# EFFECT OF ABATTOIR WASTEWATER ON THE METABOLIC STATUS OF COWPEA SEEDLINGS

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## Article Info

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## ABSTRACT

The increasing demand for meat production has led to the proliferation of abattoirs with subsequent environmental pollution. The aim of the study was to determine whether abattoir waste water (AWW) might serve as an inexpensive fertilizer. This was done by investigating the performance of cowpea seedlings grown in 1.6 kg of soil treated with varying amounts (20.0 ml, 40.0 ml, 60.0 ml, 80.0 ml and 100.0 ml) of abattoir waste water. The result indicated that at 20 ml of AWW, there was no significant (P<0.05) increase in the levels of total carbohydrates, glucose, protein, and amino acid as compared to the control and other treatments. However, with the exception of glucose, the content of these macromolecules in the cowpea seedlings increased significantly (P<0.05) at 40 ml of AWW as compared to the control and other treatments. Generally, the chlorophyll content of cowpea seedlings grown in soil treated with AWW increased at various concentrations of AWW except at 80ml and 100ml relative to the control. Moreover, the alpha amylase activity increased significantly at all levels of AWW except at 20 ml and 100 ml compared to the control, while starch phosphoryase activity increased significantly (P<0.05) at 40ml of AWW. Although the impact of AWW on the metabolism of macromolecules in the cowpeas seedlings is not clear, it was clearly observed that AWW positively affected the chlorophyll content and the activities of starch degrading enzymes at lower concentrations (20 ml and 40 ml). Since chlorophyll content and the activities of starch degrading enzymes are indicators of plant growth, it can be concluded that moderate concentration of AWW can improve plant growth.

## INTRODUCTION

Nigeria has over the years witnessed an increase in population. This makes it imperative to increase agricultural production to ensure food security for the nation. The need to ensure that meat production meets the need of the people led to the proliferation of abattoir houses both in urban and rural areas (Ezeoha and Ungwuishiwu, 2011). Abattoirs have been reported as sources of environmental pollution (Neboh *et al.*, 2013). These abattoirs lack waste treatment facilities, wastes from these abattoirs are deposited on the land or channelled into water bodies which culminate in environmental contamination (Ogbonna *et al.*, 2012). The abattoir waste water (AWW) contains undigested materials called paunch manure, undigested feed, undigested protein, excess nitrogen from digested protein, residues from digested fluids, waste minerals, worn-out cells from intestinal linings, mucus, bacteria and foreign matter such as dirt consumed (Robinson *et al.*, 1971; Ezeoha and Ungwuishiwu, 2011 ).

After meat processing, the abattoir waste water (AWW) containing these contaminants–is drained into surrounding soil environments (Amisu *et al.*, 2003). The improper disposal of abattoir waste water containing paunch manure, waste minerals and excess nitrogen from digested protein, may cause oxygen-depletion in the receiving environment, introduce toxic chemicals into the soil, or disrupt the soil ecological balance (Nwachukwu *et al.*, 2011; Ubwa *et al.*, 2013; Williams and Dimbu, 2015). AWW contamination of soil could degrade soil fertility due to accumulation of certain nutrients and heavy metals that may lead to low productivity (Rabah *et al*., 2010). On the other hand, the nitrogen, minerals, and organic material in AWW could be beneficial to the growth of food crops. The aim of this study was to determine the possibility of using AWW as source of organic fertilizer.

