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Research Article

PHYTOTOXIC EFFECT OF TANNERY EFFLUENTS ON SEED GERMINATION AND SEEDLING GROWTH OF COWPEA [*VIGNA UNGUICULATA* (L.) WALP.]

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ABSTRACT
Experiments were performed in petri dishes using different dilution levels of untreated tannery effluent (UTE) and treated tannery effluent (TE) (25, 50, 75 and 100 %) to know the effect on seed germination and seedling growth in cowpea [*Vigna unguiculata* (L.) Walp.]. The effluent as such appeared dark grey in color with a foul smell. UTE showed several fold increase in BOD and COD values compared to TE, which showed decreased level of pollutants except sulphates. Results showed a reduction in the level of pollution parameters to a large extent; several parameters are far greater than the permissible limits in the TE. Cr, for instance, displayed a 16.6 fold decrease after treatment, but it is still 70 times higher than the permissible values. The germination percentage decreased with increase in concentration of tannery effluent. The different concentrations of tannery effluents treated seeds also showed reduction in root length, shoot length and vigour index whereas fresh weight and dry weight was increased in UTE and TE treated groups except 50% UTE treated group with marginal decrease.

Keywords: *Tannery Effluents, Seed, Germination, Growth, Toxicity*

INTRODUCTION
The tannery industry is one of the oldest industries in India. India is the largest market for hide and skin. The leather is tanned by chrome and vegetable tanning process. The effluent of tannery industries containing chromium (Cr) is discharged on land surface and it is used for irrigation purposes. In the hide or skin processing, supplied quantity of chemicals (except Cr) about 15% are consumed and the unconsumed chemicals are either discharged into tannery effluent or as solid waste. In case of Cr about 70% of the offered amount is taken up by the hides/skins and about 30% remains unexhausted and goes into effluent waste water as well as into solid waste (Nath *et al.*, 2009).
Tannery industries let out 25- 40 litres of effluents and 40 grams of chrome salt for every kilogram of processed hides and skins and about 4-5 litter chrome waste water is produced per kg hide and skin. Spent chrome tan liquor is acidic and greenish in nature with a pH 2.6 to 3.8. It contains about 500 to 5500 mg/L trivalent Cr.
Total suspended solids and biochemical oxygen demand (BOD) ranges were reported to be 1500 to 2400 mg/ L and 800 to 3500 mg/ L respectively (Chakraborty *et al.*, 1965). The high level of nutrients in the effluent has been reported to inhibit seed germination and seedling growth at lower dilution of effluents, which might be due to the presence of excess amount of dissolved solids, chlorides, sulphides, Cr, high BOD and chemical oxygen demand (COD) in the effluent (Mishra and Bera, 1995). The direct interaction of metal with cellular components can initiate variety of metabolic responses finally leading to a shift in the development of the plant (Van Assche and Clijsters, 1990). Cr toxicity produces chlorosis and necrosis in plants (Cervantes *et al.*, 2001). Several polluting metals and compounds are discharged into the water streams by tanneries.

Cr (VI) is highly soluble and more bio-available than the less soluble Cr (III). Cr (> 2 ppm) has been reported to be inhibitory for plant growth. In addition, its presence in excess amount within the plant causes stunted growth, poor development of root and discoloration of the leaves (Pratt, 1966). Cr toxicity in plants leads to reduction in photosynthesis and changes in chloroplast structure (Vazquez *et al.*, 1987). Cr phytotoxicity exhibits inhibition of seed germination, degraded pigment status and nutrient imbalance as a consequence of which the yield and productivity of crop is greatly affected (Barcelo and Poschenrieder, 1997). Transport of Cr from the root is very slow (Skeffington *et al.*, 1976), accounting for the low levels of Cr in the apical part of plants. Evidently, the element enters the vascular tissue with difficulty; but once it enters, it can be rapidly transported. Toxic heavy metals are mostly absorbed and get accumulated in various plant parts as free metals which may adversely affect the plant growth and metabolism (Barman and Lal, 1994) and their accumulation is biomagnified at different trophic levels through food chains (Rai *et al.*, 2002).

The use of industrial effluents for irrigation has emerged in the recent past as an important way of utilizing waste water, taking the advantage of the presence of considerable quantities of N, P, K and Ca along with other essential nutrients (Niroula, 2003).

