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Morphological And SEM (EDX) Studies Of Finger Millet (*Eleusine Coracana* (L.) Gaertn.) Under Different Concentrations Of Sugar Mill Effluent

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Abstract

Objective: Water is vital components of life support system and the quality of the water pivotal role in the maintenance of aquatic health. Sugar mill refinery effluent containing some toxic substances put forth a huge impact on watershed management and crop plants. Toxic metals are hazardous which can stimulate morphological and histological changes in finger millet (*Eleusine coracana* (L.) Gaertn.). Hence, the present study aimed to analyze the yield and surface morphological changes in the finger millet crop. **Materials and Methods:** The healthy seed of finger millet variety (CO-13) was taken. Finger millet was cropping with different concentrations (10, 25, 50, 75 & 100 %) of sugar mill effluent. The seeds irrigated with tap water were treated as control. After, the SEM images of the finger millet after growing in 10 g of sugar mill treatment, respectively. Although the SEM images show nuclear precipitation of metals, the percentage of elements in the *Eleusine coracana* was analyzed by EDX. **Result:** The highest plant growth was analysed in 10 % of sugar mill effluent concentrations. A scanning electron microscope equipped with an energy dispersive X-ray spectrometer was used to observe and analyse the morphology of the isolates incubated in the presence of Mn, Zn, P, K, Mg, Fe, Zr, S and Cu in the root and leaf of *Eleusine coracana* (L.) Gaertn.

Key words: Sugar mill effluent, *Eleusine coracana*, SEM (EDX), Toxic substances.

1. INTRODUCTION

Water is an integral component of the life support system, and the quality of that water is crucial to maintaining the wellbeing of aquatic life. Unfortunately rapid industrialization, population explosion and non-judicious use of natural resources have resulted in many fold increase in water pollution besides sewage, industrial effluent, agricultural runoff and other household residues. Finger millet (*Eleusine coracana*) one of the most important millets, was traditionally used to prepare flour based products. The millet carbohydrates consist of free sugars, starch and non-starch polysaccharides. The millet starch consists of amylose and amylopectin fractions, normally present in the ratio of 25:75. Many of the starch granules of the millet are compound and rigid in nature and this contributes towards the nutritional advantages of the millet food in terms of slow digestion of its carbohydrates or lower glycemic index of the millet meal (2). Therefore Scanning electron microscopy with Energy-dispersed X-ray analysis (SEM/EDX) was commonly used for morphological and elemental compositional analysis of precipitated chemical compounds (3 and 4).

Based on the above analysis, in this paper morphology and chemical compositional analysis was performed using SEM-EDX for identification of lead (Pb) and investigated concentration of Pb with the physicochemical parameters in the sugar mill effluent industry. The utilization of sugar mill effluent for irrigation of crop plants is one of the highly beneficial solutions for prevention of pollution. The present investigation has been carried out on the utilization of sugar mill effluent on finger millet (*Eleusine coracana* (L.) Gaertn.) with the yield, SEM (EDX) analysis.

2. MATERIAL AND METHODS

The present research work was carried out to study the utilization of industrial effluent on crop plants in different concentrations of sugar mill effluent on yield and SEM-EDX studies of finger millet in field conditions.

2.1. Field Experiments

2.1.1. Preparation of test solution

The collected effluent from the outlet of sugar mill effluent was considered as 100 per cent raw effluent. Different concentrations (Control, 10, 25, 50, 75 and 100%) of sugar mill effluent were prepared a fresh by using tap water, whenever necessary. They were used for both germination studies and pot experiments.

Control: Tap water
10% : 10 ml effluent + 90 ml of water
25% : 25 ml effluent + 75 ml of water
50% : 50 ml effluent + 50 ml water
75% : 75 ml effluent + 25 ml water
100% : Raw effluent.

2.1.2. Laboratory Experiments

Two gram seeds of finger millet were taken into a pot. Plant seeds were sowing on equal distance in pot and sugar mill effluent was taken in different concentrations. Then the water was poured into the container and left there for 24 hours so that seeds could start germination. After, that the water was drained out and the data was recorded. Parameter like germination percentage was recorded on different periods of growth. The first recording was done after 12 hrs and subsequent recordings were taken after a one day interval till 15^{th} day. Visible radical growth and emergence of hypocotyls and the cotyledons were noted to determine germination.

