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EFFECTS OF PHARMACEUTICAL EFFLUENTS ON GERMINATION, GROWTH AND DEVELOPMENT OF *AMARANTHUS HYBRIDUS* L

Nwadinigwe, A. O., Amadi, C. C., *Amujiri, A. N., Nwadinigwe, C. C. and Ozokolie, C. B
Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria
Correspondence: angela.amujiri@unn.edu.ng

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ABSTRACT

The growth and development of *Amaranthus hybridus* polluted with pharmaceutical effluents were studied. Preliminary experiments were carried out in the Laboratory and Screen house with Paracetamol (P) and Amoxicillin (B) effluents. Distilled water served as the control. Media utilized for the main experiment was remediated with poultry manure and compost from *Delonix regia* leaves. Results from the preliminary experiments in the laboratory showed that seedlings germinated on effluent P had significantly ($p < 0.05$) higher shoot length, root length and percentage germination. Results from screen house experiments showed yellowed, stunted seedlings for the polluted treatments at week 3; control had significantly ($p < 0.05$) the highest leaf area, shoot and root lengths. Seedlings grown on effluent P had the lowest growth parameters**.** In the experiment with remediated soil, both polluted and unpolluted plants showed morphological similarities. T_3 (Soil + Poultry manure + effluent B) recorded the highest mean fresh weight of leaves, stem and root at week 7. T_2 (Soil + Poultry + effluent P) recorded the highest mean leaf area, plant height, stem girth and root circumference at week 10. *A. hybridus* adapted to and utilized pharmaceutical pollutants better while under natural attenuation (outdoor, with access to unsterilized soil and direct sunlight). These findings suggest caution in the use of effluents in agricultural practices.

Key words: *Amaranthus hybridus*; pharmaceutical effluents; seed germination; seedling growth; pollution; *Delonix regia* compost

INTRODUCTION

Amaranthus hybridus L. (family Amaranthaceae) is a cosmopolitan species. In some parts of the world, it is classified as a weed, but in West Africa, it is cultivated for its edible leaves (vegetable), cereal from the ground seeds and its vigorous growth (Farrukh *et al.,* 2003). Vegetables have enormous potential as a good source of animal fodder, minerals, proteins, vitamins, iron and calcium which have marked health effects (Atayese *et al.,* 2009; Islam *et al*., 2015). Specifically, *Amaranthus* species are a good source of medicine (the root juice reduces blood pressure and cholesterol level), flavour, dietary minerals, vitamins and the high protein seed offers excellent possibilities for improving human nutrition especially in the third world countries (Atayese *et al*., 2009). The species can be grown both outdoors and in green houses for market consumption. It grows in a wide range of climatic conditions and in some cases the seeds are sown inside half-inch-deep soil inside perforated baskets under controlled watering and transplanted some weeks after germination. Nowadays, following the high recommendation of *Amaranthus* species in diet (Atayese *et al.*, 2009), it is cultivated using various effluents or processing liquids to quicken high productivity. Normally, effluents are released either into the environment (directly on the land) or into water body after treatment in developed countries and they can be purposely used for irrigation during scarcity of water (Islam *et al.,* 2015). Unfortunately, in developing countries, generated effluents are mostly discharged without treatment on the land surface as well as in the water body through open and covered routes (Farid, 2003). Untreated effluents generated by industries are one of the major sources of pollution (Huma *et al.,* 2012). Different complex chemical compounds and toxic materials such as organic matter and heavy metals have been reported in various effluents (Idris *et al*., 2013; Nwachukwu *et al.,* 2013).

*Author for correspondence

Commercial cultivation of crops following high rate of unemployment is receiving increasing attention. However, due to high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and industrial waste is widely practised as a major and economic source of nutrients and organic matter for growing crops by poor farmers (Jamal *et al.,* 2002). The use of such waste water (untreated) for irrigation system definitely provides considerable quantities of N, P, K and Ca along with other essential nutrients to enhance the fertility of soil but introduces alien substances (toxicants) that alter both human and environmental health (Niroula, 2003; Bakare *et al*., 2009; Yasmin *et al.*, 2011; Otuu *et al.,* 2014; Obidiegwu, 2018). Living cells of plants especially leafy vegetables have high metal accumulating capacity which cause reduction of cell activities, growth inhibition and diseases in plants; and can be transferred to organisms through food chain, thereby posing health risk to the organisms along the food chain after long-term consumption (Cui *et al.,* 2004; Islam *et al.,* 2006; Kabir *et al.,* 2008; Farooqi *et al.,* 2009; Islam *et al.,* 2015). Therefore, detailed scientific study on the plant responses is necessary before any specific waste can be used for irrigation of a particular crop. This study investigated the effects of pharmaceutical effluents on the germination, growth and development of *A. hybridus*.

