Poll Res. 42 (1) : 88-93 (2023)

ISSN 0257–8050

DOI No.: http://doi.org/10.53550/PR.2023.v42i01.014

EFFECT OF LEATHER INDUSTRIAL EFFLUENT ON THE GERMINATION AND SEEDLING GROWTH OF SELECTED MEDICINAL PLANTS

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(Received 22 August, 2022; Accepted 11 October, 2022)

ABSTRACT

This study evaluated the effect of leather industrial wastewater on the germination and seedling development of three medicinal plants. *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens* are the medicinal plants that were selected for this study. Leather tanning includes different procedures which required large quantities of water so the amount of wastewater production by this industry is dangerously high. Different steps in the tanning process like liming and chrome tanning and rechromming produce an immense amount of effluent water which contains many hazardous chemicals. the chemical contents present in the tanning industrial effluent is potential to cause a serious threat to the ecosystem. The current study is aimed to evaluate the effect of leather industrial effluents on the germination and seedling development of three selected medicinal plants. Before the germination experiment, the effluent sample collected from a leather industry located at Pullepady, Kochi Eranakulam. Kerala, India was analyzed for different chemical and physical parameters. After the analysis of effluent water, it was used to conduct a seed germination experiment on 3 test plants. The concentrations of effluent used were 10%, 20%, 40%, 60%, 80% and 100% distilled water was used as control after 7 days of germination treatment germination percentage, Vigour index, and Phytotoxicity were calculated. The study reveals that even at the lowest concentration effluent sample adversely affects all three selected medicinal plants, and the negative effects intensified as the concentration of the effluent sample increases. At 100 % concentrations, all three test plants failed to germinate. *Emilia sonchifolia* is found to be most sensitive to tanning industrial effluent compared to *Andrographis paniculata* and *Tridax procumbens*.

KEY WORDS : Leather tanning industrial effluent, Physico-chemical parameters of waste water, Seed germination experiment, *Andrographis paniculata*, *Emilia sonchifolia*, *Tridax procumbens*

INTRODUCTION

The era of industrialization begins with a colossal amount of hazardous risks to the ecosystem with it. As the human population increases year by year the industrial sectors are compelled to produce more and more products to fulfil the need of the exploding population. As industrial production increases exploitation of natural resources and disposal of a large number of pollutants to the ecosystem is also increased. The leather tanning industry is one of the major industrial sectors that cause the reach of large amounts of dangerous pollutants including heavy metals to the biosphere. Most of the steps of leather tanning need large quantities of water which will become perilous effluents even after the wastewater treatment and when it reaches the ecosystem it can adversely affect both flora and fauna. India is one of the major leather-producing counties. In developing countries

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like India and Bangladesh Chromium based leather tanning is still widely used because of its low cost and higher quality of leather as a result large amount of Chromium may reach to ecosystem every year. From ancient times medicinal plants have very important role in the health care of local communities. 85% of the world's population is still dependent on medicinal plants to treat different disease conditions as a primary health care mode (Pfeifer *et al.*, 2016). since the development of scientific research identification and analysis of herbal substances for the development of modern medicines had a prolific rise. and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer and Brönstrup, 2014).

Andrographis paniculata (Burm. F) Nees is a widely used medicinal plant with an extremely bitter taste. It is an annual herbaceous plant that belongs to the family Acanthaceae. Ayurvedic, Unani and Chinese medicine its been used for the treatment of diabetes, respiratory tract infections, bug bites, and fever. The major bioactive chemical present in *A. paniculata* is Andrographolide which shows anticancer properties in many investigations. *Emilia sonchifolia* (L.) DC is a medicinal plant commonly known as Lilac tassel flower and it belongs to the family Asteraceae . In Ayurvedha it is been used for the treatment of respiratory diseases and fever. In Brazilian folk medicine, *Emilia sonchifolia* is used to treat asthma, rheumatism, and wounds, and skin rashes. In countries like Bangladesh, Thailand, and Malaysia *Emilia sonchifolia* is a common leafy vegetable. *Tridax procumbens* Linn., originated in tropical America and belongs to the family Asteraceae. The common name of this plant is coat buttons or Tridax daisy. Even though it is a common weed plant studies show that it contains many secondary metabolites which have potential medicinal properties like antimicrobial, anti-inflammatory, antidiabetic, and anti hepatic properties (Beck *et al.*, 2018) . Traditionally this plant has been used by different communities to treat fever and skin irritations

India is the third largest producer of leather in the world having about 3000 tanneries with an annual processing capacity of 0.7 million tonnes of hides and skin (Roy *et al.*, 2015). Leather industrial wastewater is having a noxious effect on the ecosystem because it contains many dangerous chemicals. In India, the Leather industry is the major source of chromium pollution in water bodies. In Kerala, most of the leather tanning industries are located in the Eranakulam district. Since leather tanning requires a large amount of water most of the leather industries are located near water bodies. The current study aimed to evaluate the physico-chemical characteristics of industrial effluent samples from Leather tanning industries located in Ernakulam District, Kerala, India and to study the effect of effluent sample on the germination and development of seedlings in three medicinal plants *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*

