NULLIFYING THE TOXICITY OF DISTILLERY EFFLUENT BY BIOADSORBENTS

GOWSALYA DEVI A.P.[1*] AND V. MARIAPPAN[2]

[*] Department of Botany, The Standard Fireworks Rajaratnam College for Women, Sivakasi, TN –India- 626 123
Department of Botany, Raja Doraisingam Govt. Arts College, Sivagangai, Tamilnadu – India – 630 561.

Abstract

The physico-chemical characteristics of distillery effluent was toxic as it contained high values of EC, TDS, TS, cations, sulphate, sodium, potassium, calcium, magnesium and nitrate. A high value of BOD and COD was noted and it is devoid of DO. The impact of distillery effluent on the morphometric, biochemical and enzymatic characteristics of the vegetable crop was analyzed after 90 days. The reduction in all the growth characteristics and biomass accumulation was parallel with reduction in carbohydrate and pigment content. The protein content and nitrate reductase also decreased with increasing concentration of distillery effluent. An increased leaf nitrate, free amino acids, L-proline, peroxidase and catalase indicated the stress nature of the plant. Applications of low cost environment friendly bioadsorbent (*Azolla*) ameliorate the stress caused due to toxicity of distillery effluent. A significant increase in germination percentage, growth, biochemical and enzymatic characteristics was noted in the bioadsorbent treated experimental plant than when they were treated with various concentration of distillery effluent alone.

Key words: *Azolla*, bioadsorbent, distillery effluent, toxicity.

I.INTRODUCTION

Sugar industries create significant impact on Indian economy and in particular the rural economy. In India, the distillery industries use molasses which is the mother liquor left after the crystallization of sugarcane juice as a raw material for the production of alcohol. In India, the contribution of distillery waste to organic pollution is approximately seven times more than the entire Indian population. The effluent discharged from distillery industries constitutes nearly 15 times the total alcohol production.Distillery effluent is highly polluted due to the presence of high load of organic and inorganic content and is highly acidic. Many workers have shown deleterious effects of distillery effluents on plant parameters at higher concentrations [1,2,] But the present study focused on the application of cheap and naturally available bioadsorbent (*Azolla* biomass) for the morphometric, biochemical and enzymatic assessment of C*yamopsis tetragonoloba* (L.) Taub irrigated with various concentration of distillery effluent.

Azolla is a free floating water fern, which occurs in the symbiotic association with N_2 fixing blue green alga *Anabaena Azollae.* It has high rate of N_2 fixation making its biomass rich in nitrogen and protein [3]. It is mainly used as green manure in agriculture and also used for bioremediation of waste water. The dried *Azolla* biomass can be cultivated easily as it has very less generation time, larger biomass production, relative higher capability of pollutant uptake, and better purification effects due to direct contact with contaminated water.

II. MATERIALS AND METHODS

Source of Distillery Effluent

The distillery effluent was collected directly from the outlet of the industry which was used by the farmers of that area for irrigation purposes. Effluent samples were collected in bottles which were thoroughly cleaned by repeated washing with distilled water and rinsed thrice with the sample water before collection. The effluent was stored in the dark at room temperature. The distillery effluent collected was analyzed as per the method of [4].The effluent was diluted to different concentration such as 20%, 40%, 60%, 80% and 100% (v/v).

Seed Collection and treatment

Healthy and viable seeds of C*yamopsis tetragonoloba* (L). Taub selected for present study, were procured from a seed vendor, certified by Tamilnadu Seed Certification Department, Rajapalayam, Tamilnadu. Seeds were surface sterilized with 0.1% mercuric chloride for 2 minutes and rinsed twice with distilled water. To evaluate the germination efficiency, spread the seeds on the sterilized petridishes lined with filter paper and irrigated with 5ml of different concentration of distillery effluent. Triplicates were maintained for each concentration along with control.

