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Effect of Cassava Processing Effluent on Seedling Height, Biomass and Chlorophyll Content of Some Cereals

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Abstract: Growth studies of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum* were carried out using cassava effluent. Effect of effluent at different concentrations on seed germination, growth of seedlings, stem height, number of leaves and biomass of the seedlings of the three different cereals was investigated. The effluent was inhibitory to seed germination and seedling growth of the cereals, which was also reflected in their biomass and number of leaves. *Pennisetum americanum* was at first tolerant, but its germination and growth was later inhibited by the effluent application. The effect of the effluent became more pronounced by continuous application, which resulted in withering of the plants.

Key words: Cassava effluent, growth, biomass, number of leaves, seedlings, chlorophyll content

INTRODUCTION

Man's industrial and economic activities in his immediate environment has brought about improved living conditions, however, these activities produce wastes which inevitably get disposed on land and this has negatively shifted this ecological balance thus threatening his own existence on the planet.

Nigeria, the world's largest producer of cassava, *Manihot esculenta* (Crantz) (FAO, 2004) very unfortunately, has consistently generated so much waste from cassava mills which are usually discharged on land or water indiscriminately and this in turn, affects the biota especially in the southern part of the country where most of the mills are located. The cassava tuber consists of about 15% peel and 85% flesh. For use as human food, the peel is invariably removed and only the flesh is utilized. Both peel and flesh contain significant amounts of hydrocyanic acid which is highly toxic to humans and animals. This is the reason why cassava tuber usually has to pass through several detoxification processes before it is safe for human or animal consumption (Onwueme and Sinha, 1991).

Compounds that are generally toxic to living organisms will also at toxic concentrations prevent germination as well as inhibit growth. Disposal of effluents and wastes is a source of concern to environmentalists and although there are reports on effect of palm oil mill effluent on soil properties (Lim, 1987), the effect of tannery effluent on seed germination (Karunyal *et al*., 1994), contaminating effect of spent engine oil on plant growth (Anoliefo and Edegai, 2001) and heavy metal accumulation in vegetables grown in mine wastes (Cobb *et al.,* 2005), literature is scanty on the effects of cassava effluent on plant growth. In a laboratory experiment, Olorunfemi *et al*. (2007), reported inhibitory effect of cassava processing effluent on the germination of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum*. The effect of cassava effluent on the growth of *Zea mays*, a cereal cultivated mostly in the southern part of Nigeria, where cassava is widely processed and two cereals widely cultivated in the north, *Sorghum bicolor* and *Pennisetum americanum* is the focus of this study. In addition, the biomass and chlorophyll contents of the cereals were examined.

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MATERIALS AND METHODS

Seeds of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum* and fresh cassava processing effluent were collected from Uselu market in Benin city, situated between 6°15′ N and 5°25′ E. The effluent was analysed following the methods outlined by AOAC (1980).

Germination Studies

The same batch of seeds was used throughout. The seeds then were sorted, cleaned and tested for viability using the method of Idu and Olorunfemi (1998). Germination studies followed the methods of Olorunfemi *et al*. (2007).

Growth Studies

Two sets of experimental pots were used for the growth experiments following modified methods of Karunyal *et al*. (1994). Pots of 21 cm diameter were filled with garden soils collected from University of Benin Farms in the proportion of sand, loam and farmyard manure 1:2:2. A set were irrigated with the effluent-water mix at concentrations of 25, 50, 75 and 100% (v/v) effluent everyday for 10 days while control soils were irrigated with 1 L of deionized water. Three replicates of the soil samples were air-dried at 80°C for elemental analysis.

Another set of pots were filled with garden soil in the proportion of sand, loam and farmyard manure 1:2:2. Seeds of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum* were sown in the pots and watered everyday for 20 days. After 20 days, the seedlings were thinned to 10 plants per pot and allowed to grow for another 10 days and watered in similar manner as was done during the initial 20 days to allow for habituation of the seedlings prior to experimental treatment. The 30 day-old healthy seedlings were irrigated with the effluent-water mix at concentrations of 25, 50, 75 and 100% everyday for 10 days, while control plants were irrigated with water only. One hundred milliliter of effluent-water mix and water were used for irrigating treatment and control plants respectively. Plant samples were taken and analysed for different parameters thereafter.

Total Chlorophyll and Protein

Total chlorophyll was estimated using the method of Arnon (1949) and total protein was determined according to the method of Lowry *et al*. (1951).