## MATERIALS AND METHODS

### Collection of Materials

Dry, brown seeds of *Vigna unguiculata* (L) were obtained as a single batch from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Seed viability was determined by floatation. All seeds that floated on water were discarded and those that remained at the bottom of water deemed potentially viable. The sandy loam soil (sand 84 %, silt 5.0 %, clay 0.4 % and organic matter 0.6%, pH 6.1) was obtained from the nursery beds of Delta State University farm project. Abattoir waste water (AWW) was obtained from local slaughterhouses in Obiaruku, Delta State, Nigeria. It was collected from four different sites and pooled together. The waste water was filtered to remove solid materials as well as suspended animal fats and oil. The physicochemical properties of the waste water are shown in Table 1:

**Table 1.** Physicochemical properties of abattoir wastewater use

| Parameters | property/values |
|---|---|
| Appearance | Turbid |
| Temperature | 29 ± 2°C |
| pH value | 5.6±0.3 |
| Conductivity (μScm $^{-1}$) | 35.0±1.0 |
| Turbidity (NTU) | 8.3±0.1 |
| Total dissolved solids (mgl $^{-1}$ ) | 740±11.7 |
| Biological oxygen demand (mgl $^{-1}$) | 48± 2.3 |
| Chemical oxygen demand (mgl $^{-1}$) | 150±10.1 |

Values are expressed as means ± SD

### Experimental Design

Filtered soil (1.6 kg) was added to each of 100 small size-planting bags (1178.3 cm$^3$, 15 cm deep) and divided into six groups of five bags each. Each bag (Groups 1 to 6) was treated with 00.0 ml, 20.0 ml, 40.0 ml, 60.0 ml, 80.0 ml and 100.0 ml respectively of abattoir waste water (AWW). Group 1 had 100 ml of tap water while tap water was added to the respective treatments to make them up to 100 ml. Cowpea seeds were sown, at five seeds per bag, and allowed to grow for twelve days before analysis. During the experiment 80 ml of water was added to each treatment daily to keep the soil moist. The entire set up was done under laboratory conditions.

### Determination of total carbohydrate, reducing sugar, amino acid, total protein and chlorophyll

Leaves from each plant (1.0 g) were homogenized with methanol (2:1 v/v), then filtered and the residue was re-extracted. The sugar was determined as described by Tietz (1982). The protein content was determined using another portion of the 70 % methanol extract, after removing the organic portion under pressure and the remaining aqueous portion diluted with 0.01 N HCl and used for the determination of total free amino acid by ninhydrin after deproteination with 5 % trichloroacetic (TCA). Total protein was determined by the method of Lowry *et al*. (1951). A portion of the homogenate was then centrifuged at 2500 rpm for ten minutes. The solutions were diluted to equal volumes and the content of the chlorophyll was determined by the method of Lichtenthaler (1987).

### Assay for alpha -amylase activity and determination of starch phosphorylase activity in the cotyledons of cowpea seedlings.

The alpha-amylase activity was determined by the method of Gupta *et al*. (2003) using the supernatant obtained from the homogenization and centrifugation of 0.5 g of cotyledons from each sample. The activity was calculated using the formula proposed by Xiao *et al* (2006). Starch phosphorylase activity was assayed according to the method of Singh and Steinnes (1976).

## RESULTS

The results of this study show that abattoir waste water (AWW) had varying effects on the metabolism of the various macromolecules depending on the concentration (Table 2). At 20 ml of AWW, there was no significant increase in the levels of total carbohydrates, glucose, protein, amino acid and chlorophyll as compared to the control. However, with the addition of 40 ml of AWW total carbohydrates, protein, amino acid and chlorophyll increased significantly while glucose significantly decreased compared to the control. At 60 ml AWW, total carbohydrates decreased significantly, protein increased significantly, and glucose decreased non-significantly. Meanwhile, amino acid and chlorophyll increased non-significantly. Total carbohydrates decreased significantly, protein increased significantly, amino acid and chlorophyll contents decreased non-significantly while glucose contents of the cowpea increased non-significantly at 80 ml of AWW. Moreover, at 100 ml of AWW, total carbohydrates, protein, amino acid and chlorophyll of the cowpea seedlings decreased significantly while glucose significantly(P<0.05) increased compared to the control. Table 3 shows that alpha amylase activity increased significantly at all levels of AWW except at 20ml and 100ml at which there was non-significant increase and non-significant decrease respectively as compared to the control. Moreover, contamination of soil with AWW resulted in a significant (P<0.05) increase in starch phosphorylase activity at 40 ml of AWW, non-significant increase at 20 ml, and non-significant decrease at 60ml, 80 ml and 100 ml of AWW.