Seedling treatment

In the first set of experiment, Surface sterilized seeds were sown and allowed to grown in different earthen pots containing uniformly mixed garden soil (sand, red soil and black soil (1:1:1 ratio)). After 10 days, seedlings of C*yamopsis tetrogonoloba*(L). Taub were treated with various concentration of distillery effluent (20%, 40%, 60%, 80% (v/v)). After 10 days of effluent treatment, various morphometric, biochemical and enzymatic characteristics were analyzed. *Azolla*, an aquatic fern was grown in plastic troughs and were then harvested and allowed to dry, powdered and used as bioadsorbent. In the second set of experiment, 10 days old seedlings of C*yamopsis tetrogonoloba* (L). Taub were treated with bioadsorbent- *Azolla* biomass (5gm) integrated with various concentration of distillery effluent. Triplicates were maintained for both control and experimental plants.

Growth and Biochemical parameters.

Various morphometric characters such as root length shoot length, fresh and dry biomass after 10 days of sowing were measured. The germination percentage, shoot length, root length, fresh weight and dry weight [5] The plant vigour index [6] were calculated. Twenty days old plants of C*yamopsis tetrogonoloba* (L). Taub were used for analyzing the biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid [7] Total soluble sugar [8] Protein [9] free amino acid content [10] Proline [11] leaf nitrate [12] invivo nitrate reductase activity [13] Catalase activity [14] Peroxidase [15]

Statistical analysis

Growth parameters were determined with ten independent replicates. Biochemical characters and enzymatic assay were carried with five replicates. The data were reported as mean ± SE (Standard Error) and in parentheses represent the percent activity.

III. RESULTS AND DISCUSSION

The physico chemical characteristics of the distillery effluent are presented in Table-1. It is clear that, the distillery effluent was dark brown in colour and pH was acidic (4.2). The total solids and total dissolved solids were 42,400mg/l and 36,200mg/l in the distillery effluent. An elevated level of EC (16,450 micromhos/cm) indicated high concentration of ionic elements. Due to heavy load of organic and inorganic matter, tremendous amount of BOD (32,300mg/l) and COD (57,164mg/l) was observed with respect to lack of dissolved oxygen. High amount of plant nutrients such as K, P, Na, C, Mg and sulphate ions resulted in the high toxicity level of distillery effluent.

Effect of four different concentrations (20%, 40%, 60% and 80% (v/v) of distillery effluent on the growth, biochemical and enzymatic activities are represented in Table 2 to 5.The result showed that the morphometric characters such as root length, shoot length, fresh and dry weight decreased with increase in the concentration of distillery effluent. Similarly chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid content diminished with increasing concentration of the effluent. Total soluble sugar, protein and *invivo* nitrate reductase activity showed a declining trend with increasing concentration. In contrary leaf nitrate, free amino acids, proline content and the activities of antioxidant enzymes such as peroxidase and catalase were increased.

Germination percentage was reduced to 10% in 80% (v/v) concentration and beyond that in 100% (v/v) concentration, inhibition of germination was observed which coincides with the findings of [16] in C*yamopsis tetrogonoloba* (L). Taub irrigated with electroplating industry effluent. At 80% (v/v) concentration of distillery effluent, the root length was reduced to 43% and maximum reduction in shoot length was observed at 80% (v/v) concentration as 54%. The inhibitory effect of distillery effluent on seed germination, seedling growth and development of phytotoxic effect is an outcome of its high salt content. [17].

Distillery effluent contained 6,200mg/l of TSS and 2,432mg/l of total hardness which lead to inhibition of root and shoot growth at higher concentration of the effluent which is correlated with the findings of [18] in wheat, peanut and green gram. A reduction in root and shoot length in horse gram was reported by [19] with increasing concentration of distillery effluent which affects the respiration of root, which in turn reduce the oxygen and increase CO_2 concentration that cause less aeration and retard the growth of the plant. In the present study, the degree of inhibition on the growth of C*yamopsis tetrogonoloba* (L).Taub increased with increase in concentration of the effluent when compared to control. A maximum reduction of fresh weight (49%) and dry weight (45%) was observed in 80% (v/v) concentration. The pronounced inhibition of shoot and root growth were the main cause for the decrease in fresh and dry weight of seedlings which is similar to the findings of [20] in *Cicer arientinum*. Inhibition of biomass accumulation was directly related to the photosynthetic process at higher concentration of effluents [21].