2.2. SEM-EDX Analysis of *Eleusine coracana*

Eleusine coracana sample was harvested after 90 DAS of growing control, 10% and 100% of the sugar mill effluent mixed in pots and dried at 70°C. The powder was put on aluminum straps and coated with carbon before it was analysed by a scanning electron microscope connected with energy dispersive X-ray spectroscopy (SEM-EDX).

The percentage of the plant surfaces were calculated from at least three areas of scanning. A scanning electron microscope (SEM) connected to an energy dispersive X-ray (EDX) was used to study the images of the plant surface and the percentage of elements. SEM image of the *Eleusine coracana* after its growth in 1.00 kg-1 of sugar mill effluent treated for 90 DAS. Although the SEM images show nuclear precipitation of metals, the percentages of elements in *Eleusine coracana* were analysed by EDX.

SEM photographs were carried for the samples, using SEM model JEOL-JSM-6390 LV attached with energy dispersive X-ray unit, with an accelerating voltage of 20 kV.

2.3. Statistical analysis

The statistical analyses of experimental results were carried out as per the procedure given by (1 and 5). Split analysis was done to find out the interaction between the varieties by using the statistical package-irri stat. Each set of parameters was analysed using ANOVA and the Least Significant Difference (LSD) test at the 95% probability level (With SPSS 10.5 EV software). All the data were given as mean of 3 assays. The level of significance was calculated at P value < 0.05%.

3. RESULT AND DISCUSSION

3.1. Yield parameters

The effect of different concentrations of sugar mill effluent on yield parameters of finger millet is presented in Table 1.

Highest number of ear head/plant (6.5) 100 seed weight (1.85) and yield (4562.87 kg/ha) was observed at 10 per cent concentration of sugar mill effluent. The lowest number of pods/plant (1.0), 100 (g) grain weight (1.18) and yield (836.28 kg/ha) was observed at 100 per cent concentration of sugar mill effluent. The F values were found to be significant at P < 0.05, level.

Table 1. The efficacy of sugar mill effluent on yield parameters of finger millet (*Eleusine coracana* (L.) Gaertn.)

Effluent conc. (%)	Yield parameters		
	No. of ear head per plant	100 (g) grain weight/plant	Yield per hectare (kg/ha)
Control	5. 0 ± 0.11	1.70 ± 0.12	3569.1 ± 48.64
10	6.5 ± 0.12	1.85 ± 0.12	4562.87 ± 49.90
25	4.5 ± 0.09	1.68 ± 0.09	2877.1 ± 39.4
50	3.0 ± 0.08	1.55 ± 0.08	2026.2 ± 35.4
75	2.5 ± 0.07	1.42 ± 0.07	1255.5 ± 31.9
100	1.0 ± 0.06	1.18 ± 0.05	836.4 ± 28.72

3. 2. Scanning electron microscopic observation

Table 2 shows the scanning electron microscope (SEM) connected to an energy dispersive X-ray (EDX) which used to study the images of the plant surface. The SEM images of the *Eleusine coracana* (L.) Gaertn. after growing in 10 g of sugar mill treatment, respectively. Although the SEM images show nuclear precipitation of metals, the percentage of elements in the *Eleusine coracana* was analyzed by EDX. The EDX spectrum showed foreign metal ions like accumulations by *Eleusine coracana*.

Scanning micrographs of root and leaf clearly revealed proper arrangement of cortex and vascular bundles (Xylem and phloem). However, gradual increase of 10 per cent effluent concentration indicated that formation of both organelles.

From the SEM picture (Plate 1 and Fig. 1) it is clear that peculiar feature of xylem and phloem is gradually distorted with increase in the concentration from 100 per cent effluent concentration. However, most acute condition was recorded from 100 per cent effluent concentration. On the other hand, shoot organelles showed much lower severity and complete destruction of organelles was observed at 100 per cent effluent concentration. Almost similar deformation was recorded for root structure also. This damage is perhaps may be due to ROS production.

The results obtained from Atomic Absorption Spectrophotometry indicated that the amount of sugar mill effluent accumulated in the root of *Eleusine coracana* was much higher (100 per cent) concentration 30, 60 and 90 DAS than in its leaf (Table 2). This might be due to the less mobility of toxic heavy metals within the plant body. Though retarded plant growth was observed for heavy metal treated plants, but this did not interfere with the completion of plant development and they survived even under a considerable pollution load. Hence, it could be stated that these heavy hazards treatments did not interfere with the completion of plant development.

