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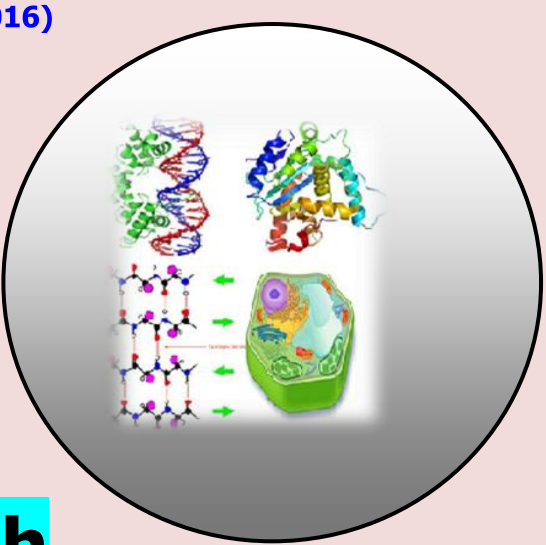

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RESEARCH PAPER

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Impact of Anaerobic and Aerobically Treated Brewery Wastewater on Seed Germination and Growth of Cowpea, Mustard, Pearl millet and Sorghum

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ABSTRACT

A lab scale experiment was conducted to assess the effects of anaerobic and aerobic treated brewery wastewater on seed germination and growth parameters of selected crop species like cowpea (Vigna unguiculata), mustard (Brassica nigra), pearl millet (Pennisetum glaucum) and sorghum (Sorghum bicolor). Two wastewater samples were collected from effluent treatment plant of brewery factory i.e., 1) effluent of up-flow anaerobic sludge blanket reactor (UASBR), 2) effluent of tertiary clarifier (ETP). Study was conducted with five types of water treatments – tap water as control, UASBR50 (50% UASBR effluent + 50% distilled water), UASBR 100 (undiluted UASBR effluent), ETP 50 (50% ETP effluent + 50% distilled water), ETP100 (undiluted ETP effluent) with three replications in completely randomized design. Experiment was conducted in petri plates for period of 5 days and parameters evaluated include germination percentage, GS (Germination Speed), MGT (Mean Germination Time), seedling length, SVI (Seed Vigor Index), PI (Phytotoxicity Index) and biomass. Study revealed that almost all parameters decreased with increase in wastewater concentration in all four tested crops. UASBR50 and ETP 50 promoted growth of cowpea and mustard, whereas ETP 50 showed positive effect on germination parameters of pearl millet. Sorghum growth was promoted by control, indicating that it is sensitive to brewery wastewater application. Both undiluted effluents showed negative impact on various parameters of four tested crops. Hence from this study, it can be concluded that dilution of wastewater and selection of suitable crop species plays vital role when wastewater is considered for agriculture.
Key words: Brewery wastewater, Cowpea, Germination, Mustard; Pearl millet and Sorghum.