MATERIALS AND METHODS

Amaranthus hybridus seeds, poultry manure and leaves of *Delonix regia* were obtained from Strada Agro Ltd., Nsukka, poultry farm at Isi-uja, Nsukka and Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, respectively. The two effluent samples: Amoxicillin (B) and Paracetamol (P) were collected from two production plants at the pharmaceutical company in Emene, Enugu at the point of discharge from the plants just before it reached the drainage channels.

Twelve Petri dishes were placed on the laboratory bench. They were lined with Whatman's filter paper and moistened with drops of effluent P (Paracetamol), as collected. Ten *A. hybridus* seeds were sown on each Petri dish. This was repeated using effluent B (Amoxicillin) and distilled water which served as the control. The experiment was carried out in three replicates and they were left to stand for seven days (duration for the germination experiment) with occasional moistening of the Petri dishes with the respective effluents and distilled water. At the end of the experiment, records were taken of the percentage germination, leaf area, final lengths of plumule and radicle.

For the second phase, garden soil was sterilized by heating to a temperature of 121^{o}C. Two kg of sterilized soil was mixed with 250 ml of effluent P and the resulting paste was transferred into perforated planting bags. The process was repeated for effluent B and the control. Ten seeds of *A. hybridus* were sown in each bag and after germination, they were thinned to two seedlings per bag. The experiment was watered every two days with 100 ml of the respective effluent and distilled water. The potted plants were placed in a screen house in order to strictly regulate the watering of the plants with the appropriate effluents. This experiment was carried out using the completely randomized design. Each treatment had 5 experimental units and 3 replicates. The experiment lasted for 3 weeks and records were taken every week of shoot length, shoot circumference, leaf area, root length and root circumference.

The third experiment was conducted outdoors in the Botanical Garden. Natural attenuation involved setting up the experiment in the open environment to receive natural sunlight, air and water, planting with unsterilized soil and transplanting into soil mixed with poultry manure and compost separately. The seeds of *A. hybridus* were broadcast into this medium and rain-fed. The reason for keeping the nursery out in the open environment was to encourage seedling development and to initiate natural attenuation. Garden soil was mixed with poultry manure in a ratio of 4 kg: 1 kg. Another set of garden soil was mixed with fresh leaves of *Delonix regia* in a ratio of 8 kg: 1 kg. These were left to stand and watered twice a week, for three weeks after which they were put in planting bags. The experiment was carried out using the randomized complete block design (RCBD) consisting of seven treatments: T_1 (Soil + Poultry manure + Water), T_2 (Soil + Poultry manure + Effluent P), T_3 (Soil + Poultry manure + Effluent B), T_4 (Soil + *Delonix regia* leaves + Water), T_5 (Soil + *Delonix regia* leaves + Effluent P), T_6 (Soil + *Delonix regia* leaves + Effluent B) and T_7 (Soil + Water - control). Each treatment was replicated six times. Three-week-old seedlings from the nursey were transplanted into these media, one plant per bag. The potted plants were placed out in the open garden and watered with 100 ml of their respective effluents every two days, throughout the experiment. The following parameters were assessed during experiment: plant height, root length, leaf area, number of leaves, stem circumference, root circumference, fresh and dry weights of leaves, root and inflorescence. The above parameters were recorded using four (4) plants per treatment at 7 weeks after germination when the plants started showing reproductive organs; and at 10 weeks, when the plants had produced fruits.

DATA COLLECTION

$$\text{Percentage germination (\%)} \quad \frac{\text{Total number of germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

Plant height/ length of plumule was measured with the aid of a meter rule from the soil level to the plant apex (tip of the inflorescence) and recorded in centimetres. Leaf area was determined by measuring the leaf length and leaf width with a meter rule and multiplying by the leaf area constant, 0.75 (Francis *et al.,* 1969). Number of leaves was determined by counting the leaves and leaf scars from fallen leaves. Root length / length of radicle was measured from the base of the stem to the main root or radicle. Stem and root circumference were measured by wrapping a measuring tape around the plant part and reading off the measurement.

Weight analysis was carried out on four seedlings harvested at random from each treatment. Each plant was carefully uprooted, rinsed with water, blotted dry, separated into stem, leaves, root, inflorescence and then weighed separately while still fresh. To find the dry weight, the separated parts were dried at 80^{o}C for 2 hours in a Gallenkamp oven and then weighed again (Ologundudu and Adelusi, 2013).