But there can be both beneficial and damaging effects of waste water irrigation on crops including vegetables (Saravanmoorthy *et al.*, 2007; Raman *et al.*, 2002). Therefore, it is necessary to study the impact of these effluents on crop system before they are recommended for irrigation (Thamizhiniyan *et al.*, 2009).

Much research has been conducted to determine the toxic effects of Cr on different plant species. However, little information is available on the ecological effects and toxicity of tannery effluents on some of the pulse plants (Leguminaceae), which are widely cultivated around the world as crop species. The heavy metals accumulated by pulse plants can threaten the health of human and cattle by entering their food chain as food and fodder respectively.

One such crop is cowpea (*Vigna unguiculata*), which is very rich in protein and one of the most economically and nutritionally important indigenous legume produced throughout the tropical and sub-tropical parts of the world. The present investigation has been carried out to study the effect of UTE and TE collected from the Common Effluent Treated Plant (CETP) on seed germination and early growth of *V. unguiculata.*

MATERIALS AND METHODS

The effluent samples for the present study were collected from a tannery before and after they passed through a common Effluent Treatment Plant (CETP) and referred to as untreated tannery effluent (UTE) and treated tannery effluent (TE) respectively. Prior to sample collection, the sample bottles were thoroughly washed with acid and then with distilled water and air dried. The samples were collected from tanneries located at Ranipet, Tamilnadu, India. The effluent samples thus, collected after labeling were stored at 4°C until further use to avoid changes in its characteristics. The effluent samples were tested at the Tamil Nadu Pollution Control Board, Chennai, India for their physicochemical properties. Seeds of *V. unguiculata* were procured from the Agricultural Co-operative society, Tamil Nadu, India. The healthy and uniform seeds were cleaned with normal water and then surface sterilized with 0.1% $HgCl_2$ for few minutes. Then, the seedlings were continuously washed with distilled water for the removal of $HgCl_2$ traces. Ten seeds were placed equidistantly on soaked filter paper spread on cotton beds in petri plates. The seeds were irrigated with equal quantity of different dilution of effluent samples and the seeds irrigated with distilled water were taken as control. The number of seeds germinated was recorded after 48hrs. The germination percentage, root length, shoot length, vigour Index, phytotoxicity percentage, fresh weight were recorded after seven days and dry weight of seedlings was recorded after keeping them in hot air oven at 80˚C for 24 hrs.

Statistical Analysis

The data were analyzed by the Students 't' test as described by Fischer (1950) and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The basic tool for the evaluation of complex industrial waste toxicity is the physico-chemical analysis and toxicological bioassay. The UTE and TE contained much higher amount of total dissolved solids (TDS), BOD and COD due to extensive use of salts mainly sodium chloride, sodium sulphate, Cr, etc. during transformation of raw hide/skin into leather. The high TDS is due to use of large quantities of salts. Salts are very much soluble in water and chemically stable under aquatic conditions, making it effectively impossible to remove them easily from effluent. The higher TDS is toxic to aquatic life by causing osmotic stress and affecting the osmoregulatory function of the organisms (McCulloch *et al.,* 1993).

High BOD indicates poor dissolved oxygen (DO) in the effluent. On the other hand, high COD is due to high amount of organic compounds in the effluent which is not affected by the bacterial decomposition. Although, the Cr concentration in the effluent was 3.5 mg/L, but continuous discharge of Cr in effluent even at low concentration can cause toxic effect to aquatic life by disrupting food chain (Fent, 2004; Kannan and Upreti, 2007). Due to the presence of various chemicals in industrial wastewater, chemical and physical tests alone are not sufficient when assessing the potential effect of wastewater in aquatic and terrestrial biota. Environmental toxicity assessment of industrial wastewater was evaluated with a range of test models which include bacteria, algae, plants, and fishes (APHA, 1998). However, the use of plant seed germination test to evaluate toxicity of industrial effluents has been considered as one of the simplest and short-term methods (Wang, 1990; Wang and Keturi, 1990a). Any inhibitory substance in the growing environment can affect the seed germination process. The results of physicochemical analysis of effluent samples are presented in Table 1. The effluent as such appeared dark grey in color with a foul smell. UTE showed several fold increased BOD and COD values compared to TE, which showed decreased level of pollutants except sulphates. Results in Table 1 though, showed a reduction in the level of pollution parameters to a large extent, several parameters are far greater than the permissible limits in the TE. Cr, for instance, showed a 16.6 fold decrease after treatment, but it is still 70 times higher than the permissible values. The pattern of seedling growth in control and different concentrations of effluents after 24 and 72 hrs is given in Figure 1 & 2 and Table 2. The various treatments of UTE and TE were found to be toxic to most growth parameters, because germination percentage, shoot length, root length and vigour index were affected. Germination percentage, root and shoot length and vigour index decreased in all TE and UTE treated groups. However, fresh and dry weight was increased in all UTE and TE groups except 50% UTE group with marginal decrease. Our findings are also in accordance with the results obtained by Singh and Swami (2014) on the effect of utilization of distillery industry wastewater for feasibility and toxicity assessment using *Zea mays* and *Triticum aestivum*. The decrease in germination percentage, root length and shoot length of seedlings treated with different dilutions of effluent concentration over the control was attributed to the presence of various chemicals present in the effluents. This may be due to change in the osmotic relationship of the seed and water. The seeds require higher amount of water uptake during the germination under the salt stress due to the accumulation of the soluble solutes around the seeds which increases the osmotic pressure. This causes excessive uptake of the ions which results in toxicity in the plant (Jones, 1986). These observations are in conformity with previous report on the effect of tannery effluent on mung bean (*Vigna radiata*) (Kumari *et al.*, 2014).

