Potential Use Of A Mix Of Fly Ash And Diluted Distillery Effluent In Agriculture: A Case Study On The Vegetative Growth And Photosynthetic Pigments Of The Ornamental Plant, Calendula Officinalis

S. N. C. Ray*, N. Ranjan, K. Kumari*** & R. C. Sinha******

* (Centre for Environment & Nature Conservation, Deptt. of Zoology, Patna University, Patna-800005,
**(Centre for Environment & Nature Conservation, Deptt. of Zoology, Patna University, Patna- 800005
***(CSIR- NEERI, Nehru Marg, Nagpur 440020, Maharashtra, India
****(Centre for Environment & Nature Conservation, Dept. of Zoology, Patna University, Patna- 800005

Abstract

The disposal of post methanated diluted effluent (PMDE) from the distillery and fly ash from the thermal power plants has assumed a cosmic dimension in India because they are produced in huge quantities. Thus, their gainful utilization is imperative. In the present paper their gainful utilization for sustainable development has been discussed herein. The results have revealed that the electrical conductivity and water retention capacity have increased in the fly ash amended soil without any marked change in the pH. Vegetative growth such as root length, number of branches, leaves and leaf area, shoot length and number of flowers have increased in fly ash amended soil. Similarly, the total chlorophyll, carotenoids and ascorbic acid are more in fly ash amended soil which clearly indicate that a mix of fly ash and diluted distillery effluent plays a significant role in the vegetative growth and in the synthesis of photosynthetic pigments. The results have also revealed the ratio of chlorophyll: carotenoid decreases as temperature increases in April in contrast to ascorbic acid. Another interesting observation has been in the present study that maturity (flowering) of the plant is earlier in the fly ash amended soil. It is suggested that a mix of fly ash and diluted distillery effluent can be used as a potential bio- fertilizer replacing chemical fertilizer eventually bringing another 'green revolution' in India.

Keywords- Ascorbate, Bio-fertilizer, Calendula, Carotenoids, Chlorophyll, Fly ash, PMDE.

1. Introduction

Molasses (one of the important by-products of sugar industry) is the chief source of production of alcohol in distilleries by fermentation method. Approximately, 40 billion litres of waste water annually discharged in distilleries known as raw spent wash (RSW) which is characterized by high biological oxygen demand (BOD: 40,760- 45,000 mg/l), chemical oxygen demand (COD: 100,000-112,000 mg/l) (Table-1). In the distillery industries, every litre of alcohol produced about 15 litres of spent wash is released as waste water. It has undesirable color and foul smell [1]. Discharge of raw spent wash into open land or in a nearby water bodies results in a number of environmental, water and soil pollution including threat to crops and animal lives. Hence, the Indian distilleries have been directed by Ministry of Environment & Forests, Govt. of India to achieve zero discharge into surface water from Dec, 2005 and the distillery units have to opt for any one of the three: (1) Ferti-irrigation, (2) Bio- composting and (3) Incineration. Most of the distillery units in India have opted for ferti-irrigation. In ferti-irrigation, the yield of mustard seeds, *Brassica compestris* have increased by 30% when compared with the control using di-ammonium phosphate (DAP) and urea [1] and the yield of wheat (*Tritiuim aestivium*) increased by 33% [2] suggesting that diluted distillery effluent is capable of replacing the application of chemical fertilizer when used under controlled conditions without having any adverse effect on the soil and ground water quality. India has witnessed a tremendous increase in food grain production after the post-green revolution. Now, large scale efforts are on for agro-based industrialization to utilize the agro-based wastes and to add value to agriculture. The major agro-based industries are the distilleries, sugar mills, pulp and paper mills, textiles which produce such a large quantity of waste waters that the nature system is tillable to assimilate it. The production of alcohol bears immense significance as a raw material for rapidly advancing chemical industry, its export potential and as a readily available source of energy. Therefore, in the present scenario as well as for future, demand for alcohol will increase in the country and so also the number of distilleries producing alcohol. As such, waste water from the distilleries shall also increase and therefore, the use of diluted distillery effluent should be used for ferti-irrigation as an alternative of chemical fertilizer because of its high content of N, P, K as macronutrients and organic matter [1].