TABLE 1: Physico chemical characteristics of Distillery Effluent

S.No	PARAMETERS	VALUES/CHARACTERS
1	pH	4.2
2	Color	Dark brown
3	Temperature	30^0C
4	Electrical conductivity	16450
5	Total solids	42400
6	Total dissolved solids	36200
7	Total suspended solids	6200
8	Total hardness	2432
9	Salinity	2864
10	Sodium	580
11	Potassium	6210
12	Calcium	2070
13	Magnesium	2260
14	Sulphate	5000
15	Chloride	8530
16	Nitrogen	1254
17	Phosphate	44
18	Dissolved Oxygen	Nil
19	BOD	32300
20	COD	57164

All the values are expressed in mg/l except Temperature, pH and Electrical Conductivity (micromhos/cm)

Table 2 Effect of distillery effluent on the growth parameters of *Cyamopsis tetragonoloba (*L.)Taub

S.No	Parameters	Control (Water)	Concentration of distillery effluent			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Germination (%)	90	80	50	20	10
2.	Shoot Length(cm)	17.5 ±0.159 (100)	16.2 ±0.180 (92)	14.8 ±0.126 (85)	10.7 ±0.591(61)	9.6 ±0.172 (54)
3.	Root Length(cm)	7.4 ± 0.183 (100)	6.8 ±0.220 (92)	5.9 ±0.165 (80)	4.6 ±0.189 (62)	3.2 ±0.127 (43)
4.	Fresh weight(gm)	2.96 ± 0.159 (100)	2.59 ±0.094 (88)	1.88 ±0.091(64)	1.72 ±0.088(58)	1.46±0.098(49)
5.	Dry weight(gm)	1.86 ±0.098 (100)	1.36 ±0.080 (73)	1.28 ±0.063 (69)	1.08 ±0.038(58)	0.84±0.019(45)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

At higher concentration, the effluent showed inhibitory effect on both photosynthetic pigments and total soluble sugar. Increasing concentration of EC, TDS, cations and anions present in the effluent destabilizes the leaf chlorophyll content and the maximum diminution of total chlorophyll content was found to be 54% in 80% (v/v) concentration. The reduction in pigment content is paralleled with the findings of [22]. Reduction in total soluble sugar content was paralleled with diminution in photosynthetic pigment and a maximum reduction of 39% total soluble sugar content and a minimum reduction of 19% were observed in 80% (v/v) and 20% (v/v) concentration of the effluent respectively. Similar reduction in total soluble sugar content was reported by [23] in *Acacia auriculiformis* irrigated with winery waste water. In the present study, the reduction in protein content was very obvious with an increase in concentration of the effluent when compared to control. These results are in agreement with the findings of [24] in tomato and capsicum and in paddy irrigated with dairy effluent and [25].

A reduction in soluble protein level eventually leads to an increase in free amino acid content. The results of the study showed that a 419% hike of free amino acid content was found in *Cyamopsis tetragonoloba* grown in 80% (v/v) concentration compared to control. A similar observation was reported by [26] in *Phaseolus aureus* irrigated with distillery effluent, [27] in *Acacia mangium* irrigated with tannery effluent. The proline content was pronounced at 80% concentration and the value ranges from 140% to 259% for 20% to 80% distillery effluent concentration. Proline accumulation helps to conserve nitrogenous compounds and protect the plants against distillery effluent stress. It is also supported by [28] that proline acts as a membrane stabilizing agent under stress conditions.

Another natural response of metabolic process of plant is nitrate assimilation. The leaf nitrate contents showed a wide variation in response to distillery effluent treatment. The leaf nitrate content was increased to 193% in 80% (v/v) concentration compared to control. It was found that at 80% (v/v) concentration of the distillery effluent, the NR value significantly reduced to 30% in the experimental plant. Similar increase in leaf nitrate content, reduction in *invivo* NR activities with increase in concentration of sugarcane industrial effluent on *Abelmoschus esculentus* has been already reported in bhendi irrigated with sugar industry effluent. [29]

An enhanced peroxidise activity was observed with the increase in concentration of distillery effluent. The increase was about 4%, 8% and 10% in *Cyamopsis tetragonoloba* grown in 40% 60% and 80% (v/v) concentration of the distillery effluent respectively compared to control plants. The observed increase in peroxidase activity can be correlated with the reduction in chlorophyll content, fresh weight and dry weight. Similar result has been reported in *Lycopersicum esculentum* and *Eleusine coracana* irrigated with paper mill effluent [30] and [31] in *Cyamopsis tetragonoloba (*L.)Taub irrigated with match industry effluent. Catalase is another anti-oxidant, scavenging enzyme and it was found to be increased with the increasing concentration of the effluent. The increase of catalase activity was about 23%, 85%, 269% and 531% in *Cyamopsis tetragonoloba* while treated with 20%, 40%, 60% and 80% (v/v) distillery effluent concentration respectively. These results are line in with the findings of [32] in *Vigna mungo* irrigated with tannery effluent and [33] in green gram irrigated with electroplating industry effluent.

