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ANALYSIS OF PHYSICOCHEMICAL PARAMETERS OF TANNERY EFFLUENT TREATED WITH COIR PITH AND NAVA RASA KARAISAL AND STUDY OF TOXICITY ON PLANTS

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The raw tannery effluent discharged from tannery industries into river, lakes and coastal areas (Tiham *et al.,* 2018) poses a serious threat in terms of health, environmental hazard and hence resulting in instability of ecosystem. In India, tannery industries consume a significant amount of water in their manufacturing processes and it has been rated as the most polluting among the industrial sectors (Vijayanand and Hemapriya, 2014). It is frequently referred to as a pollutant-producing industry that creates a wide range of extremely harmful substances. Due to its high chemical content, which includes salinity, organic load, inorganic matter, dissolved solids, suspended particles, ammonia, and nitrogen, tannery waste is known to constitute a major environmental danger. The tannery waste also contains specific contaminants such as heavy metals, sulphide, chromium, chloride, sodium, and other salt residues (Yang *et al.,* 2022).The wastewater from tanneries contains acids, alkali, sulphides, surfactants, and dyes that are used to transform a hide into leather (Mariliz *et al.,* 2014). The release of this untreated tannery effluent into water bodies and soil alters their chemical and biological characteristics, which in turn results in decreased levels of available organic materials and dissolved oxygen (Noorjahan, 2014; Lakshmi *et al.,* 2015).

In coming years, threatening the very basic socio-economic development of the country with a day-by-day increase in pollution and over exploitation (Mani and Madhusudanan, 2016). Moreover, the heavy metals present in tannery effluent inhibit the seed germination and make it unsuitable for crop growth (Ayyasamy and Kaviyarasan, 2011).Thus, arises the necessity to treat the tannery effluent before discharging it into the environment. Although numerous physical and chemical methods are not sufficient when assessing the potential effects of wastewater, the biological methods are considered to be eco-friendly and cost effective (Noorjahan, 2014).

Coir pith is a dark brown color, fluffy material and a lignocellulosic waste extracted from the fiber of coconut husk. It is dumped on roadsides and in industries (Paramanantham and Ronald, 2016). Due to its high lignocellulosic bonding, it has the property of slow degradation in a natural environment, but many microbes have the ability to decompose of coir pith (Malliga and Viswajith, 2005; Malliga *et al.,*2013; Vadivudai *et al.,*2015; Tharani *et al.,* 2019). Additionally, coir pith possesses an outstanding adsorption capacity, reduces the amount of phenolic compounds entering the water bodies, and improves water infiltration and nutrient availability to crops (Kalaibharathi *et al.,* 2019). The use of coir pith is a good source of mulch for increasing water holding capacity in summer tomato cultivation (Hossain *et al.,* 2012). The largest area of coconut production occupies South Indian states, especially Kerala, Karnataka, and Tamil Nadu (Shiva Kumar *et al.,* 2017). The physicochemical characteristics of tannery effluent and the chromium levels are both dramatically decreased by the addition of coir pith (Hashem *et al.,* 2021).

Nava Rasa Karaisal (NRK) is a microbial consortium containing bacteria, fungi, actinomycetes, algae, and protozoa that act as decomposers in ecosystem (Kalaibharathi *et al.,* 2019).The bacteria (particularly indigenous (or) exogenous bacteria) and fungi population present in NRK reduce the levels of heavy metal and contaminants present in tannery wastewater (Sudhangshu *et al.,* 2015). Since NRK is prepared from the ingredients of traditional Indian rituals it poses the ability to enrich the soil and plant by providing the nutrients required for growth and also effective in protecting the young roots from soil-borne and seed-borne diseases (Kalaibharathi *et al.,* 2019). NRK-containing bacteria and coir pith applied together would function both as an adsorbent as well as an effective absorbent during the tannery waste treatment process (Josepine *et al.,* 2020).

The present study was conducted to investigate the analysis of physico chemical parameters such as pH, temperature, electrical conductivity (EC), salinity, TDS, Mg, Ca and NO_3 in untreated and treated tannery effluent. Later, the effect of treated tannery effluent on seed germination and also analyze the morphometric

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parameters when used as a source of foliar spray on *Vigna radiata* L.

Collection of samples

The tannery effluent was collected from a tannery industry near Sempattu, and coir pith was collected from coir industries near Srirangam, Tiruchirappalli, Tamil Nadu, India (Fig. 1 and 2).

