specific wavelengths, V = final volume of chlorophyll extract, W = fresh weight of tissue extracted

2.3.3. Stomatal conductance

The youngestfully expanded healthy leaves (third leaves from top) were used for the measurement of *stomatal conductance* using Delta T AP4 porometer, Delta-T Devices Ltd, Cambridge, UK. *Stomatal conductance* measurement was taken at different weather conditions during the day, including cloudy and sunny days.

2.3.4. Metals content

Grind the pea's grains and 0.5 g sample is weighted on the electronic balance. These weighted samples place in the digestion flask. After adding tri-acid into samples then put on the digestion chamber for 1 hour. The color of the sample is removed, filter these samples and make volume up to 50 ml. Then test on the Atomic absorption spectrophotometer to analyze the heavy metals copper and chromium [18, 19].

2.3.5. Crude protein contents

Firstly crush the oven dried pea's sample and took 1.0 g sample is weighted on the electronic balance. Mixture (1.0 g $CuSO_4$ + 10 g K_2SO_4 + 0.1 g SC), 10 ml H_2SO_4 and samples are added in digestion cube. Then these digestion cubes put on the digestion block for 3 hours. When 1-2 ml solution left at the end then added distilled water to make 100 ml solution by volume. Then distillation process is completed and 50 ml solution received from flask. Titrate against N/10 H_2SO_4 and note the reading.

2.3.6. Photosynthesis rate

The youngest fully expanded healthy leaves were usedfor the measurement of photosynthesis rate using IRGA. *Photosynthesis rate* measurements were taken at different weather conditions during the day, including cloudy and sunny days.

2.4. Statistical Analysis

The data collected for the selected cultivars of pea crop were analyzed using two way ANOVA in the GLM arithmetical package using R 3.0.2 [16].

3. Results and Discussion

The plant sample was collected from the selected sites and following parameters were observed as discussed below; *germination of pea seeds, plants height, chlorophyll a, chlorophyll b, carotenoids, chlorophyll a/b ratio, total chlorophyll and carotenoids/total chlorophyll ratio, grain yield, straw yield, and stomatal conductance*.

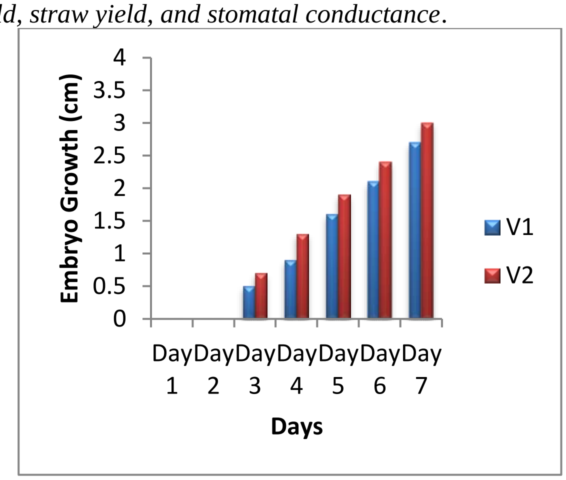

Figure 2 Germination of pea

In Figure2, represents Pea germination at outdoor conditions of the selected site (Botanical garden of Government College University Faisalabad). Germination of seeds of the selected variety *Mateur* (V2) has a better germination rate as compared to variety *Pea09* (V1).

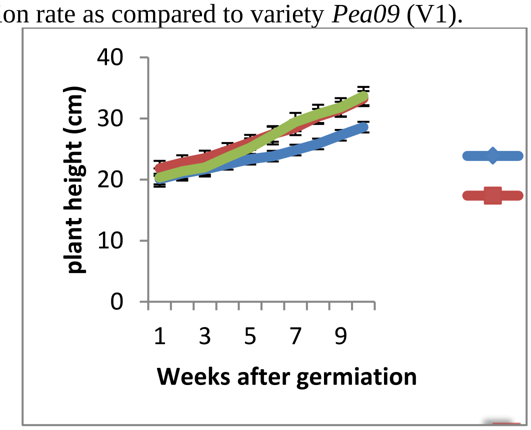

Figure 3 Plant height of Pea 09 (V1)

In Figure 3 and 4, Plant height of two varieties of a pea under three different treatments shows significant results. Plant shows maximum height by applying untreated water (T3) followed by treated water (T2) and canal water (T1). Pea plant achieved maximum plant height (35cm) under T3 irrigation followed by T2 (33cm) and T1 (29.5cm). Plants height (P and F) values of ANOVA effect on *Pea (Pisumsativum)* Table 3.

