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Assessment of pollutants in pulp paper industry effluent and their toxic effect on *fenugreek* (*Trigonella foenum-graecum* L)

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ABSTRACT

The samples of pulp paper industry, Shahjahanpur, India, were collected to assess the pollutants present in the effluent and their toxic effects on fenugreek (Trigonella foenum-graecum L). The pH of the effluent was 8.4.it had higher values of TS (2021 mg L^{-1}), TDS (1587 mg L^{-1}), TSS (77 mg L^{-1}), COD (23514 mg L^{-1}), BOD (8146 mg L^{-1}), EC (1740 ms cm^{-1}), lignin (6641ppm) and chlorophenols (512 mg L^{-1}) which were beyond permissible limits. The effluent even after secondary treatment showed the presence of various known and unknown complex pollutants. The concentrations of heavy metals in effluent were in descending order as Fe (77.54 mg L^{-1}) > Zn (47.10 mg L^{-1}) > Mn (18.27 mg L^{-1}) > As (14.02 mg L^{-1}) > Pb (12.06 mg L^{-1}) > Mg (11.69 mg L^{-1}) > Cd (7.33 mg L^{-1}) > Ni (6.22 mg L^{-1}) > Cu (5.11 mg L^{-1}) > Cr (1.03 mg L^{-1}). The accumulation of metals in root, stem and leaf of fenugreek increased with the application of effluent. The amounts of most of the heavy metals were recorded higher in stem followed by root and leaf. Among these metals, the maximum and minimum contents of Fe and Cd were recorded in various parts of plants, respectively. There were marked reductions in chlorophyll, protein and biomass production of fenugreek with the application of effluent over control.

Key words: Effluent, heavy metals, protein, pigment, fenugreek

INTRODUCTION

Pulp paper industry ranks sixth among the world's most polluting industries that generate very large-scale hazardous pollutants after paper production. Moreover, 55-60% lignocellulose waste is discharged from raw material while only 40-45% of pulp is obtained during the pulping process. Thus, the effluents discharged from these industries are heavily loaded with a mixture of several unknown organic and inorganic compounds (Das 2019, 2020). The major environmental pollutants released after secondary treatment is lignin and chlorinated phenols, while lignin is responsible for offensive colour, and also inhibits growth of phototrophic organism by reducing sunlight transfer into water. The phytotoxicity of pulp paper industry effluenton*Phaseolus mungo*, *Aestivum sativa* and *Allium cepa* was confirmed by Chandra *et al.* (2009). Heavy metal plant concentrations were considerably higher in waste water irrigated soils than in reference soil grows crops and exceeded the permitted limits. Moreover, the excess metals i.e. Cu may cause chlorosis in plant as a result of alterations in the photosystem. Therefore, a study was carried out to assess the effect of pulp paper industry effluent on fenugreek.

MATERIALSAND METHODS

The effluent samples were collected from M/s K R pulp paper mill limited, Shahjahanpur (U P). The effluents were analyzed within 48 hours for total dissolved solid (TDS), total solid (TS), total suspended solid (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (micro kjeldahl), sulphates, total phenols, lignin, phosphate and color (visual color comparison method) as per standard methods (American Public Health Association [APHA] 2012). The pH, chloride, sodium and potassium were also analyzed using the Thermo Orion (Model 960) selective ion electrode. While, the heavy metals in effluent were analyzed by the atomic absorption spectrophotometer (AAS; ZEEnit 700, Analytic Jena, Germany). A field experiment was conducted at BBA University, Lucknow, (U.P.) during rabi season of 2019 using fenugreek as test crop. The crop was irrigated with pulp paper mill effluent as and when required. The crop was allowed to grow up to 90 days. The leaf, stem and root samples

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were collected at different stages of growth. Biomass was recorded after drying the fresh harvest plant in hot air oven at 80ºC for 24 hrs. The chlorophyll a (Chl. a) and chlorophyll b (Chl. b) in fresh leaf samples was estimated by the method of Arnon (1949). While the protein content was estimated in roots, shoots, leaf using standard method (Lowry *et al.* 1951). The concentrations of metals (Cu, Cr, Ni, Pb, As, Fe, Mn, Cd, Mg, and Zn)in plant parts were determined using atomic absorption spectrophotometer in diacid digest. Turkey's test (Ott 1984) using Graph Pad Software (San Diego, California) was used for statistical analysis.