Preparation of Nava Rasa Karaisal

NRK (10 L) was made by combining five cow-based products, such as cow dung (200 g), urine (200 mL), curd (200 mL), ghee (10 mL), and four other ingredients: bananas (two pieces), besan flour (200 g), jaggery (200 g), and a handful of soil. The preparation was placed in a shaded place for 10 days and stirred well twice a day (morning and evening) for fermentation to take place. The barrel was covered with a wire mesh or net to avoid contamination from other sources (Kalaibharathi *et al.,* 2019) (Fig. 3).

Different treatments of tannery effluent

Different treatments were carried out such as 900mlTN+100mlNRK, 900ml TN+20g CP and 900ml TN+20g CP+100mlNRK in haffkine flask and incubated for 4 days. After the incubation period, the filtrate was collected on 4th day and mixed with tap water at various dilutions of untreated and treated tannery wastewater, viz., 12.5%, 25%, 50%, 75%, and 100% for further study.

Analysis of physicochemical parameters

The important physico-chemical parameters such as pH, temperature, Electrical Conductivity (EC), Total Dissolved Solids (TDS) and salinity were analyzed by Systranics Water analysis-371 in untreated and treated tannery effluent. Magnesium and calcium were analyzed by AOAC method (2000) & Nitrate was analyzed by APHA (1998).

Seed germination

The seed germination study was carried out in mung bean (*Vigna radiata* L.) using five different concentrations (viz., 12.5%, 25%, 50%, 75% and 100%) of untreated and treated tannery effluent. The seed germination and seedling growth was carried out in glass petridish using filter paper (20mm X 120mm) with thin layer of cotton at the bottom. Each petridish contained 10seeds and 5ml of untreated and treated tannery effluent dilutions and incubated at 28°C in dark conditions for 3 days.

The percentage of seed germination was observed in each test solution at 24h interval. All the experiments were done in triplicate and mean values were reported. The germination percentage was calculated using the below formula;

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Toxicity study

Sterilized soil was taken in separate plastic cups (100 g/cup) and mixed with five different concentrations (12.5%, 25%, 50%, 75%, and 100%) of treated tannery effluent and 100% of untreated tannery effluent, as well as a water control. At 24 h intervals, 20 ml of untreated and treated tannery effluent were sprayed into the respective cups. On the 5th day, morphological parameters of *Vigna radiata* L. were measured, such as growth percentage, shoot length, and root length. The average values of each experiment were tabulated after being carried out in triplicate.

In the current study, an effort was made to use coir pith and NRK to treat the tannery effluent and turn it into liquid fertilizer. After 4 days, the filtrate was collected from the treatments, and the color and odor were noted for further study (Fig. 4). The first indicator of impurities in wastewater that detract from the aesthetics of water bodies is typically their color (Josepine *et al.,* 2020). The control tannery's initial color was a dusky brown and stayed the same up to the fourth day. Due to the presence of biodegradable organic components and a substantial amount of inorganic chemicals employed in the processing, including salt and chromium, the

Fig. 1. Tannery Effluent (TN)

Fig. 2. Coir pith (CP)

Fig. 3. NRK

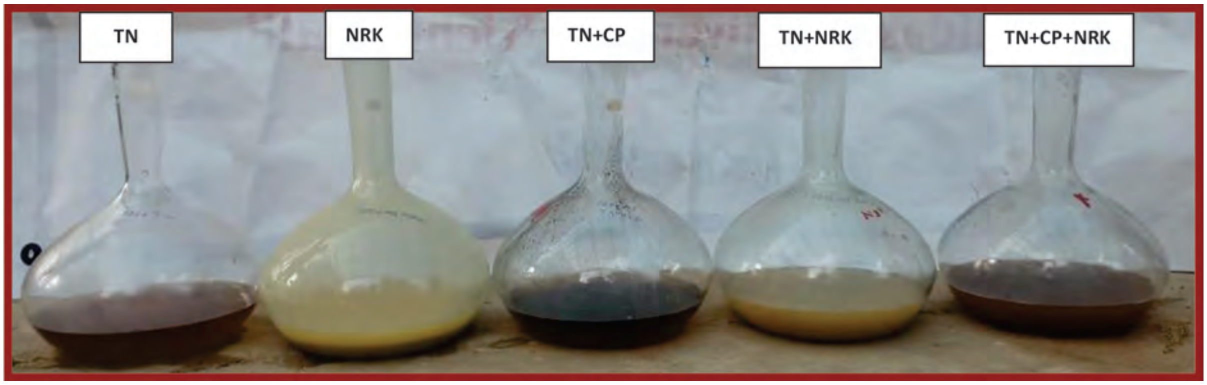

Fig. 4. Filtrate of treated tannery effluent (4th day)

effluent is colored. The putrefaction of the organic leftovers from the processed skin and hides causes the stench (Zereen *et al.,* 2013).