Table 3: ANOVA P and F values of plant height

	Plant height of V1&V2	
	P	**F**
Treatment (T)	0.083	2.61
Varieties (V)	0.001	12.10
Interaction	0.306	1.21

The significant difference is found among the treatments as well as varieties due to the enrichment of organic matters and plant nutrients in treated and untreated industrial effluents which required by growing of plants and also climatic conditions affect the varying of plants length, leaf surface, and vegetation area. Leaves of the plant having the major impingement which reduces pollution through biological activities, reduce dust concentration of air by filtration and absorption [20].

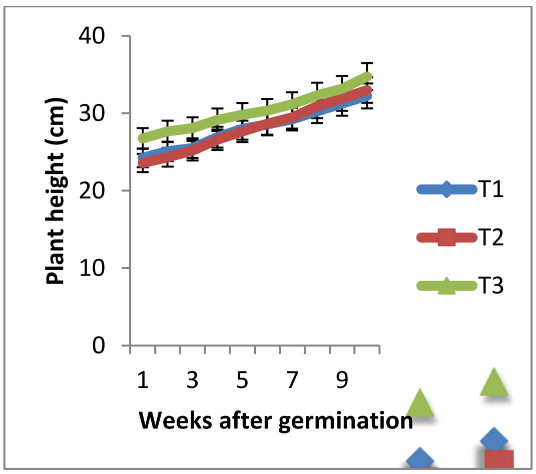

Figure 4 Plant height of Mateur (V2)

Leaves capacity as receptors also depends upon cuticular and epidermal features of leaves, phyllotaxy, surface geometry, leaf pubescence, type of canopy etc [21]. In this study, the Peas plant was maintained at same climatic and edaphic conditions and then observed the difference in plant growth may attribute to three different water treatments. The plant height of Peas decreased on treatment T1 due to the significant level of organic matters [22]. It is reported that the concentration of organic matters, essential for plant height is more in industrial effluent rather than canal water that affected on height, shoot weight, growth, and its development stage [23].