Seed germination is in fact a cumulative consequence of many physical, physiological, biochemicals, cellular and molecular events rendering the radicle able to emerge from the seed. Seeds are complimented with intracellular bodies of lipids, proteins, carbohydrates, organic phosphate and various other inorganic compounds, which facilitate the process of germination and the growth of the seedlings. These compounds are consumed during the course of germination with the help of biocatalysts like hydrolases, lipases, proteinases and phosphatases, which are released and/or synthesized *de novo* to facilitate the availability of simpler substances to the embryo, for its growth. However, in the present research, treating the seeds with different dilutions of tannery effluents resulted in a sharp decline of germination and seedling growth. The inhibitory effect of these effluents on early growth is not surprising since tannery effluents at lower and higher concentration on seed germination and early seedling growth have been reported (Mishra and Bera, 1995; Kannan and Upreti, 2008).

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Table 1: Physicochemical Analysis of Tannery Effluents

Sl. No.	Parameters	Untreated Effluent (UTE) Values	Treated Effluent (TE) Values	Discharge Permissible Limit
1	Color	Dark grey	Light yellow	-
2	Odor	Foul	-	-
3	pH	7.10	7.92	-
4	Total Suspended Solids (mg/L)	1032	186	-
5	Total Dissolved Solids (mg/L)	8640	7670	-
6	Total Solids (mg/L)	9856	7924	-
7	Sulphate (mg/L)	2675	2939	750
8	Chloride (mg/L)	1831	1685	750
9	BOD (at 27°C for 3 days) (mg/L)	1350	66	40
10	COD (mg/L)	4000	624	120
11	Nitrate (mg/L)	0.35	0.28	-
12	Nitrite (mg/L)	<0.02	0.34	-
13	Carbonate (mg/L)	<1	140	-
14	Bicarbonate (mg/L)	1370	540	-
15	Sodium Absorption ratio	23	20	-
16	Turbidity (NTU)	800	25	-
17	Total Hardness (mg/L)	1450	1290	-
18	Total Kjeldahi Nitrogen (mg/L)	224	252	25
19	Total Chromium (mg/L)	56.4	3.5	0.05
20	Copper (mg/L)	27.9	10.1	0.50
21	Iron (mg/L)	516.6	328	2.0
22	Zinc (mg/L)	15.8	13.2	2.0

Table 2: Germination Parameters of Seven Days Old Seedlings (*Vigna Unguiculata*)