Analysis of physicochemical parameters

Physico-chemical parameters of the present investigations are given in Fig. 5a-h for different treatment of tannery effluent compared with untreated tannery effluent. Environmental pollution will result from the discharge of untreated tannery wastewater with an unbalanced pH. After 4 days, the pH changed in all the treated tannery effluent, especially the pH values, which changed from initial (5.3) to final (6.38) in treated tannery effluent (TN+CP+NRK) (Fig. 5a).This could be related to the accumulation of organic acids and it also indicates the efficacy of the bacteria to biodegrade the effluent. The pH plays a large and critical role in microbial metal uptake during the breakdown of contaminants. Low or high pH tannery effluent discharge into the environment will harm the environment (Amaro de Sales *et al.,* 2021). Arasappan and Rajagopal (2015) were reported that, the neutral pH of treated tannery describes the balance of alkaline/acidic nature which is present in untreated tannery effluent. According to Arifa *et al.* (2013), various tannery effluent dilutions with water produced superior pH results of 6.9 in 40% and 7.7 in 60%. The anomalous pH of tannery effluent was similarly seen in the low-level and high-level dilution. Pretreated tannery effluent was collected at the Common Effluent Treatment Plant (CETP), Dindigul, and the pH level was found to be neutral (7.6). The pH was found to be 7.5 in 50% of the tannery effluent after diluting it with household wastewater. Low or high pH values make the water unfit for irrigation and the soil alkaline, resulting in poor crop growth and yield (Balasubramanian and Dhevagi, 2016).The temperature has reduced in all the treated tannery effluent and control on the final day (4th day) when compared to the initial day (Fig. 5b). On the 4th day, a high level of EC content was found in untreated tannery effluent (38.6µS/cm), while treating with coir pith and NRK, a reduction of EC was recorded (34.5 µS/cm) (Fig. 5c). A gradual reduction was observed in all the treatments of tannery effluent with coir pith (34.6µS/cm) and NRK (36.5µS/cm) and the minimum amount of EC was noted in the Nava Rasa Karaisal (7.57 µS/cm). These results may represent the large amounts of salts reduced during the tannery effluent treatment with coir pith and NRK. The increasing EC was recorded in different dilutions of tannery effluents; that are the highest EC (3.5 ms/cm) in 100% of tannery effluent and the lowest (0.75 ms/cm) in the pretreated tannery effluent (Arifa *et al.,* 2013). Balasubramanian and Dhevagi (2016) reported that the pretreated tannery effluent from the tannery industry contains 9.2 ds/m^{-1}, after being treated with domestic wastewater, a reduction of EC was observed (6.4 ds/m^{-1}).

Initially, a high content of salinity (20.3ppt) was found in untreated and treated tannery effluent (19.9ppt). After the incubation period, the salinity was found to be reduced (13.4 ppt) in treated tannery effluent (Fig. 5d). The addition of coir pith and microorganisms may be used to reduce the salt content which is present in the raw tannery effluent. Tanning waste's salinity may increase due to the presence of dissolved contaminants including Na+, Cl-, and NO-, making it unsuited for irrigation or consumption (Nosheen *et al.,* 2000). The pretreatment procedure of the effluent has little effect on the salts, and consequently continues to harm the ecosystem (Sampathkumar, 2001). The soil becomes more alkaline as a result of tanning chemicals including calcium chloride, sodium chloride, sodium carbonate, and sodium bicarbonate, which increases the pH of the soil (Mondal *et al.,* 2005).

On the 4th day, the maximum reduction of TDS was observed in the treated tannery effluent using coir pith and NRK (19.3 mg/L) when compared with the raw tannery effluent (22.9 mg/L) (Fig. 5e). Due to the presence of dissolved organic and inorganic salts such chloride, sodium, nitrates, nitrites, carbonate, bicarbonates, sulphate, and phosphates in high levels