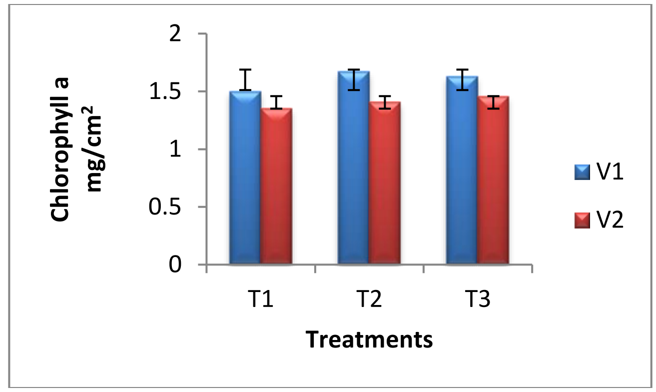

Figure 5 Effect of treatments on chlorophyll a concentration

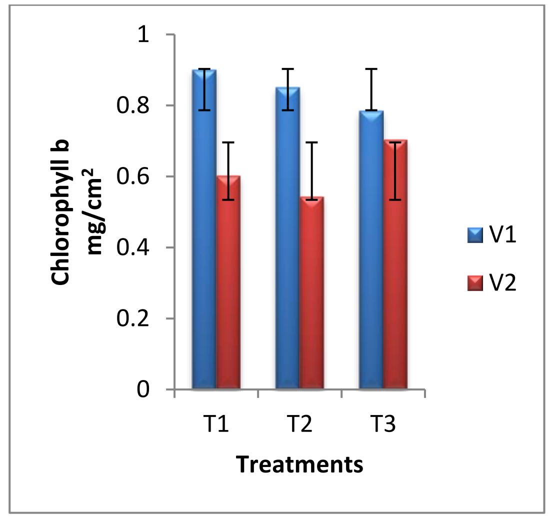

Figure 6 Effect of treatments on chlorophyll b concentration

In Figure 5 and 6, *chlorophyll a* and *chlorophyll b* were clearly shown maximum in variety (V1) as compared to variety (V2) in all three treatments (treated, untreated and canal water). The result exposed that chlorophyll contents were reduced in variety (V2). The *chlorophyll a* and *chlorophyll b* contents were observed in a floristic society comprising of the peas varieties. However, the *chlorophyll a* concentration was comparatively reduced on T1 as compared to T2 and T3. The outcomes of untreated effluent from industries were scattered in the environment and then affect the groundwater as well as the crops which were nearest to the industries. This reaction was noted mostly in some crop varieties. The pea species being acclimated for a longer period at the affected areas, this response shows the low chlorophyll concentration in the tissues.

In Figure7, maximum *total chlorophyll* was clearlyshown in variety (V1) in all treatments application (treated, untreated and control water). The result showed that total *chlorophyll* contents were reduced in variety (V2) plant.

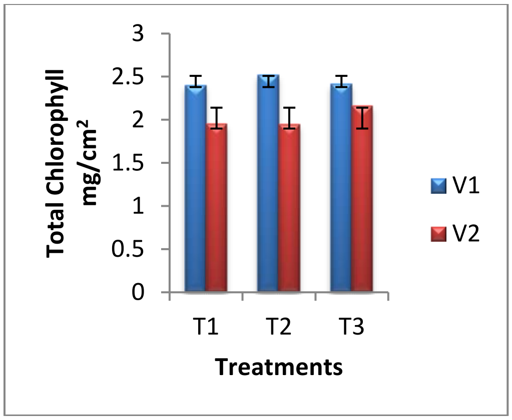

Figure 7 Effect of treatments on total chlorophyll concentration

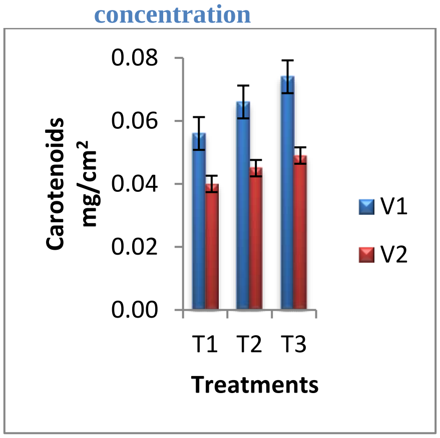

Figure 8 Effect of treatments on carotenoids concentration

In Figure8, the effect of treatments of water on plant *carotenoids* was clearly shown. *Pea09* Variety (V1) has more *carotenoids* by applying treatment T3 than the other two treatments. Overall maximum *carotenoids* were found in both varieties by applying untreated water and it was less in the other two treatments.