RESULTS AND DISCUSSION

Physico-chemical characteristics of effluent

The effluent collected from discharged drain of pulp paper mill was alkaline in reaction (pH 8.4). The effluent had higher values of TS (2021 mg L^{-1}), TSS (1587 mg L^{-1}), TDS (77 mg L^{-1}), COD (23514 mg L^{-1}), BOD (8146 mg L^{-1}), and EC (1740ms cm^{-1}). The alkaline pH might be due to the presence of residual content of sodium hydroxide and sodium sulfide utilized in pulping process of the industry (Yadav and Chandra 2018). The five days BOD/COD of the pulp paper industry effluent were 0.21 and 0.20, respectively. This revealed less degradability of pollutants present in discharged effluent. Higher TDS may be due to presence of dissolved lignocellulosic particle along with fine fibers and pith particleand adequate concentration of Na^+ and K^+ which contribute the salinity of effluent. Higher EC might be due to salt and ions content of effluent. The high concentrations of total phenols (459 mg L^{-1}), total nitrogen (141 mg L^{-1}), sulphate (1776 mg L^{-1}), phosphorus (1890 mg L^{-1}), chloride (6.15 mg L^{-1}), sodium (416 mg L^{-1}), potassium (17.65 mg L^{-1}), lignin (6641 ppm) and chlorophenols (512 mg L^{-1}) were present in effluents which wereabove the permissible limit (Table 1).

Table 1**:** Physico-chemical characteristics of discharged effluent from pulp paper industry and their heavy metals contents

Metals (mg g^{-1})	Root (Control)	Root (irrigated)	Shoot (Control)	Shoot (irrigated)	Leaf (Control)	Leaf (irrigated)
Cu	6.0± 0.32	19.55± 0.61	2.0± 0.32	16.43± 0.44	Trace	10.65± 0.32
Cr	Trace	10.44± 0.13	Trace	21.87± 0.56	1.0± 0.77	13.84± 0.13
Ni	4.0± 0.43	17.32± 0.41	3.0± 0.43	41.26± 0.89	3.0.± 0.57	14.12± 0.43
Pb	5.0± 0.52	11.13± 0.54	1.0± 0.52	19.99± 0.64	1.0.±0.90	12.31± 0.52
As	6.0± 0.51	15.22± 0.31	1.0± 0.51	22.67± 0.83	Trace	11.24± 0.51
Fe	6..0± 0.18	39.46± 0.21	5.0± 0.18	46.21± 0.19	4.0± 0.56	29.64± 0.18
Mn	5.0± 0.21	36.21± 0.39	4.0± 0.21	33.64± 0.46	3.0± 0.34	28.11± 0.21
Cd	Trace	10.33± 0.71	1.0± 0.71	11.74± 0.90	Trace	10.89± 0.71
Mg	5.0± 0.67	14.73± 0.65	3.0± 0.67	15.62± 0.23	3.0± 0.89	11.67± 0.67
Zn	3.0± 0.34	27.17± 0.33	4.0± 0.34	20.47± 0.43	3.0± 0.65	14.57± 0.34

All the values are means of triplicate (n=3) ±SD. Unit of all parameters are in mgl^{-1} except pH, color (Co-Pt. Unit) and EC (µmhoscm^{-1})

This confirmed that the secondary treatment process in industry is not adequate to degrade the all complex pollutants present in effluent, The higher values of COD and organic content were due to release of various wood extract along with chemicals used in pulping process, which makes complex compounds in effluent. Similarly, chloride was also found in effluent which is generally more toxic than sulfates to flora and fauna including microbial community. Moreover, lignin contents were noted very high in pulp paper industry effluent which might be the source of the dark color of the effluent. Besides, the discharged effluent also showed the presence of various heavy metals beyond their prescribed environmental safe limits. The presence of heavy metals along with organic compounds aggravates the toxicity and complexity of pollutants (Yadav and Chandra 2018). Furthermore, the significant amounts of Fe (77.54 mg L^{-1}), Zn (47.10 mg L^{-1}), Cu (5.11 mg L^{-1}), Cd (7.33 mg L^{-1}), Mn (18.27 mg L^{-1}), Ni (6.22 mg L^{-1}), Cr (1.03 mg L^{-1}), Pb (12.06 mg L^{-1}), As (14.02 mg L^{-1}) and Mg (11.69 mg L^{-1}) were present in effluent which are hazardous to the environment. The heavy metal sources in pulp paper industry effluent might be due to alkaline black liquor corrosion activity generated during wood digestion as it passes through iron pipes.