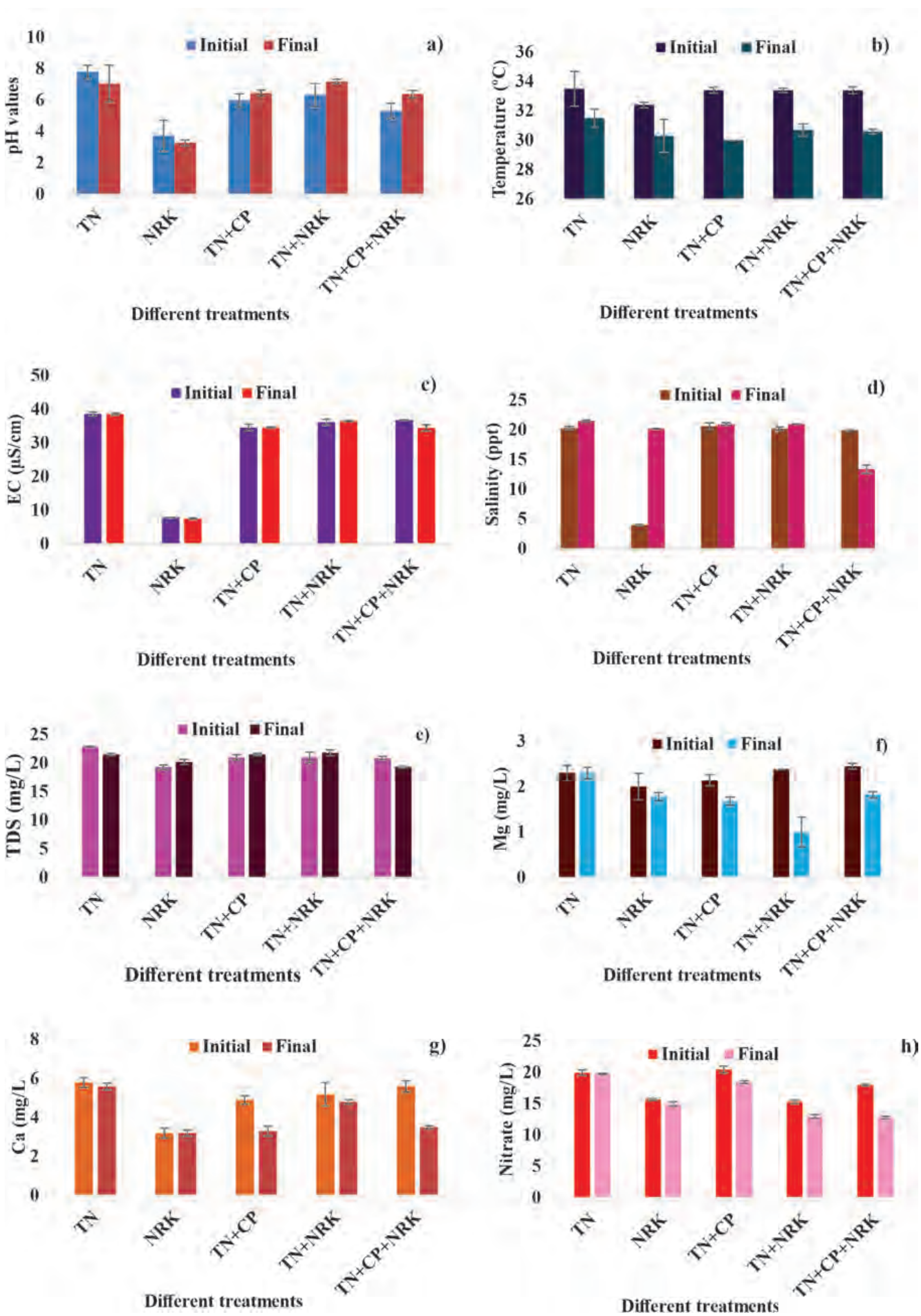

Fig. 5. (a-h) Physico-chemical analysis of untreated and treated tannery effluent a) pH, b) Temperature, c) Electrical conductivity (EC), d) Salinity, e) Total Dissolved Solids (TDS), f) Magnesium (Mg), g) Calcium (Ca), h) Nitrate (No_3)

of TDS, tannery waste should not be used as a plant fertilizer. The TDS of the effluent before the treatment was 406 mg/L, and the reduction of TDS (105 mg/L) occurred after the tannery was treated with the microbial consortium (Mythili and Karthikeyan, 2011). The raw effluent contained 200 mg/L, which was below the allowable limit; however, after treatment, the TDS level in 80% of the diluted tannery effluent increased to 4352 mg/L (Arifa *et al.,* 2013). The permissible limit of TDS (410 mg/L) was found in pretreated tannery effluent from Sharada industrial area, Kano (Ibrahim and Nafi, 2017). A high level of TDS (4950 mg/L) was detected in primary treated tannery effluent by coagulants collected from the sediment tanks of forward leathers, Nagalkeni, Chrompet, Chennai (Saritha and Meikandaan, 2013). The findings were similar to Akililu *et al.,* (2012), who reported that the pretreated tannery effluent that was collected from the outlet contained a permissible limit of TDS (319 mg/L).