Statistical analysis.

Data collected from the experiments were subjected to analysis of variance (ANOVA) and the means were compared using Duncan's new multiple-range test (DNMRT) at $p \leq 0.05$ level of significance.

RESULTS

For Petri dish experiment (Table1), results showed that the leaf area of seedlings in different treatments was the same. The percentage germination (75.00 ± 6.45 %) and shoot length (2.50 ± 0.04 cm) of the seedlings grown in Paracetamol effluent was the highest while Amoxicillin had the least. Results showed that treatment B was significantly ($p \leq 0.05$) different from treatments C and P. Treatment C had the highest mean root circumference (0.21 ± 0.01 cm) and was significantly ($p \leq 0.05$) different from P and B.

Table 1: Effects of pharmaceutical effluents on the germination and seedling development of *Amaranthus hybridus* sown in Petri dishes.

Treatment	Percentage germination (%)	Leaf area (cm^2)	Shoot length (cm)	Shoot circumference (cm)	Root length (cm)	Root circumference (cm)
C	65.00 ± 5.00^{b}	0.01 ± 0.00^{a}	2.33 ± 0.09^{a}	0.20 ± 0.01^{b}	1.55 ± 0.30^{a}	0.21 ± 0.01^{a}
P	75.00 ± 6.45^{a}	0.01 ± 0.00^{a}	2.50 ± 0.04^{a}	0.19 ± 0.00^{b}	1.60 ± 0.11^{a}	0.19 ± 0.00^{b}
B	60.00 ± 7.07^{c}	0.01 ± 0.00^{a}	2.01 ± 0.05^{b}	0.59 ± 0.41^{a}	1.14 ± 0.04^{b}	0.17 ± 0.00^{b}

Values represent means ± standard error. Means with different alphabets in the same column differed significantly using the DMRT

Key: C = Petri dish with distilled water: P = Petri dish with Paracetamol effluent; B = Petri dish with Amoxicillin effluent.

Results of the effects of pharmaceutical effluents on the seedling growth and development of *A. hybridus* grown in the Screen house showed that seedlings could not grow more than 3 cm in height and further development was limited since the polluted seedlings died off. Also, severe yellowing of the seedlings was observed in the paracetamol treatment while the control and Amoxicillin treatments showed mild yellowing. Poor seedling development was observed across the treatments.

Results showed that leaf area of treatment B recorded the highest mean at week 1 and was significantly ($p < 0.05$) different from P and C treatments. At weeks 2 and 3, B and C had the highest mean leaf area and were significantly ($p < 0.05$) different from P (Table 2). As regards shoot length, treatment B recorded significantly ($p < 0.05$) the highest mean value in week 1, but in weeks 2 and 3, B and C were significantly ($p < 0.05$) the highest. In weeks 2 and 3, P had the lowest shoot length (0.00 ± 0.00) which was significantly ($p < 0.05$) different from B and C. Treatment P recorded the highest mean shoot circumference in the first week (0.36 ± 0.18 cm) while B recorded the highest in the other two weeks (0.59 ± 0.41 cm) and (0.59 ± 0.40 cm). Treatments P and C recorded the highest mean root length in week 1 (1.23 ± 0.06 cm) and (1.15 ± 0.05 cm) while C recorded the highest mean value (1.80 ± 0.15 cm) in week 3. In week 1, the root length of B was significantly ($p < 0.05$) different from those of P and C. In week 2, P was significantly ($p < 0.05$) longer than those of B and C while in week 3 all the root lengths were significantly ($p < 0.05$) different from one another. By the third week, all plants in treatment P were dead.

Results of the root circumference showed that in week 1 there was no significant difference across all treatments. In weeks 2 and 3, all the treatments were significantly ($p < 0.05$) different from one another with C recording the highest mean root circumference (0.20± 0.00 cm) in both weeks.

Table 2: Effects of pharmaceutical effluents on the seedling development of *Amaranthus hybridus* grown in a screen house