## DISCUSSION

Although several studies have reported the negative impact of abattoir waste water on the receiving soil and water (Laukova *et al*., 2002; Amisu *et al*., 2003; Adesemoye *et al*., 2006; Neboh *et al*., 2013; Ubwa *et al*., 2013; Williams and Dimbu, 2015), some researchers have on the contrary reported abattoir waste water as a potential inexpensive bioremediation tool for soil contaminated by petroleum products (Umanu and Nwachukwu, 2010; Ogbonna et al., 2012; Umanu and Owoseni, 2013). The results of this study show that abattoir waste water (AWW) had varying effects on the metabolism of the various macromolecules depending on the concentration (Table 2). Alterations or imbalance in the metabolism of macromolecules such as sugar, protein and amino acid have been reported to be an indication of environmental stress (Hayashi *et al*., 1992; Murray *et al*., 1992; Achuba, 2006; Peretiemo-Clarke and Achuba, 2007; Al-Hawas *et al*., 2012; Olubodun and Eriyamremu, 2015). Environmental contamination or stress can result in either an increase in the levels of macromolecules (sugar, protein and amino acid) (Hayashi *et al*., 1992; Murray *et al*., 1992; Achuba, 2006; Peretiemo-Clarke and Achuba, 2007) or decrease in levels of these macromolecules(Al-Hawas *et al*., 2012; Olubodun and Eriyamremu, 2015). The metabolic imbalances observed in this study at various concentrations of AWW agree with findings of these researchers and could indicate that excess AWW can induce stress on the cowpea seedlings. The stress may take the form of oxygen-depletion, the introduction of toxic chemicals into the soil, or disruption of the soil ecological balance (Nwachukwu *et al*., 2011; Ubwa *et al*., 2013; Williams and Dimbu, 2015). However, with the exception of glucose, the content of these macromolecules in the cowpea seedlings increased significantly at 40 ml of AWW as compared to the control, indicating a better performance of the seedlings at this level of contamination.

**Table 2. Effect of abattoir waste water on the metabolism of macro molecules in cowpea seedlings**

| Amount of abattoir waste water (ml) | 0.00 | 20.00 | 40.00 | 60.00 | 80.00 | 100.00 |
|---|---|---|---|---|---|---|
| **Total Carbohydrate (mg/g)** | 31.90 ± 3.34 | 32.98 ± 3.39 | 49.00 ± 1.34ᵃ | 27.65 ± 0.82ᵇ | 22.78 ± 1.77ᵇ | 19.43 ± 1.73ᵇ |
| **Glucose (mg/g)** | 3.48 ± 0.65 | 3.78 ± 0.31 | 2.48 ± 0.59ᵇ | 3.05 ± 0.24 | 3.88 ± 0.43 | 5.33 ± 1.00ᵃ |
| **Protein (mg/g)** | 20.18 ± 1.53 | 22.10 ± 1.15 | 38.73 ± 2.12ᵃ | 27.98 ± 1.11ᵃ | 25.03 ± 1.30ᵃ | 14.85 ± 1.28ᵇ |
| **Amino Acid (mg/g)** | 4.78 ± 0.83 | 5.05 ± 0.87 | 7.20 ± 0.45ᵃ | 4.88 ± 0.54 | 4.03 ± 0.22 | 3.50 ± 0.58ᵇ |
| **Chlorophyll (mg/g)** | 251.00 ± 13.61 | 258.75 ± 18.39 | 361.25 ± 42.17ᵃ | 261.25 ± 9.46 | 246.25 ± 13.33 | 215.00 ± 4.16ᵇ |

Results are expressed as mean ± SD of five determinations. a=values significantly higher than those of control at P<0.05; b=values significantly lower than those of control; values without superscript letter are not statistically different from control.