The other waste which is produced in huge quantity is fly ash produced by thermal power plants. Over the past few decades there has been a keen interest in developing the strategies for the utilization of fly ash. The potential of fly ash as a resource material in agriculture is due to its specific physical properties like its texture, water holding capacity, bulk density, pH and contains all the essential micronutrients essential for the growth of the plant such as boron, copper, iron, manganese and lime [3], [4] (Table 2). These micronutrients are essential for the proper growth of a plant because they work "behind the scene" as activators of plant functions [5].

Recently, there have been few reports that in some states, the soil and ground water have been adversely affected due to ferti-irrigation in contrast to the observations made by [1], [2], [5] in case of United Spirit Ltd., Hathidah, Bihar. As such, it can be said that the technology of ferti-irrigation cannot be undermined but due to the ignorance of the distillery units and the complacence of the regulatory authorities permitting ferti-irrigated water more than 100 mg/l BOD in the fields might have resulted in the adverse effects on soil and ground water. It is the duty of the Regulatory Authority to abide by the Protocol of Ferti-irrigation issued by MoEF, Govt. of India. Diluted spent wash could be used for irrigation purpose without adversely affecting soil fertility [6], [7], [5], crop productivity [8], [1], [2]. The diluted spent wash irrigation improved the physical and chemical properties of the soil and further increased the soil micro-flora [9], [10], [6]. Increased concentration of spent wash causes decreased seedling growth and chlorophyll content in sun flowers (*Helianthus annuus*) and spent wash could safely be used for irrigation purpose at lower concentration [11], [8]. Mineralization of organic material as well as nutrients present in spent wash is responsible for increased availability of plant nutrients.

In view of the adverse reports received regarding ferti-irrigation, an alternative method was considered by our Centre to study, the effect of diluted distillery effluent and fly ash which are very rich in micronutrients and would serve as a very good potential bio- fertilizer. To prove this hypothesis, a mix of fly ash and diluted distillery effluent was studied on amended soil characteristics as well as vegetative growth and some biochemical constituents such as chlorophyll 'a' & 'b', total chlorophyll, carotenoids and ascorbic acid of the leaf of *Calendula officinalis* in pot conditions with a view to extrapolate the effect of a mix of fly ash and distillery effluent in the field conditions on wheat and mustard based on the laboratory results. In the field conditions, the yield of potato (*Solanum tuberosum*) increased significantly when treated with a mix of fly ash and diluted distillery effluent (Ray *et al*, unpublished data).

Chlorophyll is a complex molecule. Several modifications of chlorophylls occur among plants and other photosynthetic organisms. Chlorophyll 'a' absorbs its energy from the violet-blue and reddish orange-red wavelengths and little from the intermediate (Green-yellow orange) wavelengths. Carotenoids and chlorophyll 'b' absorbs some energy in the green-wavelength. Both chlorophylls also absorb in the orange-red end of the spectrum. Chlorophyll 'a' is reported to be thermally less stable than chlorophyll 'b' [12], [13], [14], [15], [16]. Thermal degradation of chlorophylls 'a' & 'b' in green pea was studied by [17] and reported that chlorophyll 'a' degraded 12 to 18 times faster than chlorophyll 'b' depending on temperature indicating that chlorophyll 'a' is more susceptible to thermal temperatures. Carotenoids protect chlorophylls from photo-oxidative destruction [18] and therefore, a reduction in carotenoids could have serious consequence on chlorophylls [19]. Carotenoids are effective quenchers of reactive oxygen species (ROS) and play an important role and ultimate survival of plants during stress. Decreased levels of catalase and carotenoids as well as increased levels of peroxide induced by oxidative stress reflect a general strategy required for stress and protect cells against damage.