INTRODUCTION

Industries are regarded as major polluting components among various anthropogenic activities as they release huge quantities of solid, semi-solid, and liquid wastes which pose large amounts of organic and inorganic pollutants (Uzair et al. 2009). Industrial wastewater is often considered as input of several toxicants and pollutants into aquatic ecosystems thereby affecting their functioning (Fakayode 2005, Samuel et al. 2010). Some pollutants may even reach the ground water and may adversely affect the drinking water quality. If these pollutants rich wastewater generated by industries come into contact with agricultural lands then these fertile lands will be severely affected as they may degrade soil complexes (Panasker and Pawar 2011) thus making soil unfit for crop production (Islam et al. 2006). At present, nearly 70 % of available water resources are dedicated to agriculture sector and because of growing bioenergy trends water scarcity problems are going to be high. Hence use of domestic and industrial effluents, even brackish and salty water has become point of high priority (da Costa Marques et al. 2015). But in many parts of world with water scarcity issues, application of low quality water for irrigation is in common practice. Use of low quality water for irrigation has become unavoidable due to various factors like low rainfall intensity, water scarcity, increasing demands of food due to population growth which led to agricultural expansion (Tadros et al. 2012, Alsaeedi et al. 2017). But use of this low quality wastewater can lead to harmful effects on some crops like stunted growth, decrease in yield etc. Since 1980's use of treated wastewater for irrigation is widely in practice as it is considered fertile source of various imperative nutrients useful for plant growth (Mohammad and Ayadi 2004). But at the same time, a high concentration of salinity in wastewater was found to adversely affect plant growth. Various crop species were found to be sensitive to high salinity and wastewater application, but few crops were found to withstand to these adverse conditions (Tadros et al. 2012). Sunflower seeds growth was not affected by different dosages of oilfield- produced water containing high salt concentrations and COD (da Costa Marques et al. 2015). Ramana et al.(2002) and Pandey et al. (2007) also stated that impact of wastewater application differs from crop to crop as each crop species have its own tolerance/sensitivity limits to different types of pollutants present in wastewater. In developing country like India, usage of industrial wastewater and sludge on agricultural land is under common practise and because of which toxic elements present in wastewater are transferred and accumulated in plant tissue from soil (Narain et al. 2012). Management and disposal of wastewater has become important in environmental point of view in developing countries as they may lead to pollution (Luc et al. 2006). Brewery industries release large quantities of wastewater after beer production. Brewery wastewater consists of high organic load which can cause considerable damage to lake ecology by increasing BOD and COD (Zvaura et al. 1994). Hence treatment of industrial wastewater plays important role before discharging into water streams or onto land. Beer industries follows different phases of treatment processes before discharge of wastewater and every phase of brewery wastewater treatment has its own importance as they remove specific pollutants during treatment process (Chaitanya Kumar et al. 2011). Seed germination bioassays plays important role in plant life and water acts as major environmental factor for seed germination (Salian et al. 2018). As reuse of wastewater is viable option for irrigation in water scarce regions, use of treated wastewater should be given utmost importance. Hence present study was carried out to evaluate the effects of anaerobic and aerobically treated brewery wastewater on seed germination and seedling growth parameters of selected crops like cowpea, mustard, pearl millet and sorghum.

MATERIALS AND METHODS

Wastewater collection and characterization

Brewery wastewater samples were collected from SAB Miller beer factory located near Sangareddy, Telangana (India). Wastewater samples were collected from effluent treatment plant of factory at two different points i.e., 1) effluent of up-flow anaerobic sludge blanket reactor (UASBR), 2) effluent of tertiary clarifier (ETP). Collected samples were analyzed for physico-chemical parameters using standard methods (APHA, 2005).

Seed Germination Experiment

Study comprised of five types of water treatments –tap water as control, UASBR50 (50% effluent + 50% distilled water), UASBR100 (undiluted effluent), ETP50 (50% effluent + 50% distilled water), ETP100 (undiluted effluent) with three replications in completely randomized design. Ten seeds of each selected crops like cowpea (variety: Pusakomal), mustard *(*variety*:* Kranti), pearl millet (variety: ICTP8203) and sorghum (variety: CSV-23) were placed in sterilized petri plate lined with filter paper. Five milliliter of respective wastewater was added to each petri plate and then petri plates were incubated at room temperature. Study was conducted for 5 days and following parameters were evaluated after every 24 hours.

(a) Germination Percentage – After 24 hours, number of seeds germinated in each petri plate were counted and germination percentage was calculated using following formula (Naeem et al. 2015).

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

(b) Germination Speed – It is defined as number of seeds germinated per day during the whole germination period. It was computed using the following equation (Alsaeedi et al. 2017).

$$GS = \sum (ni/ti)$$

Where, GS= germination speed
ni = number of seeds germinated on germination day
ti = number of days during germination test

(d) Seedling length- After 5 days, radical and plumule length of germinated seeds of each crop was measured using a scale. Measurement of plumule length was taken from hypocotyl base to primary leaf. Radical length was measured from primary root tip to base of hypocotyl. Radical and plumule length measurement was expressed in centimeters. Finally seedling length was calculated by adding both radical and plumule length (Dash. 2012).

$$\text{Seedling length (cm)} = \text{Radical length (cm)} + \text{Plumule length (cm)}$$

(e) Seed Vigor index – calculated by using following equation (Abdul- Baki and Anderson. 1973).

$$SVI = \text{Germination \%} \times \text{Seedling length}$$

Where, SVI = Seed vigor index

(f) Phytotoxicity index (PI) - was calculated based on root length using following equation (Rusan et al., 2015).

$$PI = 1 - \frac{RLT}{RLC}$$

Where, RLT= Root length of treated seeds,
RLC= Root length of control seeds.