Table 3 Effect of distillery effluent on the Pigment content of *Cyamopsis tetragonoloba (*L.)Taub

S.No	Parameters	Control (Water)	Concentration of distillery effluent			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Chlorophyll- a (mg/g LFW)	1.16 ±0.023 (100)	1.03 ±0.023 (88)	0.81 ±0.029 (69)	0.74 ±0.029 (63)	0.63±0.029 (54)
2.	Chlorophyll-b (mg/g LFW)	0.37 ± 0.029 (100)	0.33 ±0.029 (89)	0.27 ±0.012 (72)	0.23 ±0.012 (62)	0.19 ±0.025 (51)
3.	Total Chlorophyll (mg/g LFW)	1.53 ±0.023 (100)	1.36 ±0.017 (88)	1.08 ±0.012 (71)	0.97 ±0.046 (63)	0.82 ±0.040 (54)
4.	Carotenoids (mg/g LFW)	1.12 ±0.046 (100)	0.98 ±0.052 (88)	0.81 ±0.023 (71)	0.69 ±0.040 (63)	0.61 ±0.023 (54)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

Table 4 Effect of distillery effluent on the biochemical characters of *Cyamopsis tetragonoloba (*L.)Taub

S.NO	Parameters	Control (Water)	Concentration of distillery effluent			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Total soluble sugar (mg/g LFW)	150.3 ±7.018 (100)	122.3 ±4.79 (81)	110.8 ±3.52 (73)	103.36±1.01 (69)	91.33 ±1.61 (61)
2.	Protein (mg/g LFW)	8.21 ±0.139 (100)	6.33 ±0.953 (77)	4.68 ±0.266 (57)	3.41 ±0.109 (41)	2.90 ±0.115 (35)
3.	Amino acid (mg/g LFW)	0.59 ±0.023 (100)	0.72 ±0.052 (122)	1.41 ±0.029 (239)	1.72 ± 0.052 (292)	2.47 ±0.046 (419)
4.	Proline (mg/g LFW)	27.18 ±1.553 (100)	37.93 ±0.121 (140)	49.41 ±0.958 (181)	59.15 ±0.924 (218)	70.31 ±0.964 (259)
5.	Leaf Nitrate (mg/g LFW)	28.69 ±0.491 (100)	34.44 ±0.890 (120)	41.82 ±0.976 (146)	45.29 ±1.622 (158)	55.24 ±1.195 (193)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

Table 5 Effect of distillery effluent on the enzymatic activities of *Cyamopsis tetragonoloba (*L.)Taub

S. NO	Parameters	Control (Water)	Concentration of distillery effluent			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Nitrate reductse (µ mole/g LFW)	24.6 ±1.106 (100)	23.3 ±1.716 (95)	22.15 ±0.707 (90)	19.94 ± 0.582 (81)	17.27 ±0.769 (70)
2.	Catalase (µ mole/g LFW)	0.00065 ±0.0001 (100)	0.0008 ±0.0001 (123)	0.0012 ±0.0001 (185)	0.0024 ±0.0002 (369)	0.0041 ±0.0002 (631)
3.	Peroxidase (µ mole/g LFW)	835.2 ±4.329 (100)	854.1 ±5.894 (102)	874.61 ±2.862 (104)	900.53 ±2.925 (108)	923 ±3.512 (110)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

Effect of bioadsorbent (*Azolla* biomass) integrated with four different concentrations (20%, 40%, 60% and 80% (v/v) of distillery effluent on the growth, biochemical and enzymatic activities are represented in **Table 6 to 9**. Addition of bioadsorbent nullify the toxicity of distillery effluent and the growth parameters such as root length, shoot length, fresh and dry weight increased in all the concentration of distillery effluent compared to effluent alone treated plants. Many researchers have been reporting the removal of different pollutants by using plant based bioadsorbents [34, 35, 36] *Azolla* biomass has been used as a bioadsorbent for the removal of copper from industrial waste water [37].