Ascorbate (Vitamin C) can reach very high concentrations in chloroplasts (20-300 mM). The pool size in leaves and chloroplasts increases during high light intensity. Multiple functions of ascorbate in photosynthesis have been proposed [20], [21], [22], [23]. The total ascorbate in leaves is light dependent. Growth at high light intensity produces leaves with higher ascorbate content than low light intensity [24], [25], [26].

The aim of this study is to evaluate the effectiveness of a mix of fly ash and diluted distillery effluent on the vegetative growth and biochemical constituents of the ornamental plant, *Calendula officinalis*.

2. Materials and Methods

Calendula officinalis plants are very important ornamental plants cultivated outside as winter annual plants. It belongs to the family *Asteraceae* and is used for land scaping as a source of color in the gardens and as cut flowers. Ideal soil profiles for the growth of *Calendula officinalis* are light to sandy and moderately rich soils. The soil must be fairly moist and tolerates a pH range of acidic 4.5 to a very alkaline 8.3. It is considered as one of the most valuable medicinal plants and used in treating cancer as well as cardio-vascular diseases. It has anti oxidant activity [27]. Earthen pot having the diameter of 25 cm was carried out from December to April end.

Pot experiments with *Calendula officinalis were* carried out in 3 sets which are as follows.

Pot 1: Ordinary garden mud + water (Control)
Pot 2: Ordinary garden mud + diluted distillery effluent (BOD ≤ 100 mg/l) Experimental 1 (E_1)
Pot 3: Ordinary garden mud + fly ash in the ratio (9: 1) with diluted distillery effluent (Experimental 2) (E_2).

Soil conditions of control, E_1 and E_2 were carried out for pH, electrical conductivity (EC), nitrate and water holding capacity according to the method of [28]. Plant height was measured by measuring tape. Leaf surface area was estimated graphically by outlining the leaf on a graph paper and counting the number of squares (cm^2). The vegetative growth such as root length, shoot length, number of branches, number of leaves, leaf area and flowers were determined.

Besides the morphological parameters, biochemical parameters of photosynthetic pigments such as chlorophyll ‘a’ and ‘b’, total chlorophyll, carotenoids and ascorbic acid content of the leaves were determined. Fresh leaf samples were taken for the biochemical parameters. Chlorophyll ‘a’, ‘b’ and carotenoids were determined according to the method of [29]. Ascorbic acid was determined according to the method of [30] and nitrate by Kjeldahl method [28].

3. Results

Table 1

Characteristics of spent wash and effluent for ferti-irrigation

Characteristics	***RAW (Effluent)***	***Effluent for Ferti- irrigation***
Color	Dark- Brown	Light- Brown
Odour	Offensive	Negligible
pH	3.5- 3.7	7.1- 7.2
COD (mg/L)	100,000- 112,000	360.0 – 665.0
BOD (mg/L)	40,760- 45,000	153.0 - 290.0
Suspended Solids (mg/L)	300- 500	150.0 – 500.0
Dissolved Solids (mg/L)	8000- 11000	770.0 - 1030.0
TKN (mg/L)	100-175	1.28- 1.86
Chloride (mg/L)	4500- 6000	141.5- 177.0
Potassium(mg/L)	4500- 5500	150- 160
Sodium (mg/L)	1000- 1500	30- 40
Calcium (mg/L)	2500- 3500	85- 95
Magnesium (mg/L)	2000- 2500	75- 85

Table 2

Physico- chemical analysis of fly-ash values (Mean ± S.D.)