(g) Biomass - seedlings were oven dried at 60^0c for 48 h and their dry weight was obtained using weighing balance (Aguirre and Johnson. 1991).

Statistical Analysis

Statistical analysis of data was performed using ANOVA (Analysis of Variance). If the calculated F-ratio was significant, then Fisher's least significance test at 0.10 probability level was performed for multiple mean comparisons.

RESULTS AND DISCUSSION

Physico-chemical parameters of tap water and brewery wastewater collected from industry are represented in Table 1.

Table 1. Physico-Chemical parameters of Tap water and Brewery effluent (Mean ± SE).

Parameter	Units	Tap water	UASBR outlet	ETP outlet
pH		7.47 ± 0.1	7.37 ± 0.1	7.83 ± 0.2
Electrical Conductivity	ms/cm	0.62 ± 0.06	4.37 ± 0.54	4.04 ± 0.40
Total dissolved solids	mg/l	445.6 ± 9	2737.22 ± 256.8	2556.56 ± 214.7
Total suspended solids		5.06 ± 0.5	46.50 ± 8.8	5.50 ± 0.6
Chlorides		53.88 ± 7.04	244.28 ± 23.46	229.82 ± 23.99
Chemical Oxygen Demand		49 ± 12.58	350 ± 30.22	202.50 ± 55.83
Ammonical nitrogen		2.67 ± 0.73	66.82 ± 12.21	11.13 ± 2.73
Nitrate nitrogen		1.82 ± 0.5	7.35 ± 0.9	20.46 ± 6.5
Phosphates		0.02 ± 0.0	1.57 ± 0.4	1.25 ± 0.2
Potassium		14.87 ± 3.7	50 ± 4.5	47.20 ± 3.3
Sodium		12.00 ± 0.1	527 ± 100	416.28 ± 27.05
Total Alkalinity		128 ± 20.13	731 ± 47.7	551.2 ± 29.3
Total Hardness		134.6 ± 17.3	305.7 ± 22.3	281 ± 16.8

(**Note**: Heavy metals like Arsenic, Cadmium, Chromium, Cobalt, Lead, Nickel and Zinc are Below Detectable limit (BDL) in all analysed water sources)

UASBR outlet effluent has highest concentration of all parameters except pH and nitrate nitrogen. pH and nitrate nitrogen were highest in ETP outlet. Tap water showed low concentrations of all parameters analyzed.

Germination Percentage

Germination percentage of tested crops is represented in Figure 1.