Week	Treatment	Leaf area (cm^2)	Shoot length (cm)	Shoot circumference (cm)	Root length (cm)	Root circumference (cm)
WK1	**C**	0.03 ± 0.00^b	1.45 ± 0.10^b	0.18 ± 0.01^b	1.15 ± 0.05^a	0.16 ± 0.01^a
	P	0.02 ± 0.00^b	1.15 ± 0.14^b	0.36 ± 0.18^a	1.23 ± 0.06^a	0.16 ± 0.01^a
	B	0.06 ± 0.01^a	1.98 ± 0.20^a	0.17 ± 0.00^b	0.74 ± 0.20^b	0.16 ± 0.00^a
WK2	**C**	0.06 ± 0.01^a	2.13 ± 0.03^a	0.20 ± 0.01^b	1.30 ± 0.11^b	0.20 ± 0.00^a
	P	0.02 ± 0.00^b	1.30 ± 0.08^b	0.19 ± 0.00^c	1.60 ± 0.11^a	0.19 ± 0.00^b
	B	0.07 ± 0.01^a	2.25 ± 0.19^a	0.59 ± 0.41^a	1.14 ± 0.04^b	0.17 ± 0.00^c
WK3	**C**	0.07 ± 0.01^a	2.35 ± 0.13^a	0.21 ± 0.00^b	1.80 ± 0.15^a	0.20 ± 0.00^a
	P	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c
	B	0.07 ± 0.01^a	2.33 ± 0.13^a	0.59 ± 0.40^a	1.38 ± 0.05^b	0.19 ± 0.00^b

Values represent means ± standard error. Means with different alphabets in the same column differed significantly using DMRT

Key: C = soil + distilled water; P = soil +Paracetamol effluent; B = soil + Amoxicillin effluent

Results of the effect of natural attenuation (outdoor) on the morphological features of *A. hybridus* polluted with pharmaceutical effluents at weeks 7 and 10 are shown in Table 3. At both weeks 7 and 10, soil treatments mixed with poultry manure namely, T_1, T_2 and T_3 recorded significantly ($p < 0.05$) the highest leaf area, plant height, number of leaves and root circumference. At the same weeks 7 and 10 (Table 3), soil treatments mixed with *Delonix regia* leaves namely, T_4, T_5 and T_6 recorded significantly ($p < 0.05$) lower leaf area, plant height, number of leaves and root circumference, when compared with soil treatments mixed with poultry manure (T_1, T_2, T_3). Generally, T_7, i.e. soil and water or the control (without poultry manure, *Delonix regia* leaves or pharmaceutical effluents) produced significantly ($p < 0.05$) the least of the above vegetative parameters.

At both weeks, T_2 (Soil + Poultry manure + Paracetamol effluent) and T_3 (Soil + Poultry manure + Amoxicillin effluent) had significantly ($p < 0.05$) highest stem girth, followed by T_1 (Soil + Poultry manure + water) which was the same as T_3. The stem girth of T_4, T_5 and T_6 (treatments with *Delonix regia*) were significantly ($p < 0.05$) lower than those of T_1, T_2 and T_3. T_7 recorded significantly ($p < 0.05$) the least stem girth at both 7 and 10 weeks. At 7 weeks, T_1 and T_2 had significantly ($p < 0.05$) the longest root length, followed by T_3 which had the same length of root as T_2, T_4, T_5 and T_6 while T_7 had the shortest root length. At 10 weeks, T_6 (soil + *D. regia* + effluent B) had significantly ($p < 0.05$) longest roots (40.63 ± 4.09 cm) alongside T_1, T_2 and T_4. However, T_1, T_2, T_3, T_4, T_5 and T_7 had the same root length.

At week 7, T_1, T_2, T_3, T_4 and T_5 recorded the highest mean inflorescence length, while T_7 and T_6 recorded the lowest mean (1.33 ± 0.13 and 2.23 ± 0.15 cm). At week 10, T_1, T_2, T_3, T_4 and T_6 recorded the highest mean length of inflorescence and T_7 and T_5 recorded the lowest (13.68 ± 1.55 and 16.83 ± 1.46 cm). T_1, T_2, T_3, T_4 and T_5 were the same in length of inflorescence at week 7 but at week 10, T_1, T_2, T_3, T_4 and T_6 were the same. At week 7, T_6 and T_7 were the same while at week 10, T_5 and T_7 had similar and the lowest length of the inflorescence.

Table 3: Effects of natural attenuation and remediation on the morphological features of *A. hybridus* polluted with pharmaceutical effluents at weeks 7 and 10.