Groups	Germinat -ion %	Root Length cm	Shoot Length cm	Vigour Index	Phytotoxic -ity %	Fresh Weight g	Dry Weight g
Control	97 ± 1.9	9.6 0 ±1.4	8.60 ± 0.8	695 ± 41	-	0.65 ± 0.03	0.17 ± 0.03
25 % TE	91 ± 1.8	6.47 ± 0.4	4.77 ± 1.1*	602 ± 13	40.9 ± 9.4	1.26 ± 0.05*	0.33 ± 0.04*
50 % TE	87 ± 2.7*	3.70 ± 0.2*	6.20± 0.64*	493± 14*	62.0 ± 7.7	0.75 ± 0.06	0.19 ± 0.01
75 % TE	84 ± 1.8*	9.77 ± 2.0	7.33 ± 1.1	247 ±11*	37.8 ± 1.3	1.79 ± 0.07*	0.39 ± 0.01*
100 % TE	64 ± 3.5*	4.13 ±0.2*	4.80± 0.3*	151±5.1*	63.3 ± 3.5	1.45 ± 0.06*	0.26 ± 0.04
25 % UTE	78 ± 1.8*	5.93 ± 0.8	6.07 ± 2.0	224±8.1*	48.9 ± 3.4	1.20 ± 0.08*	0.21 ± 0.02
50 % UTE	67 ± 3.1*	5.03 ± 0.7*	6.60 ± 0.5	121±2.5*	54.2 ± 9.2	1.90 ± 0.16*	0.16 ± 0.02
75% UTE	58 ± 1.8*	3.63 ± 0.21*	5.23 ± 0.9*	37.2± 1.4*	52.8 ± 5.7	2.12 ± 0.03*	0.42 ± 0.02*
100 % UTE	40 ± 3.1*	2.83 ± 0.2*	2.90 ± 0.5*	15.3± 3.5*	75.1 ±1.3*	1.24 ± 0.08*	0.24 ± 0.03

Values are mean of three replicates ±SE
Statistically significant at 0.05 level.