Element Analysis of the Soil and Plant Samples

Samples of oven-dried plant materials (leaf, stem and root) at 80°C and soils (sand, loam and farmland manure) with and without the effluent treatment were digested and filtered following the methods of Karunyal *et al*. (1994). The concentrations of K, Na, Mg, Ca, Mn, Zn, Pb and Fe were determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

The physico-chemical characteristics of the fresh effluent (Table 1) showed that it is slightly complex with a variety of dissolved cations and suspended particles. The BOD of cassava effluent is within the permissible level of 30-100 ppm for river discharge; however the effluent is highly acidic (Table 2). The concentration profile of K, Na, Mg, Ca, Mn, Cd, Cu, Pb and Fe ions was in the order of Na>Ca>K>Mg>Pb. Fe, Cu, Mn and Cd, were low. Zinc was not detected.

The inhibitory effect of cassava effluent on the germination and growth of the cereals (*Zea mays*, *Sorghum bicolor* and *Pennisetum americanum*) in this study is consistent with results obtained for the inhibitory effect on germination of the same cereals in an earlier experiment

Table 1: Characteristics of the cassava effluent

Characteristics	Concentration (mg L^{-1})
BOD (ppm)	70.0
pH	4.60
Total solids	14,300
Sodium	120.40
Calcium	62.25
Potassium	50.90
Magnesium	25.50
Lead	9.45
Iron	2.35
Copper	1.91
Manganese	0.71
Cadmium	0.19
Zinc	Nil
Cyanide (μg mL^{-1})	0.65

Table 2: pH of soil pretreated with cassava effluent at different concentrations

Concentration (%)	pH
0	6.40
25	6.61
50	7.31
75	7.30
100	6.82

(Olorunfemi *et al*., 2007). Germination of seeds of the cereals followed the same pattern in spite of the slight differences in the composition of the effluent used in the present study. Seeds of cereals planted in the 50, 75 and 100% effluent treated soils did not germinate and no growth was observed. The seeds of *Zea mays* were most tolerant to soil treated with cassava effluent for 10 days before planting. Germination of seeds of *Zea mays* on 25% cassava effluent treated soils was inhibited until the fifteenth day of experimental period while germination of *Sorghum bicolor* seeds in the same soil was inhibited until the fourteenth day before their coleoptiles emerged above soil level. On the other hand, emergence of coleoptiles of *Pennisetum americanum* above soil level occurred just a day after those planted on control soils had emerged but the seedlings withered earlier than those of the other plants. (Table 3).

Seedlings of *Zea mays* irrigated with water grew well throughout the experimental period attaining heights that were significantly higher than seedlings irrigated with the effluent (Fig. 1). This was also apparent in the differences in the number of leaves produced by each set of the plants (Table 3). Growth of the seedlings irrigated with the cassava effluents was inhibited after which they started withering. Except for the plants irrigated with 25% cassava effluent, those irrigated with 50, 75 and 100% did not show considerable increase in the number of leaves produced compared to control plants. The number of leaves produced by 30 day old seedlings of *Zea mays*, *Pennisetum americanum* and *Sorghum bicolor* irrigated with 25% cassava effluent was significantly different from those of control plants. At higher effluent concentrations, the comparative number of leaves produced between the control plants and those irrigated with the various effluent concentrations was not significantly different. Leaf production was inhibited prior to withering of the plants.

Coleoptiles of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum* in controls soils emerged above soil level four days after planting and the seedlings grew well. Cereals planted in 25% cassava effluent treated soils did not attain significant heights compared to control plants. In *Zea mays* the mean height attained on 25% cassava effluent treated soils was 3.4 cm compared to seedlings on control soils which attained over 12 cm height. In *Sorghum bicolor*, the mean height of seedlings was 1.5 cm as against those on control soils which attained over 9.0 cm in height. Similar observations were made with *Pennisetum americanum*, the only exception being that growth and withering of seedlings occurred earlier compared to those of *Zea mays* and *Sorghum bicolor* (Fig. 1).