Chlorophyll, protein, and biomassin plants

The photosynthetic pigments in plant (Chl-a, and Chl-b), protein content and dry weight (biomass) were affectedwith pulp paper industry effluent. The results showed an increase in Chl-a, and Chl-b, content in the leaf in control.The chl a, chl b and total chl contents increased initially up to 90 days irrespective of effluent application. The values of chl a, and chl b decreased with effluent compared to control at all the stages of growth. This decrease in pigments concentration may be due to presence of several organic and inorganic pollutants. Moreover, the adverse effect on total chlorophyll may be ascribed to interference the biosynthesis of chlorophyll by different enzymatic activity. The chlorophyll sensitivity towards heavy metals in aquatic plant might be due to interaction with functional –SH group of the enzyme synthesizing chlorophyll. The protein content decreased with effluent in fenugreek plants comparison to control (Fig.1). However, the protein content increased with advanced growth irrespective of various treatments. The minimum amounts of protein in all parts of plant were noted at 30 days under different treatments. The biomass yield decreasedwith effluent application as compared to control at different stage of growth (Fig.2). There was a marked increase in biomass production with advancement of growth and maximum yield was recorded at 90 days under control treatment.

Table 2: Accumulations of metals in root, shoot, and leaf with effluent application

Parameters	Effluent (mean)	Permissible limit (EPA 2002)
pH	8.4±0.34	5-9
Color	2849±224	Dark Brown
Total solid (mg L^{-1})	2021±112	-
Total dissolved solid (mg L^{-1})	1587±30.63	30
Total suspended solid (mg L^{-1})	77±1.21	35
Chemical oxygen demand (mg L^{-1})	23514±241.00	120
Biological oxygen demand (mg L^{-1})	8146±189	40
Electrical conductivity (ms cm^{-1})	1740 ±74.00	1000
Total Phenols (mg L^{-1})	459±23.25	0.50
Total nitrogen (mg L^{-1})	141±4.10	143
Sulphate (mg L^{-1})	1776±09.70	250
Phosphorus (mg L^{-1})	1890±5.84	200
Chloride (mg L^{-1})	6.15±0.30	1500
Na^+ (mg L^{-1})	416±14.20	200
K^+(mg L^{-1})	17.65±0.70	-
Lignin (ppm)	6641±124.21	-
Chlorophenol (mg L^{-1})	512±10.13	3.0
	Heavy Metals (mg L^{-1})	
Iron	77.54±1.79	2.00 (mg/L)
Zinc	47.10±0.40	2.00 (mg/L)
Copper	5.11±0.07	0.50 (µg/L)
Cadmium	7.33±0.01	0.01 (µg/L)
Manganese	18.27±0.27	0.20 (µg/L)
Nickel	6.22±0.04	0.10 (µg/L)
Chromium	1.03±0.01	0.1 (µg/L)
Lead	12.06±0.03	15 (µg/L)
Arsenic	14.02±0.05	0.01 (ppm)
Magnesium	11.69±0.06	0.05 (mg/L)