A scanning electron microscope connected to energy dispersive. X-ray was used to study the images of the plant surface and the percentage of elements. The SEM images of finger millet after growing in 1.00 g kg^{-1} of various concentrations of sugar mill effluent at 90 DAS respectively were studied. The SEM images showed precipitation of metals in finger millet.

A scanning electron microscope equipped with an energy dispersive X-ray spectrometer was used to observe the morphology of the isolates incubated in the presence of Mn, Zn, P, K, Mg, Fe, Zr, S and Cu in the root and leaf of finger millet were analysed.

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Table 2. SEM-EDX Spectroscopy analysis root and leaf of *Eleusine coracana* (L.) Gaertn. under the sugar mill effluent treatment

S. No.	Name of the chemical elements	Root			Leaf		
		Control	Low. Con.	High Con.	Control	Low. Con.	High Con.
1	Zr	25.07	12.35	40.25	38.52	30.12	48.88
2	Zn	18.25	20.00	25.25	8.21	12.00	15.89
3	Ca	7.25	9.75	7.05	3.25	5.64	10.05
4	P	7.85	8.25	9.32	12.25	12.89	13.05
5	Fe	5.07	8.02	10.02	1.25	2.75	2.12
6	Mg	4.92	7.02	6.18	1.28	2.19	2.88
7	Mn	1.13	1.79	1.21	1.30	1.85	1.65

± Standard Error

Fig. 1. SEM-EDX spectroscopy analysis of root and leaf of *Eleusine coracana*
(Var. CO-13) under various concentration of sugar mill effluent

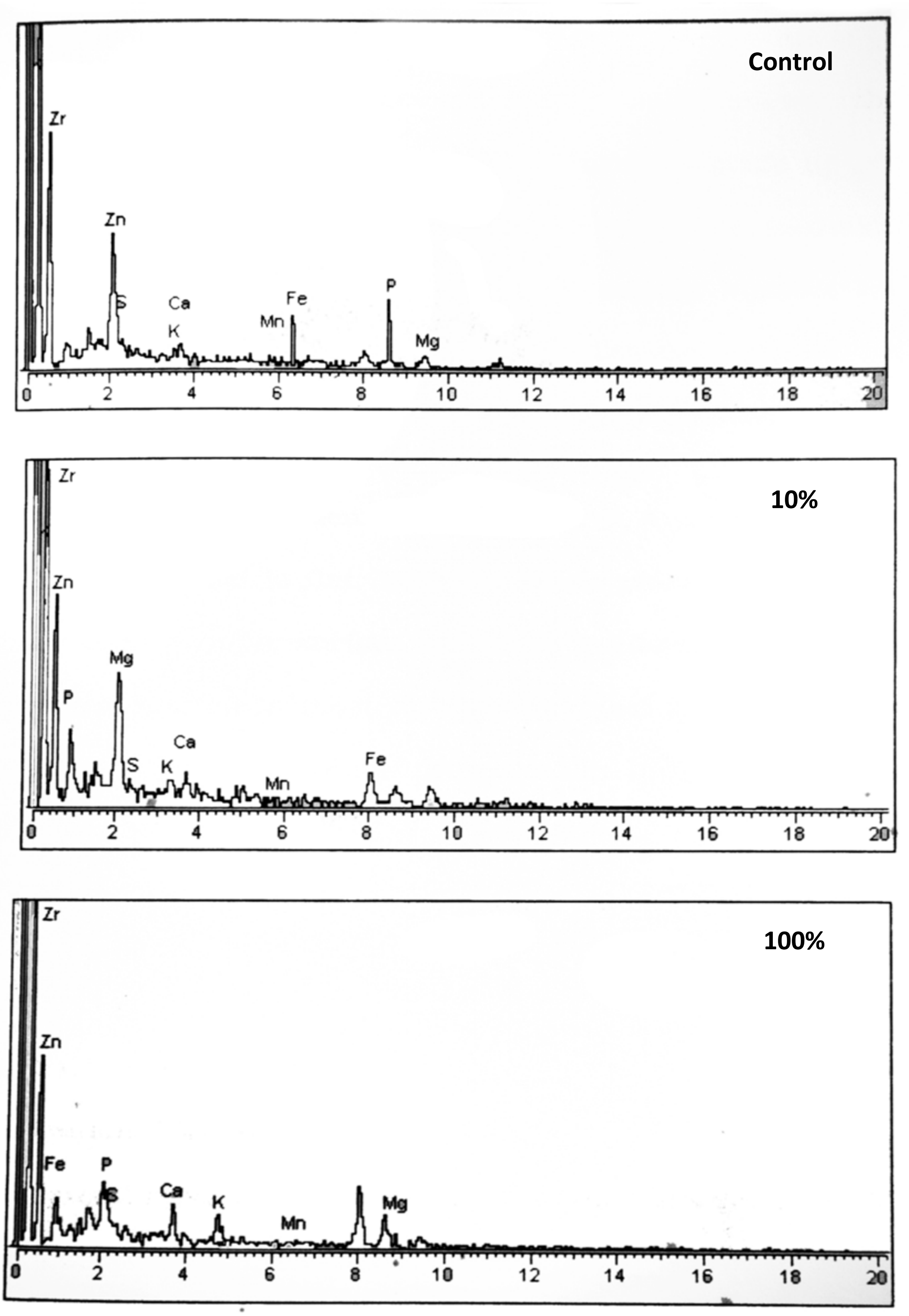
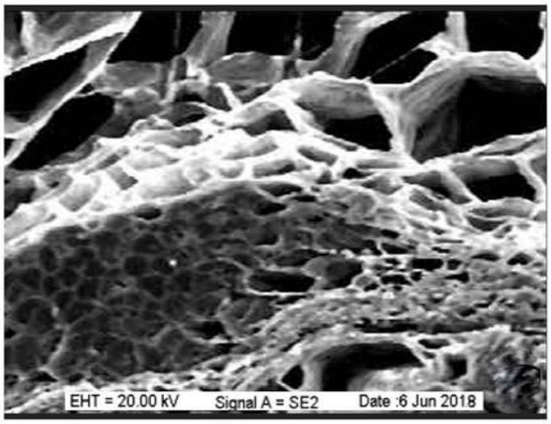