Percentage of seed germination showed increasing trend from 20% (v/v) to 80% (v/v) concentration of the effluent treated with the bioadsorbent -*Azolla* biomass. This may be due to the fact that the bioadsorbent -*Azolla* biomass released certain adsorbed products such as nutrients and growth regulators which become available to the plants. The highest root length (7.7cm) and shoot length (18.3cm) were recorded in 20% (v/v) concentration compared to effluent alone treated plants. The results of the present study clearly showed that treatment using bioadsorbent in all the four concentration facilitated the germination process, increased root length and shoot length and a huge biomass accumulation of *Cyamopsis tetragonoloba (*L.)Taub compared to the effluent alone treated plants.

Similarly chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid content significantly increased after the application of bioadsorbent in all the concentration of the effluent. A pronounced recovery was observed in 20% (v/v) concentration. *Datura* biomass – bioadsorbent has the ability to relieve the toxicity of paper mill effluent and an increase in the pigment content was reported in *Lycopersicum esculentum.* [38] Chlorophyll content significantly increased in the sugar industry effluent treated *Solanum melongena* after the addition of bioadsorbents [39].

Table 6 Effect of distillery effluent with *Azolla* on the growth parameters of *Cyamopsis tetragonoloba (*L.)Taub

S. No	Parameters	Control (Water+ *Azolla*)	Concentration of distillery effluent + *Azolla* biomass			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Germinationpercentage(%)	95	90	60	40	30
2.	Shoot length(cm)	19.2 ±0.16(100)	18.3 ± 0.18(95)	17.7 ±0.13(92)	13.5±0.17(70)	11.9 ±0.23(62)
3.	Root length(cm)	9.2 ±0.161(100)	7.7 ±0.218(84)	6.9 ±0.133(75)	6.1 ±0.141(63)	4.8 ±0.163(50)
4.	Fresh weight(gm)	4.28 ±0.13(100)	3.8 ±0.09(89)	3.41 ±0.02(80)	2.92 ±0.04(68)	2.3 ±0.06(54)
5.	Dry weight(gm)	3.23 ±0.05(100)	2.81 ±0.71(87)	2.2 ±0.07(68)	1.81 ±0.09(56)	1.41 ±0.08(44)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

Table 7 Effect of distillery effluent with *Azolla* on the pigment content of *Cyamopsis tetragonoloba* (L.)Taub

S. NO	Parameters	Control (Water+ *Azolla*	Concentration of distillery effluent + *Azolla* biomass			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Chlorophyll-a (mg/g LFW)	1.84 ±0.029 (100)	1.77 ±0.029 (96)	1.51 ±0.075 (82)	1.29 ±0.017 (71)	1.15 ±0.029 (63)
2.	Chlorophyll-b (mg/g LFW)	1.101 ±0.023 (100)	1.02 ±0.040 (93)	0.93 ±0.035 (84)	0.87 ±0.029 (79)	0.73 ±0.029 (66)
3.	Total Chlorophyll (mg/g LFW)	2.95 ±0.109 (100)	2.79 ±0.064 (95)	2.37 ±0.029 (80)	2.07 ±0.029 (70)	1.82 ±0.064 (62)
4.	Carotenoids (mg/g LFW)	1.54 ±0.04 (100)	1.48 ±0.035 (96)	1.21 ±0.04 (79)	1.14 ±0.29 (75)	1.04 ±0.29 (67)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

Table 8 Effect of distillery effluent with *Azolla* on the biochemical characters of *Cyamopsis tetragonoloba* (L.)Taub

