CHAPTER 3

MATERIALS
AND METHODS
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The effects of different concentrations of distillery effluent were carried out on: (1) Germination percentage and speed of germination index and seedling growth and dry matter transfer in two legumes and two cereals; (2) Plant growth and yield of two legumes and two cereals only.

The experiments to observe the effects of untreated, anaerobically treated and finally treated effluents (in different concentrations) of Shamli Distilleries and Chemical Works, Shamli were performed in the research plots of D.A.V. (P.G.) College, Muzaffarnagar and in earthen pots. Normal agronomic conditions were followed.

The pure (untreated) effluent was diluted using normal distilled or tap water in different concentrations, viz. 1, 2.5, 3.5, 5.0, 7.5, 10 and 25 percent etc. The distilled or tap water as such served as control. The soils (of plots & pots) were irrigated on alternate days with different concentrations of effluent to keep it saturated. The effect of distillery effluent in different dilutions was observed on four locally grown crop plants (2 cereals and 2 legumes), viz. wheat (*Triticum aestivum*, cv. PBW-343), Maize (*Zea mays* cv. Ganga 5), Mung (*Vigna radiata* cv. ML-5) and Lobiya (*Vigna sinensis* cv. Type-2).
Similarly different concentrations of anaerobically treated and finally treated distillery effluent were used for observing effects on different crop plants.

Studies were made along the following lines –

1. Effect of untreated (pure), an aerobically treated and finally treated distillery effluent (in different concentrations) on seed germination, speed of germination index (SGI) and early seedling growth of different crop plants were observed. The emergence of radicle was taken as a criterion for germination. Observations were taken daily at a fixed time. The speed of germination index (SGI) was calculated by following formula given by Carley and Watson (1968).

\[
\% \text{ SGI} = 4(7 \times 1G + 6 \times 2G + 5 \times 3G + 4 \times 4G + 3 \times 5G + 2 \times 6G + 1 \times 7G)
\]

Number of days = 7

Number of seeds = 25

1G = Seeds germinated on first day;

2G = Seeds germinated on second day and so on.

For observing the seedling growth, 10 days old seedlings were analyzed for the following parameters –

a) Seedling length

b) Root length
c) Shoot length

d) Number of lateral roots formed

e) Fresh weight of root

f) Fresh weight of shoot

g) Dry weight of root

h) Dry weight of shoot

2. Long term field experiments were performed on four different locally grown crop plants to observe the effect of distillery effluent on growth parameters of crop plants under study, such as length of root and shoot, number of leaves/plant, number of primary branches/plant, fresh weight of root and shoot, dry matter production (phytomass accumulation) and net primary productivity (NPP) etc.

A. GROWTH STUDIES:

1. Germination and seedling growth:

   For the seedling growth studies, experiments were performed in 1%, 2.5%, 3.5%, 5.0%, 7.5%, 10% and 25% concentrations of distillery effluent in selected legumes and cereals. It was diluted with distilled water to get various concentrations. The distilled water as well as normal tap water as such served as control, untreated 100% pure effluent also taken.
Uniform seeds (criteria being the size and colour of seeds) were selected; surface sterilized with 0.1% HgCl₂ solution and washed thoroughly with distilled water. In legumes these seeds were transferred on moist filter paper in desiccators containing various concentrations of sterilized effluent and pure effluent. Simultaneously, a control soaked in distilled tap water also maintained. The effluent and tap/distilled water supply to the seedling maintained through a filter paper wick dipping in effluent and water of the lower chamber of desiccators. The level of effluent in desiccators was maintained regularly. The germination and subsequent seedling growth were carried out in high as well as in dark. The emergence of radicle was taken as the criteria for germination.

In cereals sterilized and thoroughly washed seeds were kept for germination in petri plates filled with equal amount of sterilized quartz sand. The sand was irrigated on alternate days with various concentrations of distillery effluent and distilled water to keep it saturated. The studies were made in laboratory of D.A.V. (P.G.) College, Muzaffarnagar and emergence of radicle was taken as the criteria for germination.

Germination percentage was recorded after 24 hrs. For seedling growth, samples were collected on 3rd, 5th and 7th day of radicle emergence. These seedlings dissected out into radicle, epicotyl and cotyledons in legumes; into root, shoot and cotyledons in cereals.
Length, fresh weight and dry weight of these seedlings parts were recorded. In case of residual cotyledons, only the fresh and dry weight recorded. (Agarwal, 1980, Anonymous, 1985, Chang, et al. (2002).

2. Plant growth and yield:

The plant growth studies were carried out in two cereals and two legumes. For these studies, some concentrations of sterilized distillery effluent concentrations were selected for the irrigation. The soil was procured from the experimental plots of D.A.V. (P.G.) College, Muzaffarnagar and it was filled in polythene bags. Each bag was filled with two kg soil.