Characteristics	***(Value)***
pH	8.8 ± 0.42
Electrical Conductivity (µS/cm)	761.0 ± 28.0
Total Nitrogen (mg/g)	0.02 ± 0.001
Total phosphorus (mg/g)	0.14 ± 0.006
Organic Carbon (mg/g)	1.17 ± 0.59
Al (mg/g)	4615.0 ± 230.0
B (mg/g)	29.0 ± 1.26
Fe (mg/g)	4150.0 ± 207.0
K (mg/g)	9005.0 ± 450.0
Mn (mg/g)	70.0 ± 3.4
Na (mg/g)	5200.0 ± 260.0
Ca (mg/g)	82.0 ± 3.1
Cu (mg/g)	58.6 ± 2.83
Fe (mg/g)	4150 ± 207
K (mg/g)	9005 ± 450
Mn (mg/g)	70 ± 3.4
Na (mg/g)	5200 ± 260
Zn (mg/g)	82 ± 3.1

Table 3

Characteristics of soil in the experimental pot

Parameter	***Garden mud*** ***C***	***Diluted distillery effluent E_1***	***Fly-ash + Diluted distillery effluent E_2***	***C Vs E_1***	***C Vs E_2***
pH	7.73 ± 0.031	7.6 ± 0.006	7.9 ± 0.023	-1.7%	2.0%
Conductivity (µS/cm)	376.6± 10.53	400.8 ± 27.67	494 ± 6.42	6.0%	31.0%
Nitrate (mg/g)	0.38 ± 0.082	2.18 ± 0.21	1.43 ± 0.38	474.0%	276.0%
Phosphate (mg/g)	0.070 ± 0.001	0.076 ± 0.001	0.130 ± 0.002	8.6%	85.7%
Moisture content (% Change)	6.62 ± 0.04	6.42 ± 2.47	30.95 ± 2.47	3.0%	368.0%

Table 4

Vegetative growth of plant

Parameter	***Garden mud*** ***C***	***Diluted distillery effluent E_1***	***Fly-ash + Diluted distillery effluent E_2***	***C Vs E_1***	***C Vs E_2***
Length of Root (cm)	55.250 ± 3.775	84.667 ± 12.88	94.667 ± 9.452	53.2%	71.3%
Length of Shoot (cm)	8.033 ± 0.816	13.30 ± 2.066	15.450 ± 1.682	65.0%	92.3%
No. of Branches	3.0 ± 1.0	8.0 ± 2.0	25.0 ± 5.0	166.7%	733.3%
No. of Leaves	55.0 ± 5.0	140.0 ± 13.0	191.0 ± 8.0	154.5%	247.3%
Area of Leaf (cm^2)	19.03 ± 5.327	25.9 ± 5.105	29.6 ± 7.532	36.1%	45.0%
No. of Flowers	2.0 ± 1.0	3.0 ± 1.0	14.0 ± 3.0	50.0%	600.0%

Table 5

Biochemical Parameters

Parameters	***Month***	***Garden mud*** ***C***		***Diluted distillery effluent E_1***	***Fly-ash + Diluted distillery effluent E_2***	***C Vs E_1***	***C Vs E_2***
Ascorbic acid Content (mg/g of leaf tissue)	Early February	0.85 ± 0.204		0.98±0.021	1.65±0.118	15.2%	94.6%
	End of February	1.57 ± 0.373		2.47±0.233	3.40±0.325	57.1%	116.1%
	April	2.40 ± 0.694		3.41±0.239	2.61±0.691	42.3%	8.8%
Chlorophyll Content (mg/g of leaf tissue)	Early February	a	1.03±0.006	1.17±0.137	1.25±0.026	14.2%	21.7%
		b	0.36±0.034	0.42±0.041	0.47±0.012	16.7%	31.4%
		Tot	1.38±0.051	1.45±0.076	1.71±0.074	5.1%	23.3%
	April	a	0.32±0.016	0.37±0.028	0.38±0.059	16.6%	19.7%
		b	0.18±0.037	0.20±0.033	0.14±0.012	23.9%	- 23.9%