The highest content of magnesium and calcium (2.3 mg/L and 5.8 mg/L respectively) were found in the untreated tannery effluent and low level (1.82 mg/L and 3.5 mg/L respectively) in the treated tannery effluent (Fig. 5f and 5g). The microorganisms that are present in the NRK could be able to digest the calcium and magnesium using coir pith during metabolic activities. Srinivas *et al.,* (2013) strongly reported a 50% and 48% reduction in calcium and magnesium, respectively, to the tannery effluent treatment with six bacterial sps. The minimum percentage (20%) of tannery effluent diluted with the maximum percentage (80%) of freshwater contains a low level of calcium and magnesium when compared to other diluted tanneries and 100% (Arifa *et al.,* 2013). Balasubramanian and Dhevagi (2016) studied that the tannery effluent has 5.0 meq/L of calcium and 6.2 meq/L of magnesium during the time of pretreatment; once it has been treated with domestic wastewater (DWW) in the ratios of 1:3, 1:1 and 3:1, the reduction of calcium and magnesium was found in the 1:3 ratio, which is 25% of TTE plus 75% of DWW. The results of the present study agreed with the findings of Ravishankar *et al.* (2015), who suggest that the calcium (328 mg/L) and magnesium (202 mg/L) were reduced with the addition of a microbial consortium. The similar study was done by Singh *et al*. (2014), Lakshmi *et al.* (2015), Arasappan and Kalyanaraman (2015) and Kalaibharathi *et al.* (2019). The nitrate was estimated in untreated and treated tannery on the initial and final day. A large amount of nitrate was observed in raw tannery effluent (20 mg/L) on the 4th day and the drastic reduction of nitrate was estimated in all the treated tannery effluent, particularly in the combined treated tannery using coir pith and NRK (12.8 mg/L) (Fig. 5h). According to Kewish *et al.* (2015), the tannery effluent