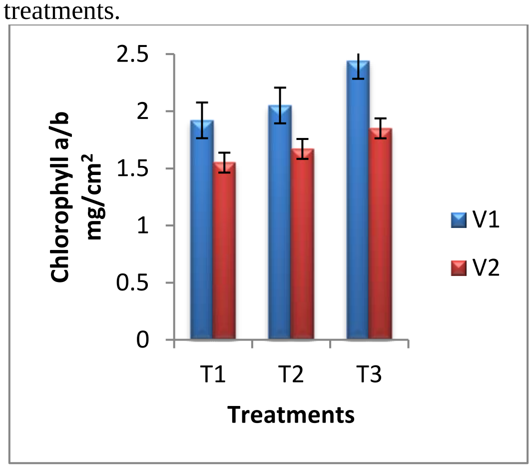

Figure 9 Effect of treatments on chlorophyll a/chlorophyll b concentration

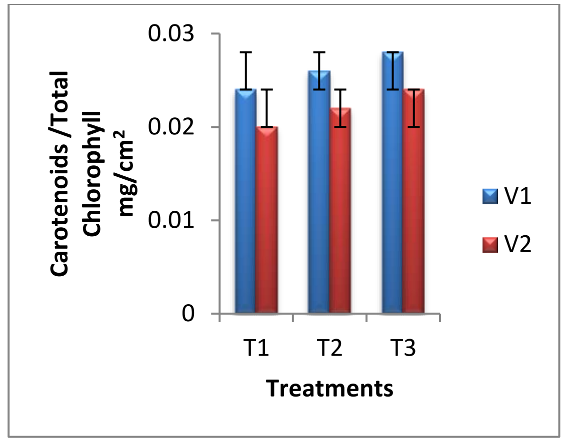

Figure 10 Effect of treatments on carotenoids/total chlorophyll content

In Figure 9 and 10, the effect of treatments of water on plant *chlorophyll* ratio *a to b*, *carotenoids* and *total chlorophyll* ratio were clearly shown. Variety *Pea09* (V1) has more *carotenoids* and *chlorophyll* ratio under T3 treatment than the other two treatments. But variety *Mateur* (V2) showed less *chlorophyll* ratio as compared to *Pea09*. Overall maximum *chlorophyll* ratio was found in T3 as followed by T2 and T1.

Table 4: ANOVA P and F values of chlorophyll Chlorophyll

	Chlo A		Chlo B		Total Chlo		Chlo A/B		Cartonides		Caro/ Total Chl	
	P	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**
Treatment (T)	0.997	0.001	0.771	0.27	0.970	0.03	0.338	1.19	0.930	0.07	0.418	0.94
Varieties (V)	0.441	0.64	0.280	1.28	0.336	1.01	0.940	0.01	0.047	4.90	0.54	4.55
Interaction	0.670	0.41	0.374	1.07	0.511	0.71	0.261	1.51	0.713	0.35	0.264	1.49

All *chlorophyll* (P and F) values of ANOVA effect on Pea *(Pisumsativum)* Table 4. The significant difference is found among the treatments and also some difference is found between the varieties.

Chlorophyll content is necessary for photosynthetic activity and air pollution is the main indicator to reduce the chlorophyll contents [24, 29]. The water pollutants decreased *chlorophyll* synthesis and increase the degradation of *chlorophyll* [1, 3, 24, 25]. It has been reported that the *chlorophyll* content should be a helpful indicator for the assessment of injury induced by untreated wastewater pollutants such as [11, 25]. During this study in which *chlorophyll a*, *chlorophyll b*, *carotenoids, chlorophyll a/b* ratio, total *chlorophyll* and *carotenoids/total chlorophyll* were also estimated on three different treatments on two varieties of pea crop. The *carotenoids, chlorophyll a/b ratio, total chlorophyll* and *carotenoids/total chlorophyll* concentration was found to be higher in treatment T3 at selected site as compared to treatment T2 and treatment T1 in both varieties. When the heavy metal settles down on the shoots and leaves, then it blocks the stomata to prevent normal gaseous exchange and apply significant bearing on photosynthesis [1, 26, 27]. Water pollutants apply additional stress on plants that can reduce plant growth and by other stresses, the productivity decreased as well [28]. These findings are in accordance with other studies [29]. The *photosynthesis* and respiration process move continuously when plants and crops inhale the CO_2 and O_2 through *stomata*. If the layer of particles closes the stomata for a longer period of time then these processes stopped and the plant may suffer mortality.

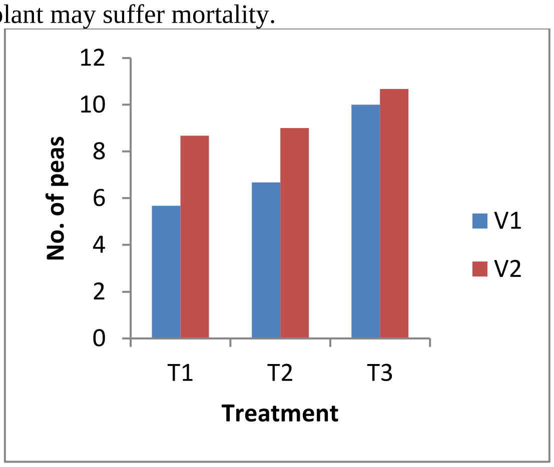

Figure 11 Effect of treatments on grains and straw yield

In Figure11, treatments of water show significant results about *grain* and *straw yield*, treatment T3 showed more yield production on two varieties then T1 and T2. Variety *Pea09* (V1) shows better results as compared to variety *Mateur* (V2) and showed maximum yield production with treatment T3 rather than treatments T1 and T2.