		Tot 0.50±0.038	0.60±0.039	0.42±0.115	20.6%	-14.5%
Total Carotenoid Content (mg/g of leaf tissue)	February	0.30±0.029	0.35±0.045	0.43±0.027	15.8%	43.62%
	April	0.22±0.026	0.14±0.032	0.13±0.029	-35.8%	-38.5%
Chlorophyll a: b Ratio	February	2.85	2.79	2.64	-2.1%	-7.4%
	April	1.78	1.85	2.80	3.9%	57.3%
Chlorophyll: Carotenoid Ratio	February	4.64	4.21	3.98	-9.2%	-14.6%
	April	2.27	4.26	3.16	87.8%	39.0%

4. Discussion

4.1 Impact on soil properties

Pots irrigated with diluted distillery effluent (E_1) were found to have slightly lower pH as compared to those irrigated with fresh water (C) whereas, the pH was slightly higher in E_2 than E_1 and C (Table 3). The higher pH in E_2 could be due to the presence of Ca, Na, Mg and OH^- and calcium oxide, a major constituent of fly- ash and forms calcium hydroxide with water and thus contributes to higher pH in E_2. With regard to the electrical conductivity (EC), there was 6% more in E_1 and 31% more in E_2 than the control (Table 3). Similar results have been reported by [30] and [31]. The nitrate content was 474% more in E_1 and 276% more in E_2 than the control (Table 3). It is known that plants take up nitrogen in the form of nitrate (NO_3^-) because nitrates are more quickly available to plants as they move through the roots and as such lesser content of nitrate in E_2 may be due to more hydraulic absorption because of higher water holding capacity in the fly ash amended soil. The phosphate content is 8.6% higher in E_1 and 85.7% in E_2 than the control (Table 3). It is known that P is essential for the nitrate absorption by the roots resulting in lesser content of nitrate in E_2. The moisture content in E_1 is 3% and in E_2 is 368% more than the control (Table 3). Fly ash decreases porosity and thus increases water holding capacity. This would facilitate the absorption of nutrients as well as photosynthetic activity. Similar findings have been reported by [5].

4.2 Vegetative growth

Table 4 shows the vegetative growth of Calendula officinalis in different amended soil under study. A mix of fly- ash and diluted distillery effluent (E_2) has a potential for improving plant growth. Shoot length has increased by 92.3% in E_2 and 65.0% in E_1 as compared to the control (Table 4) (Fig. 2). Table also shows that root length in E_2 is 71.3% and 53.2% more in E_1 than the control. Similarly the number of branches in E_2 is 733.3% and 166.7% more than the control (Table 4) (Fig. 2). The no. of leaves is 247.3% and 154.5% more in E_2 and E_1 respectively (Table 4) (Fig. 2 & 5). Similarly, the number of flowers is 600% more in E_2 and 50% more in E_1 than the control (Fig. 5) (Table 4). A balanced fertilization with diluted distillery effluent which contains macronutrients for plant nutrition is very important for the proper growth of the plants. Micronutrients in small quantities, however, their deficiencies cause a great disturbance in the physiological and metabolic processes in plants [32]. Plants rooting are very much affected by the placement of N and P fertilizers and they have synergistic effects on roots [33]. P uptake has shown that the root growth is of prime importance [34]. Adequate soil moisture, N, P and K promotes 1) root growth, 2) large abundant leaf area, 3) increases number of branches per plant. In the present study, the root length is 71.3% more in E_2 than the control and 53.2% more in E_1 (Table 4) (Fig. 2). This is due to the more P in E_2 than E_1. It has been observed in the present study that the number of flowers and leaves are more in E_1 & E_2 than the control which could be due to N & K (Table 4) (Fig. 5). Leaf area in E_2 is 45% more and in E_1 is 36.1% more than the control which could be due to N and K (Table 4) (Fig. 4). Similar reports have been made by [35]. Similarly the length of the shoot is maximum in the fly- ash and diluted distillery effluent (E_2) (Table 4) (Figs. 5 &1).. Similar observations have been made by [36] in fly- ash amended soil. The plants in pot culture experiment (E_2) produced more and large flowers Table 4 and (Fig. 5). This could be due to the micronutrients available in fly ash than the control. Similar reports have been made by [37] & [38].