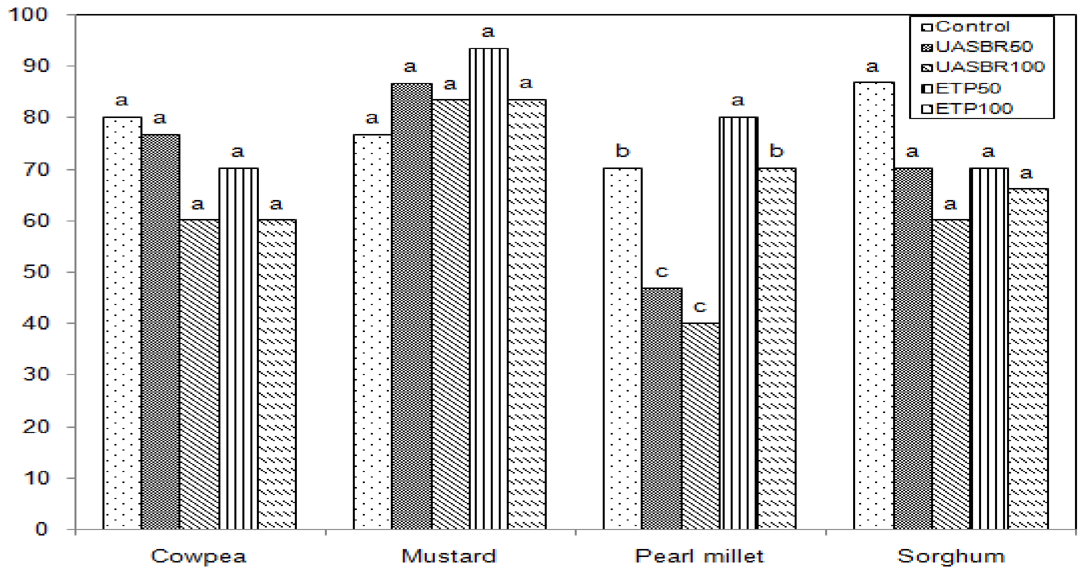

Figure 1. Effect of anaerobic and aerobically treated brewery wastewater on seed germination (%) of tested crops (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

Germination percentage of all four crops is shown graphically in Figure 1. Seed germination decreased in all four crops with increase in wastewater concentration. Similar decrease in germination percentage with increasing sugar factory effluent concentration was reported in barley seeds (Ozkara et al. 2011); in okra by exposure to marble industry wastewater (Uzma et al. 2016) and in chickpea, maize and pigeon pea by applying brewery wastewater (Salian et al., 2018) Germination percentage in cowpea, mustard and sorghum was not significantly different (p=0.10). 80 % germination was achieved with control in cowpea and in treatments highest germination % was observed with UASBR50 (76%) and lowest with UASBR100 and ETP100 treatment (60 %). In mustard, ETP50 showed highest germination with 93% and lowest germination% was recorded with control (76 %). Both undiluted anaerobic and aerobic treated brewery wastewater i.e., UASBR100 (83 %) and ETP100 (83 %) also showed positive effect on seed germination of mustard as their percentage was higher than control. Similar results were inferred by Rehman et al. (2009) in his study on brassica that 100% concentration of treated textile effluent showed highest germination % than control. In pearl millet, highest germination percentage was observed with ETP50 (80 %) and lowest was recorded with UASBR100 (40%). Seed germination in pearl millet with ETP100 (70 %) was not significantly different from control (70 %). UASBR50 and UASBR 100 showed inhibitory effect on pearl millet seed germination. In sorghum, highest seed germination percentage was observed with control (86 %) and among treatments with UASBR50 and ETP100 (70 %), whereas lowest seed germination percentage was recorded with UASBR 100 (60 %).

Mustard germination percentage was not affected by undiluted UASBR and ETP effluent. This might be due to tolerance capability of mustard to wastewater than other tested crops. Similarly, Rehman et al. (2009) reported that radish germination percentage was not affected by untreated textile effluent application while reduction in germination percentage of turnip and Brassica was observed. UASBR100 treatment showed inhibitory effect on seed germination percentage of cowpea, pearl millet and sorghum. This might be due to high sodium concentration of UASBR effluent as shown in Table 1. Elevated sodium concentrations were found to decrease final germination percentage in common bean seedlings (Rahman et al. 2008, Alsaeedi et al. 2017). Baghel et al. (2008) reported that undiluted distillery effluent resulted in complete failure of germination in Pea seeds. High salt concentration in irrigation water may induce osmotic stress and may affect the germination and growth of plants as it inhibits water uptake by seed (Dash, 2012). But mustard showed good response with undiluted effluent than other tested crops, this might be due to its tolerance capacity to salts. This statement was in line with Ashraf (2004), who stated that response to osmotic pressure induced by salts in irrigation water may vary from species to species.

Germination Speed

GS is defined as number of seeds germinated per day during the whole germination period. Computed values of GS are presented graphically in Figure 2.