Seeds of two legumes viz. *Vigna radiata*, *Vigna mungo* and two cereals viz. *Triticum aestivum* and *Zea mays* were sown in these polythene bags and allowed to grow under field conditions. These bags were irrigated with some concentrations of effluent whenever necessary. Simultaneously, a control set of each crop was maintained and irrigated with tap water. For the growth studies plant samples were taken on 30th and 60th days of seedling emergence. The day of 35 to 100% emergence was taken as starting day. Root and shoot length, nodule number, fresh and dry weight per plant were recorded in these legumes and in cereals spikelet number, grain number, weight etc recorded.

For studies on yield parameters, plants were harvested at 90th day of emergence. In case of crops certain yield parameters such as number of pod or ear per plant, pod cover weight, number
of seed per pod, seed weight and seed/grain weight per plant were recorded. The dry samples of these crops were used for quantitative estimation of total N, total P and total heavy metal contents. Thus the effect on some yield parameters of different crop plants under study were evaluated, viz. –

a) Days to first flowering

b) Days to first fruiting

c) Length of pod or ear

d) Seed output or seed yield (No. of pods or ears/plants, No. of seeds/pod or ear).

e) 100 seed weight

f) Biological yield

g) Harvest index = \frac{\text{Seed yield}}{\text{Biological yield}} \times 100 \text{ and expressed as percentage}

Effect of distillery effluent on the reproduction capacity of crop plants was also studied. Reproductive capacity was measured as product of average seed output and average percent germination of seeds collected from plants of various effluent concentrations (Salisbury, 1942; AOAC (1990).
Like wise effects on some biochemical attributes like stomatal resistance and leaf temperature of different crop was also studied. (Johanson, 1940, Change et al., 2002.)

In the same manner certain biochemical changes due to the use of untreated, anaerobically treated and finally treated distillery effluent in different concentration was also studied based on Arnon (1949), APHA (1985). Allen et al (1986), Sadasivam and Manickam (1996).

ESTIMATION OF PIGMENT CONTENTS

200 gm of fresh weight of tissue of experimental plant material was homogenised with 8.0% acetone, centrifuge at 4000 rpm, for 5 minutes, Final volume made to 10 ml with acetone, absorbance of the extracts read at 645 nm for chlorophyll and 665 n.m. for carotenoid. These pigment contents were estimated by the formulae of Arnon (1949).

pH Studies:

In the present study one part of extract and 5 parts of water was used based on Piper (1950) and Jackson (1973).

Leaf pH was measured by a pH meter using a glass electrode (Chang (1968), SSSA, (1996)).
PROTEIN ESTIMATION

For protein estimation digestion of legume plant material was done according to Snell and Snell (1954) and later estimation of the digest done with Nesslers reagent of Koch and Meekins formulae (Oser, 1965). Amount of total protein was calculated by using a calibration curve (Fig. 1.).

CARBOHYDRATE ESTIMATION:

Estimation of Sugar was done by Pavys method (Sadasivam and Manickam, 1996). Here 100 gm of fresh material crushed with lead acetate powder and then heated and than filtered. H₂S gas passed to precipitate lead. Lead was removed and than solution made 100 cc. It is then filtered and filtrate taken in burette and than by using Pavys solution A and B in beaker titration done to find out total sugar level in plants.

Age affected study were done based on Leopold (1961) Wieglan and Namken (1966) and Lindo and Nooden (1977).

Like wise experiments were performed to note the effect of untreated (Pure) distillery effluent on characteristics of sandy soils like temperature, water holding capacity, soil pH etc and than on growth of plant. For this sandy soil was thoroughly irrigated with pure effluent and then was left for 2-3 months. After 2-3 months effect on different soil characteristics was studied. Side by side this soil was used to grow plant to observe fertility of soil.
DETERMINATION OF SOIL pH:

In the present study one part of soil and 5 parts of water was used (Piper, 1966). Soil pH was measured by a pH meter using a glass electrode (1:5 soil: water). The over dry weight of the soil of a unit volume was estimated. In the field moist samples were used for the determination of soil pH.

Water holding capacity was determined by using perforated/Granular brass boxes (Piper, 1950); SSSA (1996).

SAMPLING AND ANALYSIS OF SOIL:

Soil samples were collected from the natural sites near distillery factory. Plastic bags (18 cm height, 12 cm diameters) were used for pot culture. Pots were supplied with various concentrations of distillery effluent (untreated pure effluent) 1, 2.5, 3.5, 5.0, 7.5, 10 and 25 % of with tap water. Daily about 200 ml of treated effluent was added to the respective pot, similarly pots with 200 ml of tap water served as control. Experiments were performed during crop season up to 4 months harvesting the crop soil sample were taken, air dried, sieved and analyzed for pH, water holding capacity, soil temperature (Jackson 1973), Piper (1950) and Allen et al. (1986).

All data on growth, yield and biochemical components were statistically analyzed wherever possible based on Peterson (1939), Rao (1952), and Mishra and Mishra (1989).
Figure-1: Calibration Curve