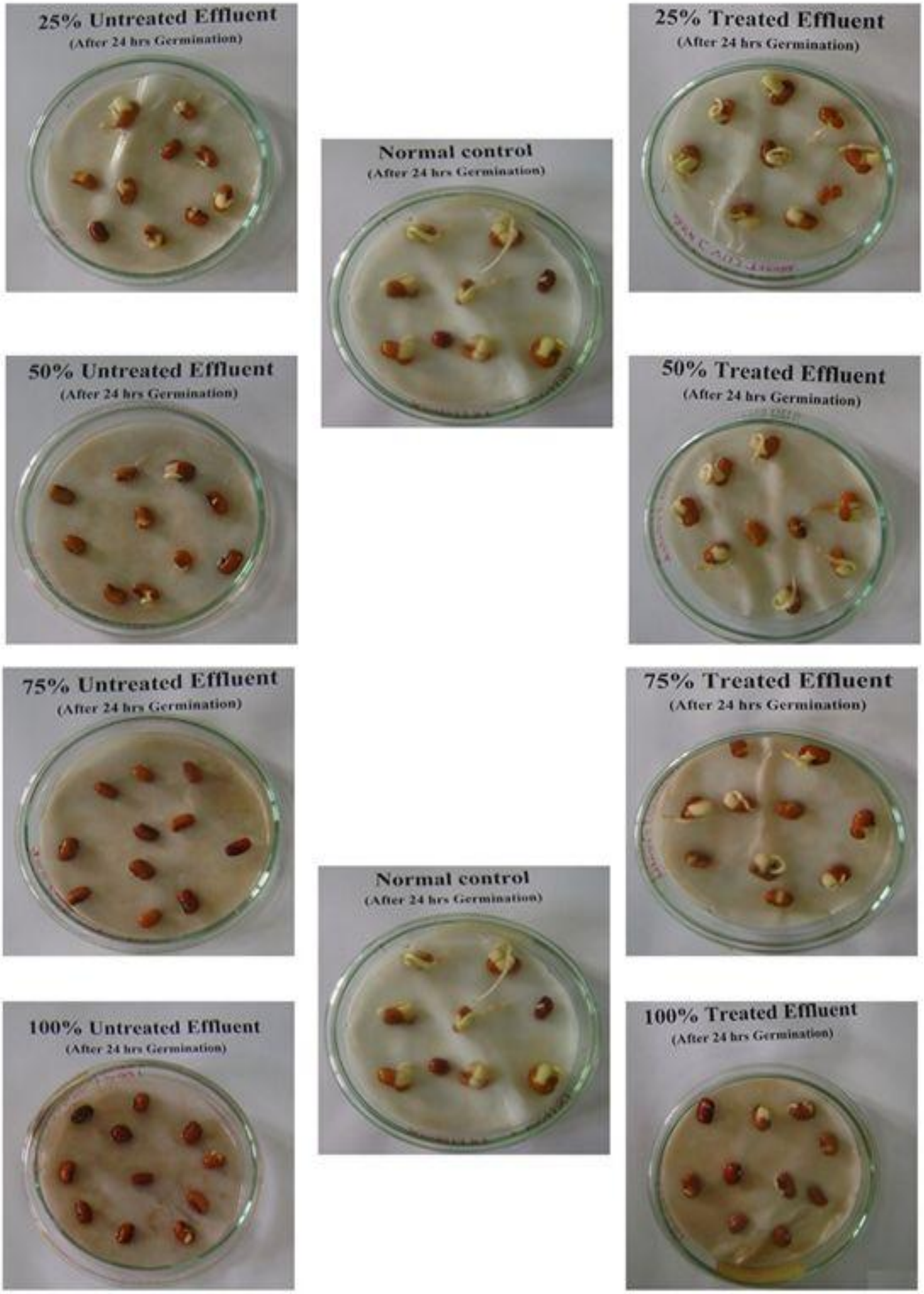

Figure 1: Effect of Different Dilutions of UTE and TE on *V. unguiculata* Germination after 24 Hrs

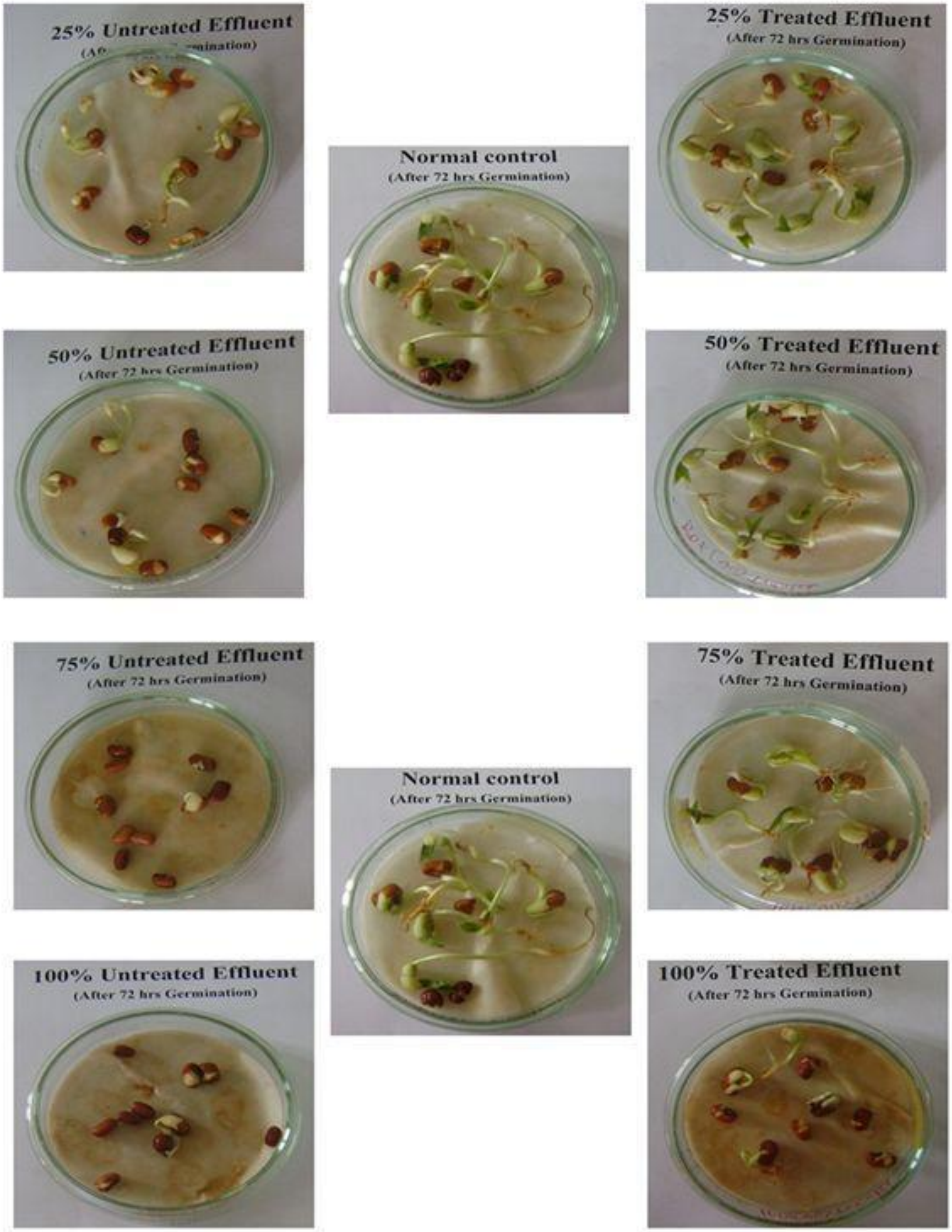

Figure 2: Effect of Different Dilutions of UTE and TE on *V. unguiculata* Germination after 72 Hrs