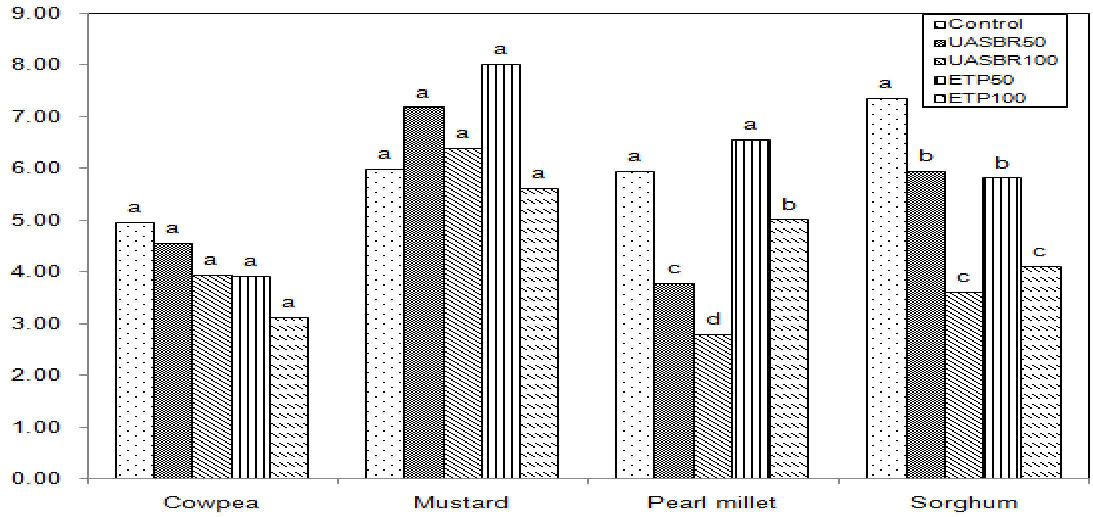

Figure 2. Effect of anaerobic and aerobically treated brewery wastewater on germination speed of tested crops (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

In cowpea and sorghum, highest GS was achieved with control (4.9 and 7.3) respectively and among treatments highest GS was recorded with UASBR50 effluent (4.5 and 5.9) respectively. In cowpea, lowest GS was recorded with ETP100 (3.1) and in sorghum with UASBR100 (3.6). Highest GS in pearl millet was achieved with ETP50 (6.53) and it was not significantly different from control (5.9) at 0.10 probability level. Lowest GS in pearl millet was recorded with UASBR100 (2.7). In mustard, highest GS was observed with ETP50 (8.0) and lowest GS was recorded with ETP100 (5.6). ETP100 showed inhibitory effect on germination speed of cowpea and mustard, UASBR100 on GS of pearl millet and sorghum. High sodium levels present in undiluted brewery effluent might have led to decrease in GS in all four crops. A similar negative effect on GS of common bean seedlings was reported by Alsaeedi et al. (2017). Delay in Germination speed can be due to concentration of Na^{+} and Cl^{-} ions in plant tissues which causes inequity in uptake of nutrients from water or soil and thereby causing poisonous effect (Mena et al. 2015).

Seedling length

Seedling lengths of four crops are shown graphically in Figure 3. Individual values of radical and plumule lengths are presented in Table 2.

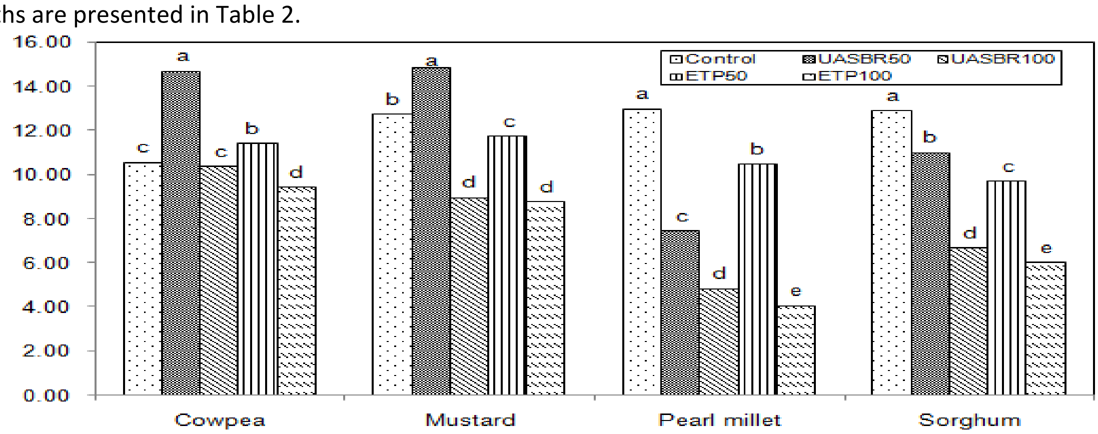

Figure 3. Effect of anaerobic and aerobically treated brewery wastewater on seedling length (cm) of tested crops. (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