MATERIALS AND METHODS

The study was designed to assess the impact of Lather tanning industrial effluent on seed germination and seedling development of *Andrographis paniculata Emilia sonchifolia* and *Tridax procumbens.*

Collection of effluent samples from the leather tanning industry

The leather manufacturing company is located at Pullepady, Kochi Eranakulam. Kerala, India is dealing with all beam house operations including liming procedures, chrome tanning, and dyeing so the factory is potential to produce an immense amount of wastewater every year and the effluent must be loaded with large quantities of harmful chemicals like heavy metals. Effluent samples were collected in sample good quality plastic bottles which were filled leaving 2 inches for thermal expansion. The sample bottles were placed in an ice chest to maintain a 4 ^{o}C temperature. The collection of Leather tanning industrial effluent was done in February 2022.

Physico-chemical analysis of effluent and water sample

The effluent sample analysis was done following the standard methods of (APHA, 1998). By using digital portable water analysis kit (model pp9040) physico-chemical characteristics like pH, EC, TDS (total dissolved solids), Total hardness, Total alkalinity, chloride, DO (dissolved oxygen), BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) Were analysed. Quantification of Chloride was done by titration with Silver nitrate as titrant based on the Mohr method (Shukla and Arya, 2018). Fluoride content in the sample was determined by titration with Lanthanum nitrate solution (Eriksson and Johansson, 1970).

Chemicals and Reagents

Silver nitrate ($AgNO_3$, 99%), Nitric acid(HNO3 70%), Sodium chloride (NaCl 99%), Calcium Carbonate ($CaCO_3$, 99%), and Sodium hydroxide (97.0%) were purchased from Sigma-Aldrich chemicals privet Limited, Anekal Taluk, Bangalore. Calcium picrate ($C_{12}H_4CaN_6O_{14}$ 99%) was purchased from Musechem, Fairfield, New Jersey, United States. Methylene blue, Lanthanum(111) nitrate ($(La(N0_3)_3$. 6H2), 99.990%) and 2-Morpholinoethanesulfonic acid sodium salt (MES Na) from Nice Chemicals Private Limited, EdaPPally Ernakulam, Kerala, India. Preliminary Analysis.

Metal estimation

In a 500 ml beaker, 100 ml of Effluent sample were taken and 5 ml concentrated HNO_3 was added. Using a hot plate sample in the beaker allowed to evaporate dawn to 20 ml. 5 ml HNO_3 was added after cooling the sample. Then the sample was filtered to remove any insoluble materials which may clog the atomizer. By adding distilled water the volume of the sample made up to 100 ml and use for Atomic absorption spectrometry according to the manufacturer's instruction manual for the quantification of Chromium.

Collection of seeds

The Seeds were collected from healthy-looking plants which were grown in the botanical garden, Sree Narayana College Nattika. Thrissur, Kerala, India. Uniformly looking seeds were selected for the experiment. All the seeds were treated with 0.02% Triton-X solution to avoid contamination. After decontamination using 0.02% Triton –X seeds were washed several times with distilled water.

Seed Germination experiment

Seed germination studies were conducted in March 2022. Sterilized Petri dishes were used for the germination experiment. selected concentrations of Leather tanning industrial effluent water were 10%, 20%, 40%, 60%, 80% and 100 %. Distilled water was used as a control. Petri dishes were lined with Whatman's No. 1 Filter paper. 20 seeds of each three medicinal plants were placed in each Petrides. An equal amount of varying concentrations of the effluent sample were added to labelled Petri dishes. Triplicates of each treatment including control were maintained at 28 ^{o}C. Treated seeds were kept under observation for 7 days. on the 8 th, day germination percentage, radical, and hypocotyl length were noted. The germination percentage (GP) was calculated as the following

Germination percentage = (g/20)*100

As g is number of germinated seeds and 20 is the number of seeds used for the germination experiment.

The length of the Radicle and hypocotyl were measured and expressed in centimeters. the sum of Hypocoltyl length and radical length were represented as seedling length and used for further calculations as follows

Vigour Index = Seedling length * Germination percentage Phytotoxicity = (Radicle Length of control – Radicle length of test)/ Radicle length of control

Statistical analysis

All the data derived from the experiment were statistically analyzed for the calculation of standard error. The data expressed in this study are the mean of 3 replicates.± Standard error.