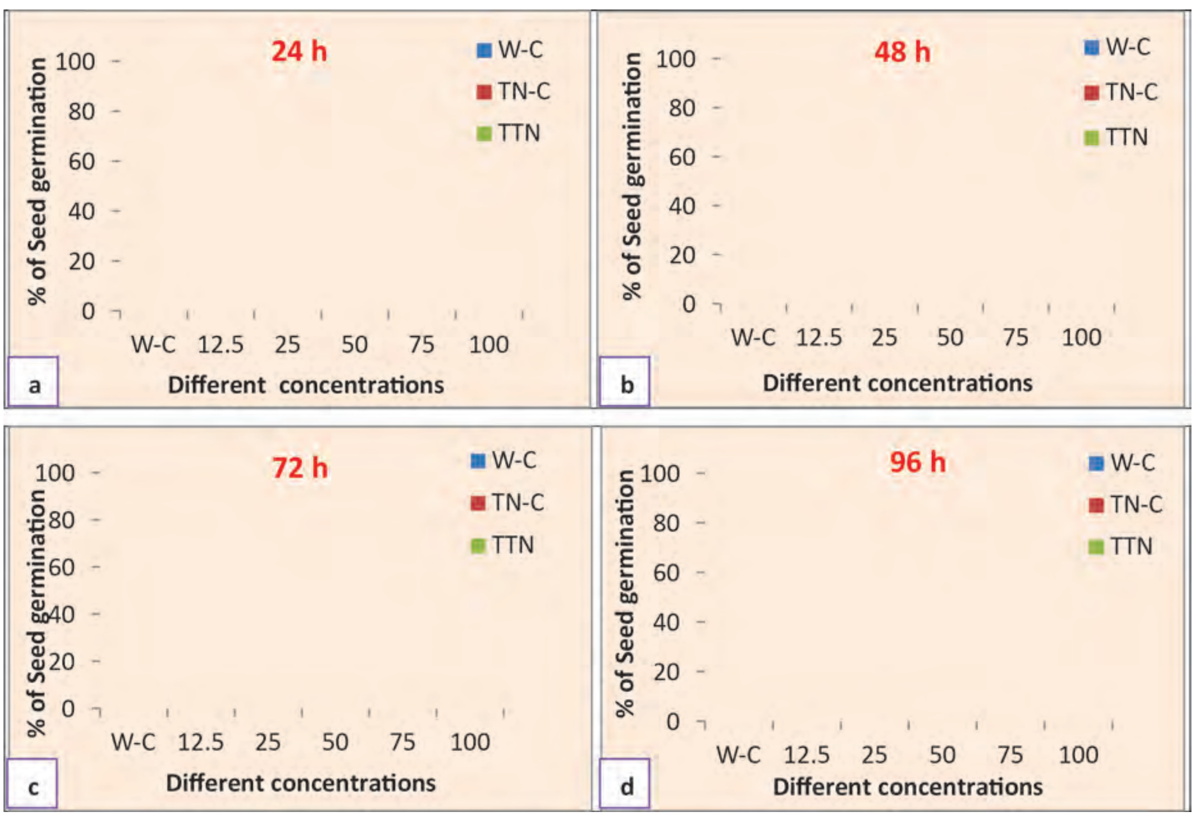

Fig. 6. (a-d) Effect of untreated and treated tannery effluent on seed germination (%) of *Vigna radiata* L.

contains the highest amount of nitrate (27mg/L) before the treatment; whereas in the present study the nitrate content was utilized by the microbial consortia could indicate the metabolic activities.

Seed germination of *Vigna radiata* L.

The various dilutions of untreated and treated tannery wastewater (12.5%, 25%, 50%, 75% and 100%) were prepared for analysis of seed germination on *Vigna radiata* and to observe the growth percentage at 24h intervals. The germination of green gram was started and showed a maximum in water control when compared to treated tannery effluent. The seeds germinate 100% and 80% in the treated tannery effluent at lower concentrations (12.5% and 25%, respectively), whereas the highest concentrations of 75% and 100% suppress the germination of seeds at 96h. The highest concentrations of raw tannery effluent (75 and 100%) inhibited the germination of seeds after 24h (Fig 6 a-d and Fig. 7). The increased concentration of untreated and treated tannery effluent decreased the percentage of seeds that germinated due to the heavy metal stress. The highest shoot and root length was recorded in water control and 12.5% followed by 25%, 50%, 75%, and 100% concentrations of effluent. The maximum shoot length (14.2 mm) and root length (26.8 mm) was found in treated tannery effluent (12.5%) when compared with untreated tannery effluent. The highest concentrations (75% and 100%) were found to arrest the shoot and root length in both untreated and treated tannery effluent (Table 1).Mythili and Karthikeyan, 2011 reported higher concentration of the tannery effluent proved to be toxic as there was 100% inhibition of seed germination**.** The lowest concentration (25%) of treated tannery effluent using *Lyngbya* sp. BDU 90901 and coir pith showed effective seed germination of *Vigna radiata* L. (Lakshmi *et al*., 2015).

According to Balasubramanian and Dhevagi (2016), seedlings treated with 75 times diluted tannery effluent had a higher germination percentage, while seeds treated with undiluted tannery effluent delayed in germination. As per Seal *et al.* (2015) all of the seeds grew better at a 20% effluent concentration, possibly as a result of the growth-promoting properties of the nitrogen and other mineral components found in the effluent. Singh *et al.* (2014) reported that the effects of different concentrations (5, 15, 25, 50, 75 and 100%) of diluted tannery effluent mixed with Ganga