4.3 Photosynthetic pigments

In the present study Table 5 shows chlorophyll a is 21.7% more in E_2 and 14.2% more in E_1 than the control (Table 5). The higher chlorophyll in E_2 is due to the presence of high N, K and Mg which are present in the diluted distillery effluent and P from fly ash resulting in higher content of chlorophyll a in E_2. Chlorophyll b is 31.4% more in E_2 and 16.7% in E_1 than the control (Table 5). The higher content of chlorophyll b in E_2 is due to higher P content in fly ash amended soil. Similar reasons may be assigned for higher total chlorophyll content in E_2. The carotenoids are the accessory pigments ubiquitous in photosynthesis. These pigments participate in light harvesting and fulfill photo- protective function as well as stabilize the pigment protein complexes of the photosynthetic apparatus. The carotenoids are helpful in stressful conditions especially during high photo- flux and high temperature.

4.4 Ratio of Chlorophyll a: b

Ratio of chlorophyll a:b in the present study decreased in the month of April when the temperature was higher than February (Table 5). Chlorophyll ‘a’ has been reported to be thermally less stable than chlorophyll ‘b’ [12], [13], [14], [15], [16]. Since chlorophyll ‘a’ degrades faster than chlorophyll ‘b’ as a function of temperature, therefore, the ratio of chlorophyll ‘a’ to ‘b’ decreases with increasing temperature in April in contrast to the findings of [39].

4.5 Ratio of chlorophyll: carotenoid

Similar to the trends in chlorophyll a: b, the ratio of chlorophyll:carotenoid has also decreased in the month of April (Table 5), with the exception in E_1 where the ratio has insignificantly increased.(Table 5). This could be due to the higher total chlorophyll content in E_1 than C and E_2 (Table 5). It is well known that chlorophyll as compared to carotenoids is thermally less stable and as such it would be logical to expect lower ratio in the month of April than in the month of February.

4.6 The role of Ascorbic acid on photosynthetic pigments

Oxygen is potentially toxic and even more so when combined with light pigments and electron transport activity, such conditions are provided in the chloroplasts. Photosynthesis releases oxygen, absorbs light and carries out electron transport in the chloroplast, therefore, needs protection from reactive oxygen species. Antioxidants and free radical scavengers are needed to deal with these toxic products of photosynthesis [21], [22], [23]. Ascorbate is the most abundant soluble antioxidant in chloroplasts. Interestingly in mammals, the eye is amongst the tissues containing the highest ascorbate concentration [40] which suggests that ascorbate is particularly important in situations where cells contain pigments designed to absorb light effectively.

Given its abundance and the importance of plants as dietary source of ascorbic acid for humans (who are unable to synthesize it), it is very surprising that very little is known about the ascorbate metabolism in plants. As such, the role of ascorbate in photosynthesis and photo- protection has been studied in *Calendula officinalis* as a function of diluted distillery effluent and a mix of fly ash and diluted distillery effluent as a bio fertilizer.

In the present study, the ascorbate concentration in E_2 is 94.6% more and 15.2% more in E_1 than the control (C) in early February when the temperature is relatively less but in the end of February the ascorbate concentration increases by 116.1% in E2 and 57.1% in E1 than the control (Table 5). But in the mid- April when the temperature is high, the increase in E_2 is 8.8% and in E_1 it is 42.3% which is more than the control. The parallel increased levels of total chlorophyll in E_1 and E_2 than control in February with respect to April is suggestive of the fact that, ascorbic acid is related to photosynthesis for photo- protection against reactive oxygen species.