In all four tested crops, seedling length decreased with increase in effluent concentration. Similar results observed in barley root growth by application of sugar factory effluent (Ozkara et al. 2011), in okra seedlings by using marble industry wastewater (Uzma et al. 2016), in wheat cultivars (Kaushik et al. 2005), in black gram seedlings (Wins and Murugan. 2010) and in vegetable crops like radish, turnip and brassica by using treated and untreated textile mill effluent (Rehman et al. 2009). Seedling length in cowpea was highest with UASBR50 (14.7 cm) and lowest was with ETP100 (9.4cm). In mustard, highest seedling length was observed with UASBR50 (14.8 cm) and lowest was with ETP100 (8.7 cm). In pearl millet and sorghum, seedling length was highest with control (12.9 cm for both crops) and lowest seedling length was recorded with ETP100 (4 and 6 cm respectively). When compared to aerobically treated wastewater, anaerobically treated brewery wastewater which was diluted i.e., UASBR50 showed promoting effect on seedling lengths of cowpea, mustard and sorghum. In cowpea, UASBR 100 (10.33 cm) also showed promoting effect on seedling length and it was not statistically different from control (10.53cm). In contrast, seedling length of pearl millet was promoted by ETP50 (10.46 cm) than UASBR50 (7.43 cm). Presence of nutrients (N, P, Ca, Zn, Mg, etc.) in treated effluent or lower concentrated effluent might have played a key role in promoting seedling length, whereas at higher concentrations the nutrients become toxic to crops and inhibit the growth of seedling (Anupama, 2011, Uzma et al. 2016). High sodium concentrations of undiluted brewery effluents may be attributed to negative effect on seedling length of four crops. Similar report was given by Khajeh-Hosseini et al. (2003) and Naeem et al. (2015) who stated with increasing levels of salinity there was decreasing trend of seedling length. Similar salinity effect on Leucaena seedling length was reported by using textile mill wastewater by Tadros et al. 2012. But Azizullah et al. (2012) stated that impact of wastewater on crops cannot be attributed to single factor as different chemicals present in effluents can act positively, synergistically, or sometimes even antagonistically. Hence, they concluded that reported effects of wastewater on plants might be considered as overall net effect of various substances occurring in waste water.

Table 2. Effect of anaerobic and aerobically treated brewery wastewater on radical and plumule lengths of crops under study.

Treatment	Cowpea		Mustard		Pearl millet		Sorghum	
	Radical length (cm)	Plumule length (cm)	Radical length (cm)	Plumule length (cm)	Radical length (cm)	Plumule length (cm)	Radical length (cm)	Plumule length (cm)
Control	5.4[c]	5.1[c]	5.1[c]	7.6[a]	8.7[a]	4.2[a]	8.4[a]	4.5[b]
UASBR50	7.2[a]	7.5[a]	8.5[a]	6.3[b]	4.8[c]	2.6[c]	6.2[b]	4.8[a]
UASBR100	5.4[c]	4.9[d]	4.9[d]	4.0[d]	2.8[d]	2.0[d]	3.1[d]	3.6[c]
ETP50	5.7[b]	5.7[b]	5.3[b]	6.4[b]	7.0[b]	3.4[b]	5.0[c]	4.6[b]
ETP100	4.9[d]	4.5[e]	4.5[e]	4.2[c]	2.5[e]	1.5[e]	2.6[e]	3.4[d]
CD ($p > 0.10$)	0.11	0.15	0.16	0.18	0.14	0.11	0.15	0.15

(Note: CD =Critical Difference, Means with different letters are significantly different from one another at 0.10 level of probability within different treatments)

Seed Vigour index

The computed values of SVI are represented in Figure 4.