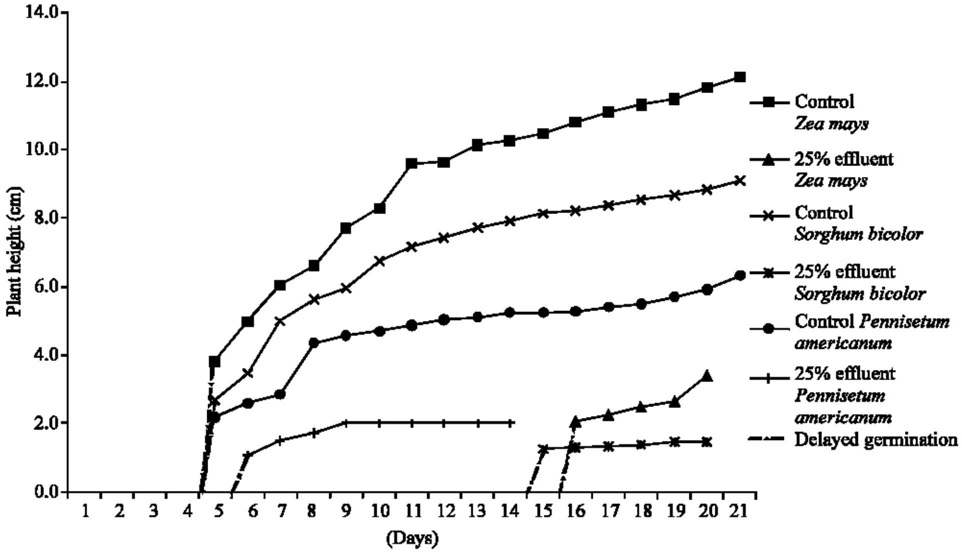

Fig. 1: Comparative effect of soil pretreated with cassava effluent on plant height of *Z. mays*, *S. bicolor* and *P. americanum*

Table 3: Effect of soil pretreated with cassava effluent on leaf number

	Leaf No.					
	Zea mays		*Sorghum bicolor*		*Pennisetum americanum*	
	0	25	0	25	0	25
Day	(%)					
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00	1.66±0.33	0.00±0.00
5	0.67±0.33	0.00±0.00	2.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
6	1.67±0.33	0.00±0.00	2.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
7	2.00±0.00	0.00±0.00	2.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
8	2.30±0.33	0.00±0.00	2.33±0.00	0.00±0.00	2.00±0.00	1.00±0.00
9	3.00±0.00	0.00±0.00	2.66±0.41	0.00±0.00	2.00±0.00	1.00±0.00
10	3.00±0.00	0.00±0.00	3.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
11	3.00±0.00	0.00±0.00	3.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
12	3.00±0.00	0.00±0.00	3.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
13	3.00±0.00	0.00±0.00	3.00±0.00	0.00±0.00	2.00±0.00	1.00±0.00
14	3.00±0.00	0.67±0.33	3.00±0.00	1.00±0.00	2.33±0.33	0.00
15	4.00±0.00	1.00±0.00	3.00±0.00	1.00±0.00	3.00±0.00	0.00
16	4.00±0.00	2.00±0.00	3.00±0.00	1.00±0.00	3.00±0.00	0.00
17	4.00±0.00	2.00±0.00	3.67±0.33	1.00±0.00	3.00±0.00	0.00
18	5.00±0.00	2.00±0.00	4.00±0.00	1.00±0.00	3.00±0.00	0.00
19	5.00±0.00	2.00±0.00	3.00±0.00	1.00±0.00	3.00±0.00	0.00
20	5.00±0.00	0.00	3.00±0.00	0.00	3.00±0.00	0.00

Values are means of 3 replicates, 10 days after addition of effluent
0±0.00 = No germination and growth
0 = Withering

The concentration of sodium and potassium in the soils pretreated with cassava effluent increased with effluent concentration (Table 4). There was no significant increase in the concentration of magnesium with effluent concentration. The concentration of iron and manganese in the effluent treated soils did not show a uniform increase, rather a decrease in concentration of these elements were obtained.

Table 4: Element analysis of soil treated with cassava effluent at various concentrations

	Concentrations (mg g^{-1} dry weight)				
Treatments	Mg	Na	K	Fe	Mn
0	4.86±0.32	1.54±0.03	1.85±0.27	0.34±0.33	0.18±0.02
25	5.34±0.33	1.71±0.04	2.60±0.34	0.55±0.33	0.13±0.12
50	5.36±0.07	1.86±0.07	3.52±0.12	0.41±0.24	0.10±0.05
75	5.86±0.02	1.98±0.24	3.76±0.23	0.41±0.14	0.09±0.07
100	5.85±0.24	2.31±0.32	4.93±0.04	0.41±0.07	0.13±0.16

Values are means of 3 replicates, 10 days after addition of effluent

Table 5: Effect of cassava effluent on biomass

	Biomass (g $plant^{-1}$)				
Plant	Control	25 (%)	50 (%)	75 (%)	100 (%)
Zea mays	11.92	8.69	0	0	0
Sorghum bicolor	09.94	9.57	0	0	0
Pennisetum americanum	16.13	9.98	0	0	0