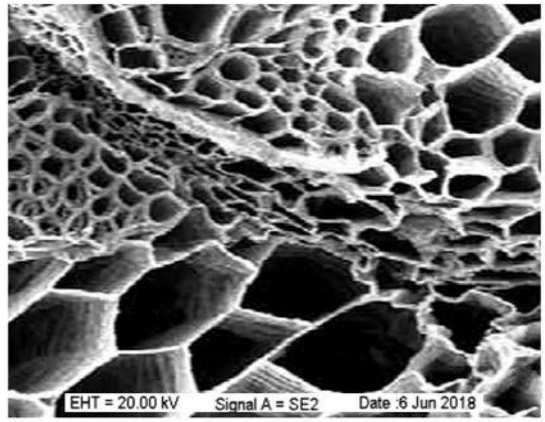
PLATE 1

SEM-EDX MICROGRAPH OF CONTROL, 10 AND 100% SUGAR MILL EFFLUENTCONCENTRATION ROOT AND LEAF CROSS SECTION OF

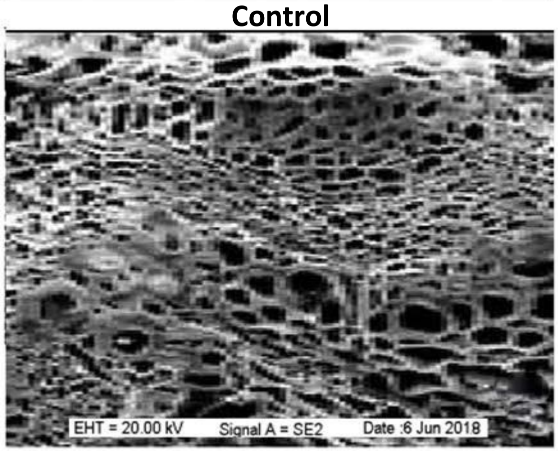
***Eleusine coracana* (Var. CO-13)**

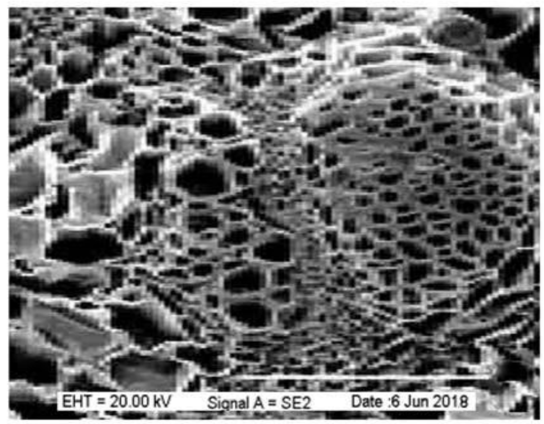
Root *Leaf*





Control **Control**





10% **10%**