However, in E_2 the ascorbic acid has decreased from 3.34 mg/ gm of tissue to 2.61mg/ gm of tissue in April with respect to the end of February. This could be due to the fact that because of early maturity (flowering) (Fig. 5), the onset of senescence was earlier in E_2 than others. As such, it is concluded that ascorbic acid plays an important role in scavenging the singlet oxygen species produced especially when strong light is absorbed by the leaves of *Calendula officinalis*. During late March there is a transformation from vegetative growth to flowering stage as well as the high temperature and as such, the plant undergoes a stressful condition resulting in the increase in the ascorbate content so as to enable the plant meet the stressful conditions as anti- oxidant thus playing the role of photo- protection. Similar reports have been made by [40], [41], [5].

In the present study, two interesting findings have been observed. Firstly, the flowering is about 20 days earlier in E_2 as compared to E_1 and control. This is due to the presence of Boron and Zn [42] present in the fly ash (Table 2) and absent in the distillery effluent (Table1). Secondly, the ratio of chlorophyll ‘a’ and ‘b’ as well as the ratio of chlorophyll: carotenoids decrease as the temperature increases in the month of April. This could be due to the photo- adaptation and photo- protection of chlorophyll b and carotenoids. The reasons have been assigned earlier in the discussion.

In view of the above results, a mix of fly ash and diluted distillery effluent can be used as potential bio- fertilizer. It is well known that even the Cobra poison can be therapeutic if it is used in a proper way. As such, the technology of ferti- irrigation and a mix of fly ash is essential because both the wastes are produced in huge quantity and if they are mixed in a proper proportion that could be used as a source of macro and micro nutrients and act as potential bio fertilizer for any crop. With the growing concern for the degrading environment due to excessive waste production, there can be no better alternative than to make a good use of the resource material present in the wastes for beneficial use rather than treat them as wastes and throw them unutilized ignoring the concept of "Waste to Wealth". In our opinion the Regulatory Authorities should go for *"Glocalization instead of Globalization"*. Whatever feed- back we got during the All India Training Programme, the technology of Reverse Osmosis and Incineration of the treatment of distillery wastes has not been successful. Thus, it is suggested because a mix of fly ash and diluted distillery effluent has given an encouraging result and if used as a potential bio-fertilizer would bring *another Green- Revolution in India.*

5. Conclusion

Diluted distillery effluent and fly ash are resources and not wastes. The major characteristics that make the suitability as a bio- fertilizer because it is a mix of macronutrients such as nitrate, phosphate and potassium and organic mass which comes from the diluted distillery effluent and micronutrients such as Mg, S, Bo, Fe, Mn and Zn come from the fly ash. This paper has shown how *"Pollutants turn nutrients" proving the concept of "Waste to Wealth".*

6. Acknowledgement

The authors thank Mr. B. L. Prasad and R. Ram for their valuable support in field work.