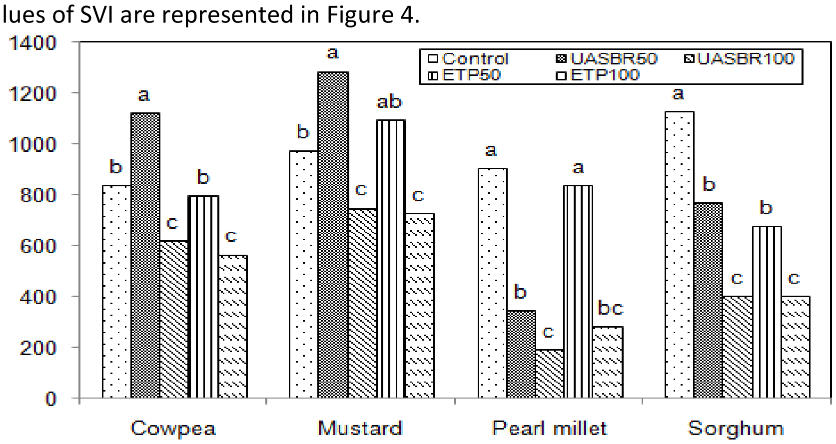

Figure 4. Effect of anaerobic and aerobically treated brewery wastewater on seed vigour index of tested crops. (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

Undiluted brewery effluent treated by anaerobic and aerobic methods i.e., UASBR100 and ETP100 showed inhibitory effect on SVI of all four tested crops. Highest SVI in cowpea and mustard were observed with UASBR 50 (1124 and 1285 respectively), whereas lowest SVI was observed with ETP 100 (565 and 728 respectively). Highest SVI in pearl millet was recorded with control (907) but SVI value of ETP50 (837) did not show any statistical significance with control. Lowest SVI in pearl millet was observed with UASBR 100 (192).

Similarly in sorghum also highest SVI was recorded with control (1128), and lowest with UASBR 100 (402). Similar to seedling length, SVI values also followed same trend i.e., except pearl millet all three crops vigour index was promoted by UASBR 50. A similar positive effect of distillery wastewater dilution on vigor index of chickpea seedlings was reported by Narain et al., (2012).

Phytotoxicity index

Phytotoxicity index values of all four studied crops are represented graphically in Fig 5.

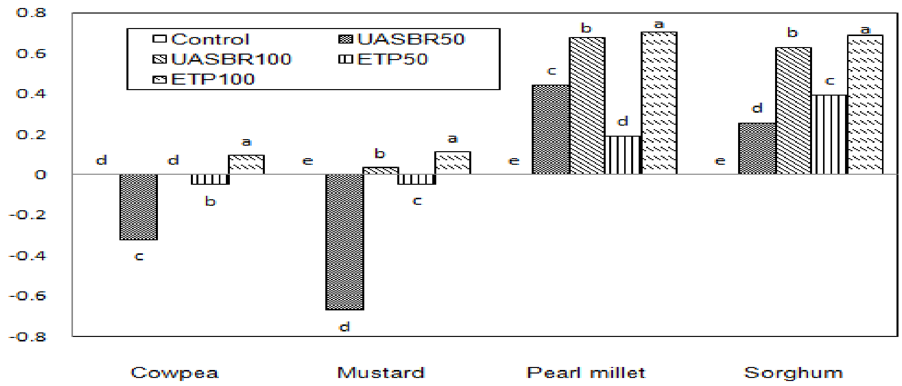

Figure 5. Effect of anaerobic and aerobically treated brewery wastewater on phytotoxicity index of tested crops. (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

Highest phytotoxic effect in cowpea (PI = 0.09), mustard (PI = 0.11), pearl millet (PI = 0.7) and sorghum (PI = 0.69) were observed with ETP100. UASBR100 also showed phytotoxic effect on mustard (PI = 0.03), pearl millet (PI = 0.68) and sorghum (PI = 0.63), but in cowpea UASBR 100 not showed any phytotoxic effect and it was not significantly different from control. Diluted anaerobic and aerobically treated brewery wastewater (UASBR 50 and ETP 50) not showed any phytotoxic effect on cowpea and mustard seedlings. But pearl millet and sorghum seedlings were adversely affected by both diluted and undiluted effluents of anaerobic and aerobically treated brewery wastewater. Similar positive effect of brewery wastewater on PI of chickpea and negative effect on PI of maize and pigeon pea was reported by Salian et al. (2018).