**Table 3: Effect of abattoir waste water on starch degrading enzymes in the cotyledon of cowpea seedling**

| Amount of abattoir waste water (ml) | 0.00 | 20.00 | 40.00 | 60.00 | 80.00 | 100.00 |
|---|---|---|---|---|---|---|
| **Amylase (mgstarch/ minmg protein)** | 1.45 ± 013 | 1.58 ± 0.13 | 1.85 ± 0.13ᵃ | 1.90 ± 0.08ᵃ | 1.70 ± 0.14ᵃ | 1.28 ± 0.17 |
| **Phosphorylase (starch/minmg protein)** | 2.03 ± 0.22 | 2.25 ± 0.26 | 2.55 ± 0.40ᵃ | 1.78 ± 0.36 | 1.76 ± 0.17 | 1.75 ± 0.13 |

Results are expressed as mean ± SD of five determinations. a=values significantly higher than those of control at P<0.05; values without superscript letter are not statistically different from control.

This may be attributed to the presence of **organic** matter and essential nutrients such as nitrate and phosphate in abattoir waste water (Tezcan *et al*., 2009; Neboh *et al*., 2013; Umanu and Owoseni, 2013) which are necessary for plant growth.

Chlorophyll is an important biomolecule that is vital for photosynthesis and plant growth (Mckee and Mckee, 1999; Nelson and Cox, 2005). Reduced chlorophyll content is an indication of environmental contamination (Agrawal, 1992; Peretiemo-Clarke and Achuba, 2007). Generally, the chlorophyll content of cowpea seedlings grown on soil contaminated with AWW increased at various concentrations of AWW except at 80 ml when compared to the control. Particularly, the chlorophyll content of the cowpea seedlings was significantly higher than the control at 40ml of AWW. This observation is contrary to what is expected as the abattoir waste water has been reported to cause degradation of soil fertility and probably leads to low productivity (Adesemoye *et al*., 2006; Rabah *et al*., 2010). On the other hand, abattoir waste water is rich in organic matter and essential nutrients such as nitrate and phosphate (Tezcan *et al*., 2009; Neboh *et al*., 2013; Umanu and Owoseni, 2013). The levels of these essential nutrients at 40 ml could enhance plant growth.

The result of this study shows that the activities of the starch degrading enzymes, alpha amylase and phosphorylase were affected by AWW ( Table 3). The two enzymes exhibited significant increases in activity at 40 ml of AWW in soil compared to the control. Alpha amylase is essential for starch degradation. Moreover, starch phosphorylation is important for plant growth, and this explains why mutant plants lacking starch phosphorylase exhibits reduced growth and accumulate nearly phosphate-free starch to a high level (Zeeman *et al*., 2007). The significant increase in these enzyme activities at this concentrations (at 40ml) of AWW suggest a positive effect of AWW on the growth of the cowpea seedlings. This is supported by the fact that organic manures such as cow dung, poultry droppings, and household refuse and effluents which are rich in organic matter and nutrients such as nitrate and phosphorus, have been extensively employed by subsistence farmers to improve crop production (Lombin *et al*., 1991; Osemwota, 2010).

Generally, the result of this study indicates that the various concentrations of AWW has varying effect on the metabolism of macromolecules and the activities of alpha amylase and starch phosphorylase in the cowpea seedlings. Nonetheless, more positive results were observed at lower concentration (40ml) while higher concentrations of AWW produced negative effects.

## CONCLUSION

The results of this study show that abattoir waste water (AWW) had varying effects on the levels of macromolecules, and the activities of alpha amylase and starch phosphorylase. Although the impact of AWW on the metabolism of macromolecules in the cowpea seedlings is not so clear, it was however clearly observed that AWW positively affected the chlorophyll content and the activities of alpha amylase and starch phosphorylase, particularly at lower concentrations (20 ml and 40 ml). Since chlorophyll content and the activities of alpha amylase and starch phosphorylase in plants are better indicators of growth than the levels of macromolecules, which predominantly increased and decreased non-significantly relative to the control, moderate level of AWW can improve plant growth.

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