RESULTS AND DISCUSSION

All Physicochemical parameters of the effluent sample were found to be exceeding the prescribed limits of the BIS Standard of effluent discharged (IS:2296-1982). Total Dissolved Solids (TDS) is the amount of total inorganic salts and dissolved particles present in a water sample according to BIS the maximum limit of TDS allowed in the industrial effluent sample is 2100 ppm but the tannery effluent which was analyzed in this study shows more than twice the amount of permissible limit. COD and BOD represent the organic compound present in a sample both of these parameters are found very high in the analyzed effluent sample as compared to the BIS standard. The hardness of a water sample represents the total concentration of calcium and magnesium. carbonates, sulfates, chlorides, and nitrates. Total hardness is found to be very high (1256 mg/l) in the effluent sample. The total amount of bases like carbonates and bicarbonates present in the sample is referred as the total alkalinity of a sample here the analysis show that the alkalinity content in the test sample is very higher than the BIS standard. Electrical conductivity is an indicator of the presence of salts in a water sample the higher EC in the tannery effluent (15.62 dS/m) indicates the elevated presence of inorganic salts and chloride

Table 1. Physico-chemical characteristics of tannery effluent sample

S. No.	Physical parameters of Tannery effluent sample	Effluent sample	BIS Standard of effluent discharged (IS:2296-1982)
1	Colour	Brown	–
2	Odour	Foul smell	–
3	TDS (ppm)	4960	2100
4	COD (mg/l)	1490.06	250
5	BOD (mg/l)	875	30
6	T. Hardness (mg/l)	1256	600
7	T. Alkalinity (mg/l)	1360	600
8	Electrical conductivity (dS/m)	15.62	8.50
9	pH	9.64	5.5-9.0
10	Chloride (mg/l)	1724.6	1000
11	Fluoride (mg/l)	4.14	2
12	Sulfate (mg/l)	1270	1000
13	Chromium Total (mg/l)	47.3	2

ions. pH is a very significant parameter of water, changes in pH can seriously affect the ecosystem. leather industrial wastewater shows alkaline ph because of the presence of carbonates and bicarbonates in high concentrations. Since in tanning process large quantities of NaCl is been used chloride content in tannery effluent is very high (1724.6 mg/l) Sulphate content in the tannery effluent is 1270 mg/l because in tanning chrome sulfate liquids are a common reagent normally used for chrome tanning.

Germination Experiment

In control, *Andrographis paniculata* shows the highest germination percentage which is followed by *Emilia sonchifolia* and *Tridax procumbens*. This data clearly shows that leather industrial effluent drastically affects the germination percentage of all three medicinal plants. 60 is the highest concentration of the effluent sample in which all plants managed to germinate. At 80 % concentration, only *Andrographis paniculata* and *Tridax procumbens* were able to initiate the germination process, at this concentration *Tridax procumbens* is found to be more resistant than compare to *Andrographis paniculata* and *Emilia sonchifolia*.. Emilia is found to be the most sensitive species at higher concentrations. At 100% all three plants were unable to germinate and develop the seedlings.

The data shows that in control *T. procumbens* is having the highest seedling length as compared to the other two medicinal plants. *Andrographis paniculata* and *Emilia sonchifolia* are having similar seedling lengths at control but as the concentration of effluent increases the seedling length of *Emilia sonchifolia* is found to be smaller than *Andrographis paniculata*. Leather industrial effluent adversely affects the growth of seedlings in all three plants and *Tridax procumbens* is found to be having the least negative effect as compared to the other two

Vigour index is a very important factor that gives insight into the ability of a seed to germinate and

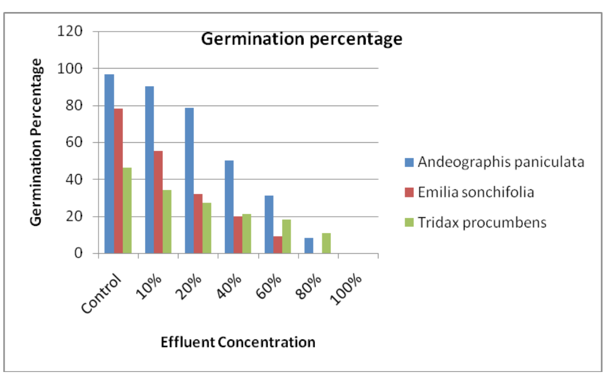

Fig. 1. Effect of leather industrial effluent on Germination Percentage

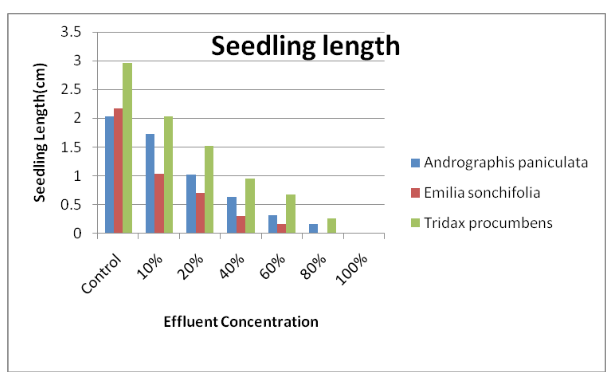

Fig. 2. Effect of leather industrial effluent on Seedling length

Table 2. Effect of leather insudtrial effluent on radical and hypocotyle length *Andrographis paniculata*, *Emilia sonchifolia* and *Tridax procumbens*