Biomass

Dry weights of studied crops are shown graphically in Figure 6.

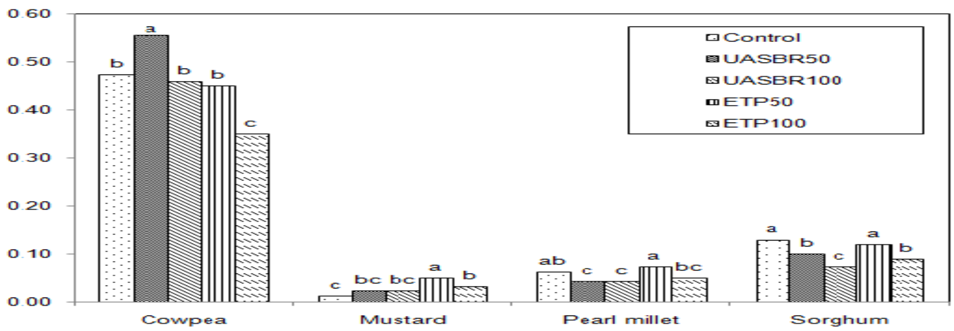

Figure 6. Effect of anaerobic and aerobically treated brewery wastewater on dry weight (gm) of tested crops. (Note: Means with different letters are significantly different from one another at 0.10 level of probability within different treatments).

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Dry weight in cowpea was highest with UASBR 50 (0.56 gm) and lowest with ETP100 (0.35 gm). Cowpea seedlings dry weight with UASBR 100 (0.46 gm) and ETP50 (0.45 gm) were not significantly different from control (0.47 gm). In mustard, highest dry weight was achieved with ETP50 (0.05 gm) and least dry weight was recorded with control (0.01gm). Dry weight in pearl millet was highest with ETP50 (0.07 gm) and least dry weight was observed with both diluted and undiluted UASBR effluent (0.04 gm). In sorghum, highest dry weight was achieved with control (0.13 gm) and it was not significantly different from ETP 50 (0.12 gm) and lowest dry weight was observed with UASBR 100 (0.07 gm). Uzma et al. (2016) reported that lettuce shoot dry weight values were lower when exposed to various concentrations of match industry effluent than control. Diluted brewery effluent showed positive biomass values when compared to undiluted wastewater in all four crops. Similar results were reported in sunflower seedlings when exposed to tannery effluent (Hussain et al., 2010).

Application of anaerobic and aerobically treated brewery wastewater at different concentrations on cowpea, mustard and pearl millet was found to be not having any negative impacts on germination and early seedling growth. But sorghum was found to be susceptible to brewery wastewater exposure at different concentrations. Rehman et al. (2009) reported that radish was found tolerant and turnip as susceptible to application of textile effluent. Similarly Uzma et al. (2016) found out that okra seedlings germination and growth was affected by exposure to marble industry effluent, but was not affected by application of match industry wastewater. Hence it is important to know the behaviour of individual crop species before using wastewater for irrigation. But as germination experiments are preliminary bioassays, further studies are recommended to know the long term effects of wastewater application on soil properties and crop growth.

CONCLUSION

Dilution of anaerobic and aerobic brewery effluent showed promoting effect on various growth parameters of cowpea, mustard and pearl millet seedlings. But growth of sorghum seedlings was promoted by control than brewery wastewater. Undiluted anaerobic and aerobic brewery effluent showed inhibitory effect on all four crops. Hence, it is evident that both anaerobic and aerobically treated brewery wastewater when diluted showed promoting effect, and undiluted effluent showed inhibitory effect on seed germination and growth parameters of all four tested crops. Thus, this study concludes that dilution of effluent and selection of suitable crop species plays major role to alleviate the negative impacts of wastewater on growth of crops.

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