All the values are three ± SD replicates

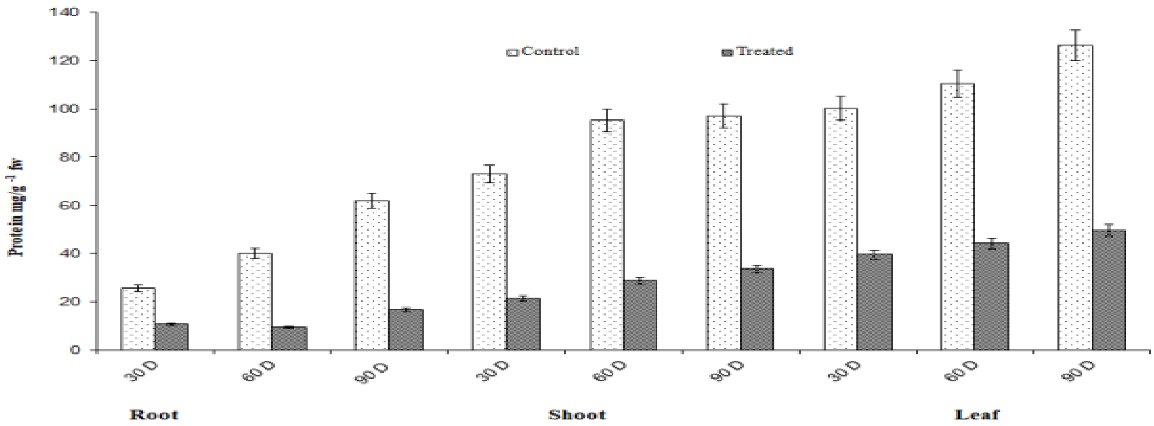

Fig.1: Effect of effluent on protein content in fenugreek plant

Accumulation and distribution of metals in plants

The accumulation and distribution of different heavy metals in different parts of plants was higher in effluent treated plants than control. The increase in concentration of heavy metals in various parts of plant was manyfold as compared to control. Among these metals, the maximum and minimum amounts of Fe and Cd in plants were recorded in shoot and roots respectively, which may be due to higher absorbing capacity of these elements by plants. The mechanism of metal absorption in various parts of the plant is significantly influenced by wastewater contents such as pH, cation exchange capability, organic matter and metal concentration. The highest accumulation of Cu in root (19.55 mg/g)> shoot (16.43 mg/g) > leaf (10.65 mg/g), while the highest concentration of Ni was in shoot (41.26 mg/g) > root (17.32 mg/g) > leaf (14.12 mg/g) of fenugreek.The toxicity of Ni in reduced the length of root and shoot, germination index, biomass, necrosis and inhibits the various physiological processes along with oxidative damage in plant. The highest concentrations of Pb (19.9 mg/g) and As (22.67 mg/g) were recorded in shoot of fenugreek, respectively. The concentration of Cr was found higher in shoot (21.87 mg/g).The significantly highest concentration of Fe (39. mg/g), Mn (36.21 mg/g) and Cd (11.74 mg/g) wererecorded in root, shoot of fenugreek. The concentration of Zn was less in decreasing order in root (27.17 mg/g) > shoot (20.47 mg/g) > leaf (14.57 mg/g).In general higher concentrations of these elements were recorded with effluent application over control.

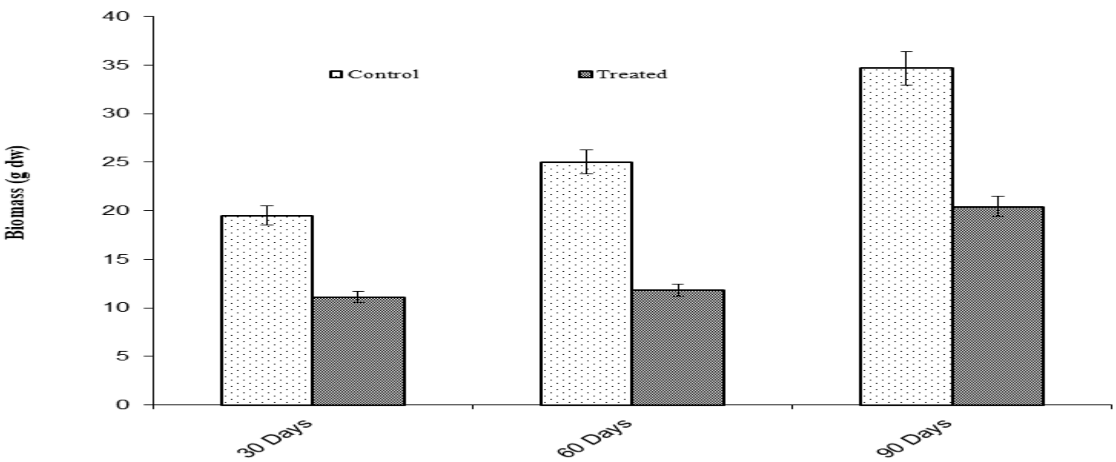

Fig.2: Effect of effluent on biomass in fenugreek plant

It may be concluded from the results that pulp paper industry effluent contained several pollutants along with heavy metals. The toxiceffect of pulp paper industry effluent on fenugreek showed a reduction in photosynthetic pigment, and accumulation of metals in high concentration.The comparative heavy metals accumulation was higher in stem. The biomass was also reduced with effluent application.There is an urgent need for the development and optimization of the tertiary stage treatment process before pulp paper industry effluent could be discharged safely into the environment.

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