Concentration of Effluent	*Andrographis paniculata* Radicle length (cm)	*Andrographis paniculata* Hypocotyle length (cm)	Emilia sonchifolia Radicle length (cm)	Emilia sonchifolia Hypocotyle length (cm)	Tridax procumbens Radicle length (cm)	Tridax procumbens Hypocotyle length (cm)
Control	1.01 ±0.2	1.03 ±0.03	1.09 ±0.02	1.08 ±0.04	1.49 ±0.20.	1.47 ±0.05
10%	0.89 ±0.7	0.84 ±0.05	0.56 ±0.1	0.48 ±0.2	1.09 ±0.07	0.94 ±0.1
20%	0.66 ±0.5	0.36 ±0.3	0.46 ±0.2	0.25 ±0.5	0.85 ±0.1	0.69 ±0.02
40%	0.41 ±0.12	0.22 ±0.1	0.19 ±0.1	0.12 ±0.03	0.51 ±0.2	0.45 ±0.4
60%	0.24 ±0.4	0.08 ±0.2	0.14 ±0.04	0.03 ±0.05	0.41 ±0.06	0.27 ±0.02
80%	0.14 ±0.32	-	-	-	0.23 ±0.03	0.03 ±0.1
100%	-	-	-	-	-	-

Values are mean of 3 replicates ± standard error

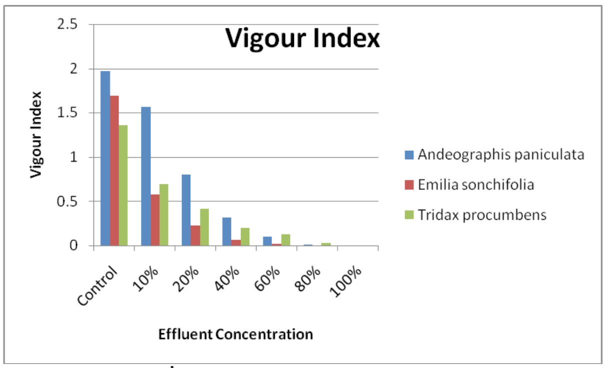

Fig. 3. Effect of leather industrial effluent on Vigour index

successfully develop into a healthy seedling which may be seriously affected by external stress conditions, in this study the stress induced by the different chemical and physical conditions caused by leather industrial effluents shows the vigour index of all three medicinal plants drastically decreased as the concentration of effluent increased. *Emilia sonchifolia* is found to have the lowest vigour index at 60 % concentration. at control, *Tridax procumbens* is the plant with the lowest vigour index than *Emilia sonchifolia* and *Andrographis paniculata*

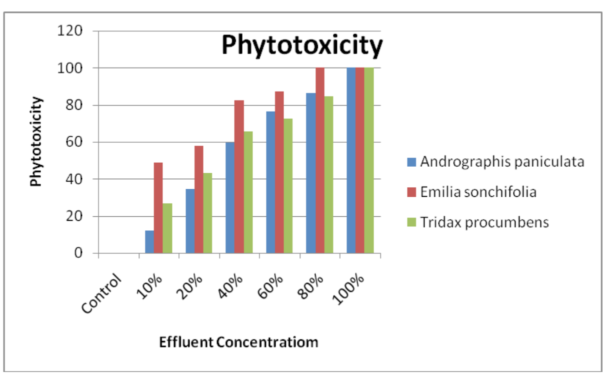

Fig. 4. Effect of leather industrial effluent on phytotoxicity

but at the highest 80% concentration it is having highest vigour index.

Phytotoxicity is the adverse effect of a specific substance on seed germination and plant growth. Here we have evaluated the adverse effect of leather industrial effluent on the germination of three selected medicinal plants. The data shows that as the concentration of industrial effluent increases the toxic effect on the three test plants increases. Even at 10 %, the effluent can seriously affect the germination and seedling development the adverse effect is high in *Emilia sonchifolia* as compared to *Andrographis paniculata* and *Tridax procumbens*. *Emilia sonchifolia* was only able to survive up to 60%, and from that the effluent is 100 % toxic. *Andrographis paniculata* and *Tridax procumbens* were managed to germinate at 80% but since the toxic effect of effluent id very high, the growth was very weak.

ACKNOWLEDGMENT

The present work was done by the support of a research fellowship (GRANT NO 08/663(0003)/ 2019) from Human Resource Development Group, New Delhi.

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