The inhibition of seed germination may be due to high level of dissolved solids, which enrich the salinity, and conductivity of the absorbed solute by seed before germination (Gautham and Bishoni, 1992). The increased salinity decrease the osmotic potential to such an extent, that retard or prevent the uptake of water necessary for germination or by toxic effects of ions on embryo viability (Llanes *et al.*, 2005). The salt content outside the seed is also known to act as limiting factor and causes less absorption of water by osmosis and inhibit the germination of seeds (Malla and Mohanty, 2005). It has also been reported that salts stress either alter metabolic pattern or does not permit the synthesis of enzymes required for seed germination (Ashraf *et al.*, 2002). Other possibility of inhibition of seed germination at higher concentration may be due to the presence of Cr in the effluent which concomitantly affected á-amylase activity in germinating seeds, as documented by previous reports in various plants (Zeid, 2001; Nath *et al.*, 2008).

Conclusion

The physico-chemical analysis of both untreated tannery effluent (UTE) and treated tannery effluent (TE) revealed that it is highly polluted and the quality can be improved by proper wastewater treatment to bring down pollutant level within tolerable limits for reuse and irrigation. This study showed that UTE and TE prevented germination of *V. unguiculata* seeds along with reduction of shoot length, root length at different concentration due to presence of variety of toxic substances. Since, UTE and TE of Central Effluent Treatment Plant (CETP), Ranipet carries higher amount of salts and Cr it may interfere with the metabolic activities of the plants irrigated with it. The benefits of wastewater use in irrigation are numerous but precautions should be taken to avoid short and long-term environmental risks.

REFERENCES

Ashraf MY, Afaf R, Qureshi MS, Sarwar G and Naqvi MH (2002). Salinity induced changes in alpha-amylase and protease activities and associated metabolism in cotton varieties during germination and early seedling growth stages. *Acta Physiologiac Plantarum* **24**(1) 37-44.

APHA (American Public Health Association) (1998). *Standard Methods for the Examination of Water and Wastewater*, 18th edition, (American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C. New York, USA).

Barcelo J and Poschenrieder C (1997). Chromium in plants. In: S. Carrali, F. Tittarelli, P. Sequi (edition) *Environmental Issues*, (Francotangati Angeli, Italy) 102-129.

Barman SC and Lal MM (1994). Accumulation of heavy metals (Zn, Cu, Cd and Pb) in soil and cultivated vegetables and weeds grown in industrially polluted fields. *Journal of Environmental Biology* **15** 107-115.

Cervantes C, Campos Garcia J, Debars S, Gutierrez Corona F, Loza Tavera H, Torres Guzman JC and Moreno-Sanchez R (2001). Interaction of chromium with micro genesis and plants. *FEMS Microbiology Review* **25**(3) 335-347.

Chakraborty RN, Singh M, Khan AQ and Saxena KL (1965). An industrial waste summery report. Part 1: Tanneries of Uttar Pradesh. *Environmental Health* **7** 235.

Fent K (2004). Ecotoxicological effect at contaminated sites. *Toxicology* **205**(3) 223-240.

Fischer RA (1950). In: *Statistical Methods for Research Workers*, 11th edition, (Oliver and Boyd, London, UK).

Gautham DD and Bishoni S (1992). Effect of Dairy effluent on wheat (*Triticum aestivum*). *Journal of Ecobiology* **4**(2) 111-115.

Jones RA (1986). High salt tolerance potential in Lycopersicon species during germination. *Euphytica* **35**(2) 575-582.

Kannan A and Upreti RK (**2007).** Impact of untreated and treated tannery effluent on seed germination and growth characteristics of mung bean (*Vigna radiata*). *Toxicology International* **14** 73-81.

Kannan AR and Upreti RK (2008). Influence of distillery effluent on germination and growth of mung bean (*Vigna radiata*) seeds. *Journal of Hazardous Materials* **153**(1-2) 609-615.