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7. Appendices

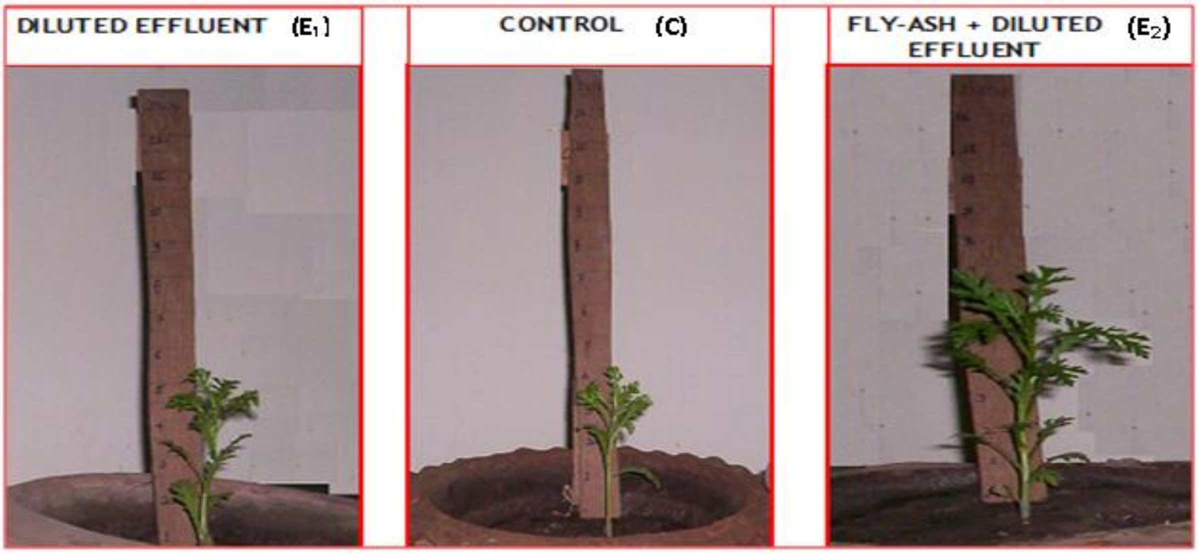

figure 1: Shows the growth of plant is more in (E_2) than (E_1) and (C). [Beginning of December, 2011 (Temp. 20 ± 1.5°C)]

figure 2: Shows more branches in (E_2) than (E_1) and (C). [Mid February, 2012 (Temp. 22 ± 2°C)]

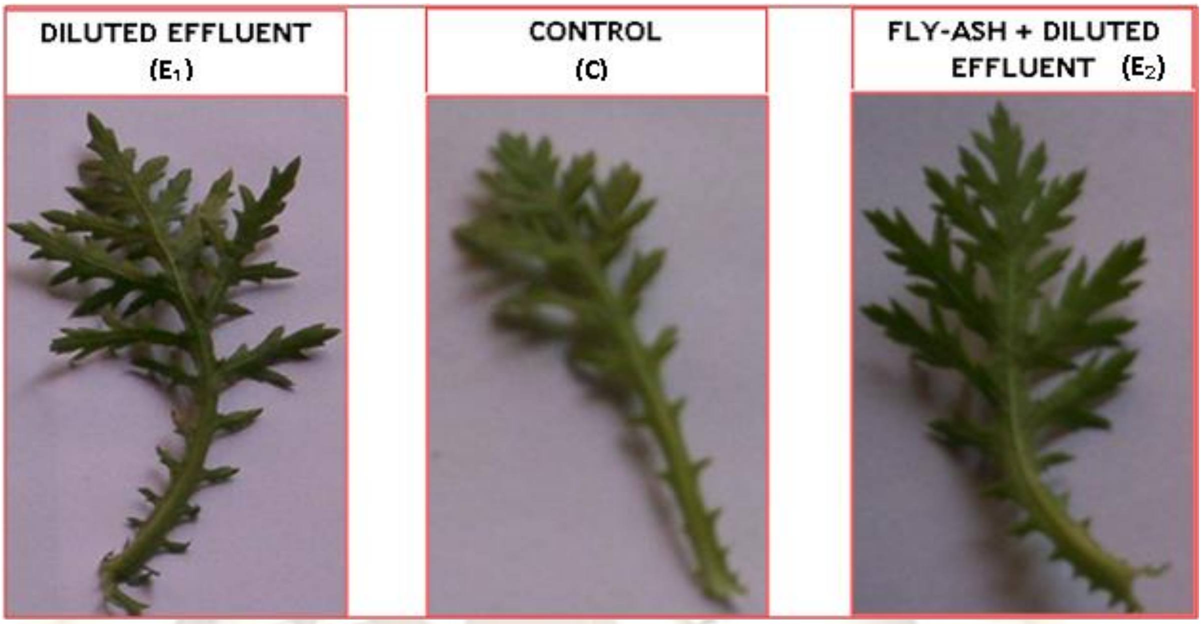

figure 3: Shows greater leaf area in (E_2) than (E_1) and (C).

figure 4: Shows the greater development in root of (E_2) than (E_1) and (C).

figure 5: Shows early flowering in (E_2) than (E_1) and (C). [Mid April, 2012 (Temp. 30 ± 2°C)]