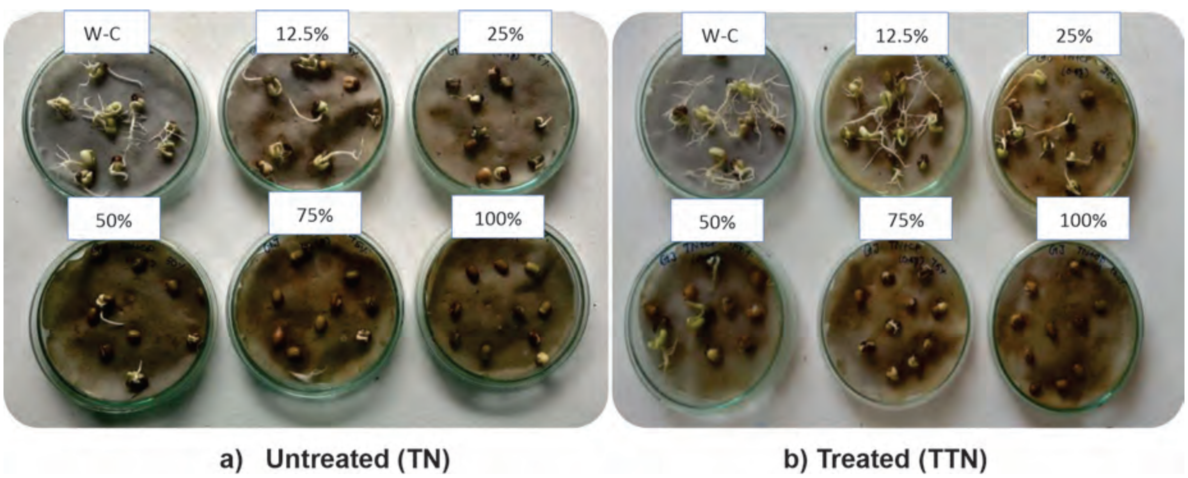

Fig. 7. Effect of untreated and treated tannery effluent on seed germination of *Vigna radiata* L. (96h)

Table 1. Effect of treated tannery effluent (TN+CP+NRK) on seed germination of *Vigna radiata* L. (96h)

S. No	Different dil. of TN & TTN (%)	Shoot length (mm±SD)		Root length (mm±SD)	
		TN	TTN	TN	TTN
1.	12.5	11 ± 1	14.2 ± 0.76	25 ± 1	26.8 ± 1.26
2.	25	5.5 ± 0.5	12.3 ±0.577	8.2 ± 1.25	16.8 ± 0.76
3.	50	2.3 ±0.57	7.2 ± 0.76	6 ± 1	7.2 ± 0.76
4.	75	-	2 ± 0.5	1.3 ± 0.57	2 ± 1
5.	100	-	-	-	-
6.	W-C	20.3 ± 0.76		34.6 ± 2.52	

W-Water, C-Control, TN-Tannery Effluent, TTN-Treated tannery, CP-Coir Pith,NRK-Nava Rasa karaisal; ± - Standard river water samples on the seed germination of *Vigna radiata* L. They concluded that the higher concentration (75% and 100%) of the tannery effluent inhibited the seed germination and growth. The different dilutions of tannery effluent (10%, 25%, 50%, and 100%) are used to stimulate the plant growth of radish (*Raphanus sativus* L.). The lowest concentration of the effluent (10%) exhibited the highest growth when compared to other concentrations studied by Kamlesh et al. (2005). Arbuscular Mycorrhizal (AM) fungi were used to study the germination of green gram (*Vigna radiata* L.) seeds at six different tannery effluent concentrations (control-AM, 50% effluent, 50%+AM, 100% effluent, and 100% + AM). Maximum shoot length, root length, number of leaves, leaf area, fresh weight, and dry weight measurements were obtained in AM + 50% treated effluent (Indira and Ravi, 2010).

Toxicity study on *Vigna radiata* L.

The toxicity study was carried out to observe the shoot length and root length of *Vigna radiata* seeds on 5th day (Fig. 8 and 9). The better results showed in 12.5%

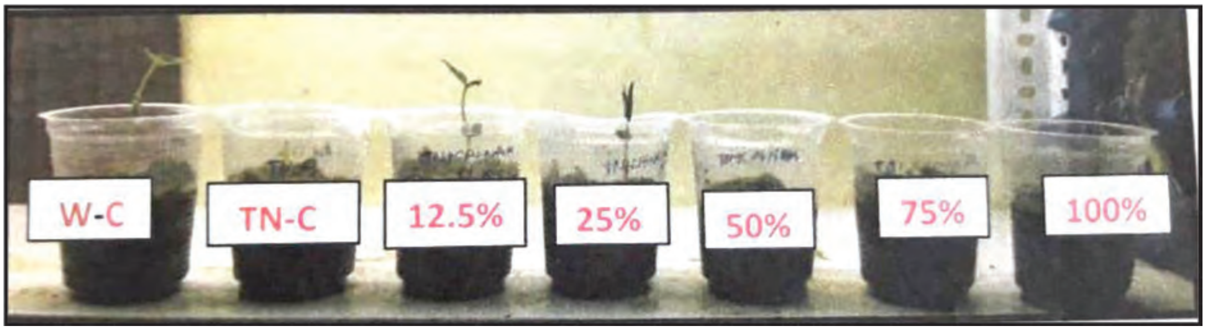

Fig. 8. Effect of treated tannery effluent (TN+CP+NRK) as irrigation on growth of *Vigna radiata* L. (5th day)