Kumari V, Kumar S, Haq I, Yadav A, Singh VK, Ali Z and Raj A (2014). *International Journal of Latest Research in Science and Technology* **3**(4) 165-170.

Llanes A, Reinoso H and Luna V (2005). Germination and early growth of *Prosopis strombulifera* seedlings in different saline solutions. *World Journal of Agricultural Science* **1**(2) 120-128.

Malla L and Mohanty BK (2005). Effect of paper mill effluent on germination of green gram (*Phaseolus aureus* Roxb.) and growth behavior of seedlings. *Journal of Environmental Biology* **26** 379-382.

McCulloch WL, Goodfellow WL and Black JA (1993). Characterization, identification and confirmation of total dissolved solids as effluent toxicants. *Environmental Toxicology and Risk Assessment*, **2**, STP 1216. J.W. Gorsuch, F.H. Dwyer, C.J. Ingersoll and T.W Lapont. Edition, (American Society for Testing and Materials, Philadelphia, P.A, USA) 213-227.

Mishra P and Bera AK (1995). Effect of tannery effluent on seed germination and early seedling growth in wheat. *Seed Research* **23** 129-131.

Nath K, Singh D, Shyam S and Sharma YK (2008). Effect of chromium and tannery effluent toxicity on metabolism and growth in cowpea (*Vigna sinensis* L. *Saviex Hassk*) seedling. *Research in Environment and Life Science* **1**(3) 91-94.

Nath K, Singh D, Verma A and Sharma YK (2009). Amelioration of tannery effluent toxicity in radish (*Raphanus sativus*) based on nutrient application. *Research in Environment and Life Sciences* **2**(1) 41-48.

Niroula B (2003). Comparative effects of industrial effluents and sub-metropolitan sewage of Biratnagar on germination and seedling growth of rice and Black gram. *Our Nature* **1** 10-14.

Pratt PF (1966). *Diagnostic Criteria for Plant and Soil*, H.D. Chapman (edition) (University of California, Division of Agricultural Science, River Side, California, USA) 115.

Rai UN, Tripathi RD, Vajpayee P, Jha VN and Ali MB (2002). Bioaccumulation of toxic metals (Cr, Cd, Pb and Cu) by seeds of *Euryale ferox salisb* (Makhana). *Chemosphere* **46**(2) 267-272.

Raman S, Biswas AK, Kundu S, Saha JK and Yadav RBR (2002). Effect of distillery effluent on seed germination in some vegetable crops. *Bioresource Technology* **82**(3) 273-275.

Saravanmoorthy MD and Ranjitha Kumari BD (2007). Effect of textile waste water on morpho physiology and yield on two varieties of peanut (*Arachis hypogea* L.). *Journal of Agricultural Technology* **3**(2) 335-343.

Singh SV and Swami VK (2014). Utilization of distillery industry wastewater as liquid bio fertilizer: Seed bioassay Test for feasibility and toxicity assessment. *International Journal of Innovative Research in Science, Engineering and Technology* **3**(10) 17020-17027.

Skeffington RA, Shewry PR and Peterson PJ (1976). Chromium uptake and transport in barley seedlings (Hordeum vulgare L.). *Planta (Berl.)* **132**(3) 209 – 214.

Thamizhiniyan P, Sivakumar PV, Lenin M and Sivaraman M (2009). Sugar mill effluent toxicity in crop plants. *Journal of Phytology* **1**(2) 68-74.

Van Assche F and Clijsters H (1990). Effects of metals on enzyme activity in plants. *Plant Cell and Environment* **13**(3) 195-206.

Vazquez MD, Poschenriender C and Barcelo J (1987). Chromium (VI) induced structural and ultrastructural changes in bush bean plants (*Phaseolus vulgaris L*.). *Annals of Botany* **59**(4) 427-438.

Wang W (1990). Toxicity assessment of pretreated industrial wastewaters using higher plants. *Research Journal of the Water Pollution Control Federation* **62** 853-860.

Wang W and Keturi PH (1990a). Comparative seed germination tests using ten plant species for toxicity assessment of metals engraving effluent sample. *Water Air Soil Pollution* **52**(3-4) 369-376.

Zeid IM (2001). Responses of *Phaseolus vulgaris* chromium and cobalt treatments. *Biologia Plantarum* **44**(1) 111-115.