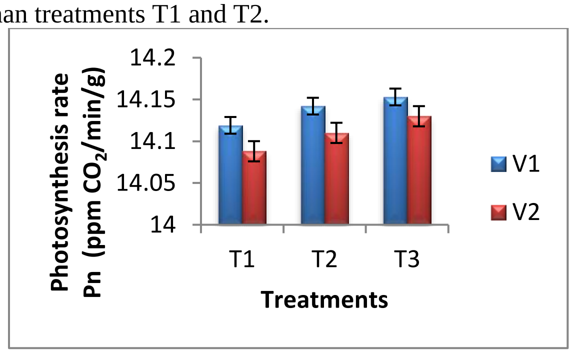

Figure 12 Effect of treatments on leaf photosynthesis rate

In Figure12, maximum leaf *photosynthesis* rate was clearly shown by variety *Pea09* (V1) under all treatments application (treated, untreated and control water). The result showed that total *photosynthesis* rate was reduced in variety *Mateur* (V2) plants. Overall applying treatment (T2 and T3) to both varieties were shown more leaf *photosynthesis* rate rather than treatment (T1).

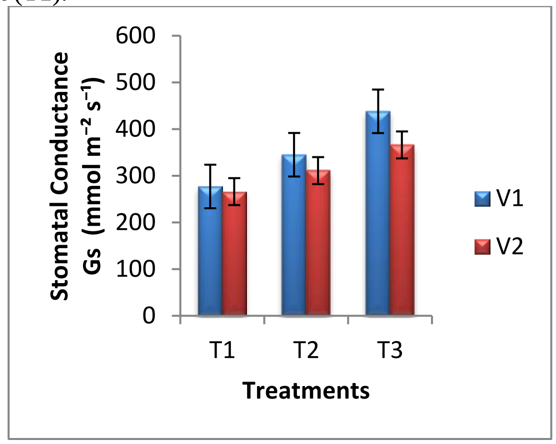

Figure 13 Sensitivity of stomatal conductance in Pea leaves

In Figure13, relationship between three treatments, T3 showed maximum *stomatal conductance* on two varieties then T1 and T2. Between two varieties the variety *Pea09* (V1) shows better results than variety *Mateur* (V2). Variety *Pea09* (V1) showed maximum stomatal conductance with treatment T3 as compared to the treatments T1 and T2.

Table 5: ANOVA P and F values of stomatal conductance and photosynthesis rate

	Stomatal conductance		**Photosynthesis rate**	
	P	**F**	**P**	**F**
Treatment (T)	0.0001	43.65	0.0001	23.48
Varieties (V)	0.006	11.34	0.0001	154.33
Interaction	0.130	2.43	3.17	0.79

Stomatal conductance and photosynthesis rate (P and F) values of ANOVA effect on Pea *(Pisumsativum)* Table 5. A significant difference of *stomatal conductance and photosynthesis rate* were found among the varieties as well as treatments.

4. Conclusion

The study exposed that wastewater coming from industrial (treated or untreated) source extensively enhanced plant growth due to nutrients, organic matter and moderate level of pollutants. Both varieties behaved similarly showing high growth increment in term of height under untreated water irrigation followed by treated industrial effluents and canal water. Particularly, variety *Pea09* as compared to variety *Mateur* exhibited marked differences in their height under different sources of irrigation. These findings suggested that higher uptake of organic matter and nutrient was observed in plants mostly under untreated industrial waste followed by treated industrial effluents. Canal water contribution to organic matter and nutrients uptake for two varieties was comparatively low. Overall growth rate of pea varieties on untreated industrial effluents was considerable but there is possibility have some health risks from production of crops instead of canal water which affects human health. Influential, treated industrial wastewater is found as a good and reliable alternative source of fresh and canal water for agriculture purposes.

5. Acknowledgement

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