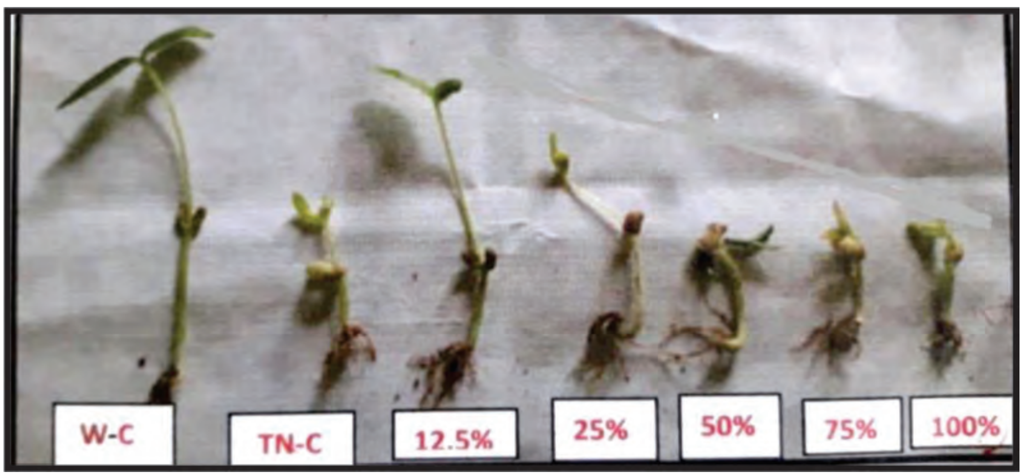

Fig. 9. Effect of treated tannery effluent (TN+CP+NRK) as irrigation on growth of *Vigna radiata*. L (5th day)

Table 2. Effect of treated tannery effluent (TN+CP+NRK) on growth of *Vigna radiata* L. (5th day)

S. No	Different dil. of TN+CP+NRK	Observation of growth (%)	No. of Leaves	Shoot length (cm)	Root length (cm)
1.	W-C	100	6	6 ± 0.81	1.16 ± 0.23
2.	TN-C	67	-	1.16 ± 0.23	1.16 ± 0.23
3.	12.5	67	2	5.3 ± 0.47	1.33 ± 0.23
4.	25	67	2	3.66 ± 0.47	1.73 ± 0.20
5.	50	67	2	2.16 ± 0.23	1.43 ± 0.32
6.	75	33	-	1.8 ± 0.23	1.33 ± 0.32
7.	100	33	-	1.16 ± 0.12	1.1 ± 0.29

W-Water, C-Control, TN-Tannery Effluent (100%), CP-Coir Pith, NRK-Nava Rasa karaisal; ± - Standard and 25% of combined (TN+CP+NRK) treated tannery effluent since the plants were healthy when compared to other concentrations. The highest shoot and root length were observed in water control (6 and 1.16cm) followed by (12.5%) treated effluent (5.3 and 1.33cm) when compared to tannery control (Table 2). When the concentration increased its reduced the percentage of growth, shoot length and root length in treated effluent. Treated tannery effluent irrigation with domestic wastewater resulted in good growth on *Tagetes erecta*, with the highest shoot and root length (10.47 and 11.22 cm) (Balasubramanian and Dhevagi, 2016). Higher concentrations (75% and 100%) of treated tannery effluent with Ganga river water completely inhibited the *Vigna radiata* L. seed germination (Singh *et al.,* 2014). Treated tannery effluent with Arbuscular mycorrhiza (AM) on *Vigna radiata* L, resulting in maximum shoot length, root length, and number of leaves (Indira and Ravi, 2010). The highest root and shoot length of *Helianthus annuus* L. (sunflower) was observed in the lowest concentration (20%) of treated tannery effluent when compared to other treatments (40%, 60%, 80%, and 100%) (Arifa *et al*., 2013). Uddin *et al.* (2014) recorded the tallest plant produced from the combined treatment of coco dust with N, P, K, S, and Zn.

The results of present study concluded that by treating the tannery effluent with coir pith and NRK, the seed germination were found to be high in 12.5% and 25% when compared to other concentrations and control of tannery effluent. Treated tannery effluent showed better shoot and root length as like water control. Hence, it is suggested that treated tannery effluent may be suitable for foliar and irrigation purpose in plant growth.

Authors' contribution

Conceptualization of research work and designing of experiments (GJ, PM); Execution of field/lab experiments and data collection (GJ, PM); Analysis of data and interpretation (GJ, PM); Preparation of manuscript (GJ, PM).

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