S. NO	Parameters	Control (Water+ *Azolla)*	Concentration of distillery effluent+ *Azolla* biomass			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Total soluble sugar (mg/g LFW)	390.4 ±3.30 (100)	370.2 ±1.525 (95)	351.4 ±1.617 (90)	340.8 ±1.909 (87)	250 ±1.069 (64)
2.	Protein (mg/g LFW)	40 ±1.528 (100)	34 ±2.00 (85)	31 ± 1.528 (78)	26 ±1.528 (65)	19 ±1.528 (48)
3.	Amino acid (mg/g LFW)	0.166 ±0.17 (100)	0.187 ±0.01 (113)	0.192 ±0.026 (116)	0.219 ±0.015 (132)	0.348 ±015 (210)
4.	Proline (mg/g LFW)	18.81 ±0,196 (100)	19.24 ±0.225 (102)	24.85 ±1.398 (132)	35.41 ±0.997 (188)	44.58 ±2.194 (237)
5.	Leaf Nitrate (mg/g LFW)	6.06 ±0.049 (100)	7.03 ±0.056 (116)	7.49 ±0.305 (124)	9.16 ±0.102 (151)	11.05 ±0.151 (182)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error

Table 9 Effect of distillery effluent with *Azolla* on the enzymatic activities of *Cyamopsis tetragonoloba* (L.)Taub

S. NO	Parameters	Control (Water+ *Azolla*	Concentration of distillery effluent + *Azolla* biomass			
			20% (v/v)	40% (v/v)	60% (v/v)	80% (v/v)
1.	Nitrate reductase (µ mole/g LFW)	29.87 ±0.765 (100)	27.2 ±3.073 (118)	26.3 ±0.839 (88)	24.6 ±0.781 (82)	22.9 ±0.961 (77)
2.	Catalase (µ mole/g LFW)	0.00045±0.0001 (100)	0.00062±0.0001 (138)	0.0007±0.0002 (156)	0.0012±0.0002 (267)	0.0024 ±0.0003 (533)
3.	Peroxidase (µ mole/g LFW)	798.61 ± 4.429 (100)	822.42 ±2.664 (103)	839.48 ±3.419 (105)	862.71 ±1.897 (108)	884.8 ±2.590 (111)

Values in parenthesis indicate percent activity with respect to control;
Value represents mean of 5 samples with their standard error (±)

An increase in total soluble sugar, protein content and decrease in free amino acid and proline after the addition of bioadsorbent in the present study indicated the active promotive nature of *Azolla* biomass on plant growth and metabolism. In *Azolla* treated *Cyamopsis tetragonoloba* L. Taub, the total soluble sugar content was recorded as 95% and 64% in 20% (v/v) and 80% (v/v) concentration of distillery effluent compared to 81% and 61% in the effluent treated alone respectively. The protein content was found to be 85% and 48% compared to 77% and 35% in 20% (v/v) and 80% (v/v) distillery effluent concentration respectively. A reduction in metal toxicity and a promotion in sugar and protein content in *Eleusine coracana* by the addition of bagasse bioadsorbent were reported by [40]

Free amino acid content was found to be reduced from 419% to 210% in 80% (v/v) concentration of the effluent. The amendment improved the quality of the experimental plant by recovering from stress and hence the reduction of free amino acids to the maximum. Similarly a reduction in proline content was also noted in all the concentration of the effluent indicated the stress relieving nature of the effluent. Accumulation of proline is also considered as an adaptive response to stress [41] and hence a reduction in proline content is an indication of stress relief.

In *Azolla* treated *Cyamopsis tetragonoloba* (L.) Taub a maximum reduction in the leaf nitrate content paralleled with an increase in *invivo* nitrate reductase activity was observed compared to effluent alone treated plants.This may be due to the bioadsorption by plant biomass to remove or adsorb toxic elements present in the effluent. Our results are in accordance with the findings of [42]. Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. Upon the addition of dried biomass of *Azolla*, to the effluent treated seedlings of *Cyamopsis tetragonoloba* (L.) Taub, these enzyme activities significantly decreased than in the effluent alone treated plants. It is paralleled with the findings of [43]

The present investigation was focussed on the application of low cost bioadsorbent in the soil to nullify the toxic effects of distillery effluent. The result of the present study clearly stated that the addition of bioadsorbent to the various concentration of distillery effluent showed improved germination percentage, root length, shoot length, biomass, biochemical parameters and enzymatic activities compared to the effluent alone treated plants. As *Azolla* biomass can efficiently remediate the toxicity of distillery effluent, it can be safely used for the irrigation purpose to promote sustainable agriculture.

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