0 = Withering

Table 6: Effect of cassava effluent on chlorophyll content

	Chlorophyll (μg g^{-1} fresh weight)				
Effluent concentration	Control	25 (%)	50 (%)	75 (%)	100 (%)
Zea mays	1.92	0.39	0	0	0
Sorghum bicolor	1.54	0.27	0	0	0
Pennisetum americanum	1.13	0.18	0	0	0

0 = Withering

The effect of cassava effluent on biomass of the experimental plants is shown in Table 5. There was a significant decrease in biomass in *Zea mays* and *Pennisetum americanum* over the control at 25% cassava effluent concentration. There was no significant difference in biomass of control over the effluent irrigated *Sorghum bicolor* plants. Plants irrigated with 50, 75 and 100% effluent concentration no sooner wilted and were not analyzed. Results obtained for the chlorophyll content of the experimental plants irrigated with various effluent concentrations showed that the chlorophyll content decreased at 25% cassava effluent concentration (Table 6) implying that the effluent inhibits chlorophyll synthesis. At higher concentrations, the plants wilted and were not analysed.

In this study, increases in some properties of cassava effluent polluted soils with corresponding decrease in plant height and dry matter yield of cereals used as experimental plants are consistent with results obtained for *Zea mays* by other workers (Ogboghodo *et al*., 2003, 2006). The rise in pH of the soils, the workers suggested, accounted for the nutrient unavailability for growth of plant and increases in some of soil properties were implied to have accounted for increased number of microorganisms which, in turn, may have fixed these micronutrients in their cells or in the soil. In a previous study of the effect of cassava effluent on germination of *Zea mays*, *Sorghum bicolor* and *Pennisetum americanum*, it was reported that the inhibition of germination of these cereals was due to the acidic nature of the effluent (Olorunfemi *et al*., 2007). In this study, like others (Ogboghodo *et al*., 2003, 2006) the pH of the soils polluted with cassava effluent increased towards neutrality. Cereals like *Zea mays* and *Sorghum bicolor* have been reported to grow successfully under a pH range 5.5-8.0 and 5.0-8.5, respectively (Onwueme and Sinha, 1991), therefore, inhibition of germination of these cereals may not have been due to the observed pH increase in the soil in this study.

The occurrence of heavy metals in soils may be beneficial or toxic to the environment. Excess of metals may produce some common effect of individual metals on different plants. The biota may require some of these elements in trace quantities but at higher concentrations there may be toxicity problems. Metal toxicity in plants has been reported by various authors (Brown and Jones, 1975; Foy *et al.*, 1978). The presence of some heavy metals in the cassava effluent in this study may as well partly account for the inhibition of germination and growth of the cereals.

Cyanide, a known metabolic inhibitor (Lehninger, 1984) is present in both the peel and flesh of cassava tuber from which the effluent is obtained during cassava processing with the concentration of the cyanogenic glucosides ranging from 10-500 mg kg^{-1} of fresh tuber, depending on the cultivars (Onwueme and Sinha, 1991). Bengtson and Cornette (1973) reported that the addition municipal compost to soils increased soil pH from 2.8-5.8 and concluded that municipal compost has a marked effect on the buffering capacity of the soil. Stevenson (1985) reported that organic matter strongly affects the soil fertility by improving the soil structure, increasing the availability of plant nutrients by acting as an accumulation phase for toxic, heavy metals. The cyanide in the effluent used for polluting the soil in this study is present in significant amount and its continuous accumulation may have presumably prevented the utilization of soil nutrients by the plants grown on the polluted soils.

In this study, the chlorophyll content and protein content of the cereals decreased significantly at low effluent concentrations. In the presence of cysteine and at low concentrations (0.1-1.0 mM), cyanide has been found to promote the germination of lettuce, *Amaranthus* and *Lepidium* by metabolizing it to cyanoalanine which is in turn converted to asparagine (Mayer and Poljakoff-Mayber, 1989). However such an interpretation requires that the protein into which the cyanide is incorporated will be specific for germination and that the amount in the seed is not inhibitory to germination. This may possibly explain why germination and growth was inhibited since the cereals were expected to absorb whatever nutrient they were supplied with.

We submit therefore that it could be that the presence of some heavy metals and the amount of cyanide accumulated in the plants may have reached toxic levels thereby causing inhibition to their growth and eventual withering.

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