Treatment	Leaf area (cm^2)	No. of leaves	Plant height (cm)	Stem Girth (cm)	Root length (cm)	Root circumference (cm)	Inflorescence length (cm)
At	**Week 7**						
T_1	54.10±2.16^a	36.75±2.36^b	93.30± 3.03^a	2.95±0.12^{b,c}	31.7±1.11^a	3.93±0.11^a	5.50 ± 0.96^a
T_2	54.23±2.01^a	47.25±2.95^a	92.78± 1.96^a	3.63±0.18^a	28.00±2.74^{a,b}	4.03±0.21^a	5.25 ± 1.11^a
T_3	51.43±2.91^a	47.00±3.58^a	91.20±3.76^a	3.38±0.11^{a,b}	24.40±0.89^{b,c}	3.83±0.13^a	3.75 ±0.25^{a,b}
T_4	36.90±3.27^b	23.25±2.29^c	73.90±5.71^{b,c}	2.58±0.21^c	21.65±0.93^{c,d}	3.08±0.19^{b,c}	4.88 ± 0.66^a
T_5	34.68±3.36^b	24.00±2.35^c	65.88±7.57^c	2.53±0.18^c	21.25±1.49^{c,d}	2.75±0.20^c	3.73 ± 0.6$^{a ,b}$
T_6	32.73±1.88^b	21.75±1.32 ^{c}	79.60±4.06^{a,b}	2.88±0.09^c	22.13±1.71^{c,d}	3.35±0.10^b	2.23±0.15^{b,c}
T_7	17.93± 1.21^c	17.00±1.73^c	48.23±2.17^d	1.70±0.07^d	17.65±0.55^d	2.25±0.13^d	1.33±0.13 ^{c}
At	**Week 10**						
T1	68.63±0.95^a	70.00±9.54^a	112.45±5.19^a	3.45±0.13^{b,c}	36.05±1.11^{a,b}	4.15±0.09^a	23.18±0.79^a
T2	68.98±2.15^a	84.00±3.11^a	116.85±0.35^a	4.13±0.14^a	31.25±1.80^{a,b}	4.45±0.16^a	21.70±1.23^a
T3	65.85 ±3.25^a	81.00±3.49^a	109.78±4.29^a	3.88±0.13^{a,b}	29.68±2.35^b	4.05±0.18^{a,b}	21.60± 1.44^a
T4	56.65±2.03^b	41.75±5.20^b	89.35±5.33^b	3.00±0.22^{c,d}	33.60±4.68^{a,b}	3.20±0.20^{c,d}	20.63±1.27^{a,b}
T5	53.83±2.05^{b,c}	41.25±5.33^b	83.10±5.77^b	2.90±0.19^d	30.05±3.21^b	3.00±0.22^d	16.83±1.46^c
T6	48.35±2.62^c	43.50±4.86^b	93.33±3.88^b	3.35±0.10^{c,d}	40.63±4.09^a	3.58±0.14^{b,c}	21.43±1.68^a
T7	35.83± 1.71^d	21.00±1.47^c	61.45±3.56^c	2.10±0.08^e	28.40±2.69^b	2.18±0.22^e	13.68±1.55^c

Values represent means ± standard error. Means with different alphabets in the same column show significant difference in means by DMRT ($p \leq 0.05$).

Key: T_1 = Soil + Poultry manure + Water; **T_2** = Soil + Poultry manure + Effluent P; **T_3** = = Soil + Poultry manure + Effluent B; **T_4** = Soil + *Delonix regia* leaves + Water; **T_5** = Soil + *Delonix regia* leaves + Effluent P; **T_6** = Soil + *Delonix regia* leaves + Effluent B; **T_7** = Soil + Water (control).

The effect of natural attenuation and remediation on the fresh weight of *A. hybridus* polluted with pharmaceutical effluents is shown in Table 4. At week 7, T_1 and T_3 recorded significantly ($p < 0.05$) the highest mean in fresh weight of leaves (21.42 ± 0.73 and 23.41 ± 1.06 g), stem (21.57 ± 1.20 and 27.01 ± 0.95 g) and root (7.10 ± 0.36 7.41 ± 0.40 g) while T_1 recorded significantly ($p < 0.05$) the highest mean in inflorescence (6.26 ± 0.47 g). T_7 (control) recorded the lowest mean in the fresh weight of leaves (5.03 ± 0.57 g), stem (5.45 ± 0.58 g) and root (1.85 ± 0.20 g) while T_5,T_6 and T_7 had the lowest mean inflorescence. At week 7, T_4, T_5 and T_6 were the same in the fresh weight of leaves, stem and root.

At week 10, T_2 and T_3 recorded significantly ($p < 0.05$) the highest mean fresh weight of leaves (32.72 ± 1.03 and 32.13 ± 0.55 g) and root (11.77 ± 0.78 and 10.32 ± 0.49 g); T_1 recorded the highest mean in inflorescence (14.59 ± 1.26 g) while T_7 recorded significantly ($p < 0.05$) the lowest mean fresh weight of leaves (7.91 ± 0.59 g), stem (7.98 ± 0.21 g), root (2.45 ± 0.18 g) and inflorescence (2.65 ± 0.23 g). At week 10, T_2 and T_3 were the same in fresh weight of leaves, root and inflorescence, while T_5 and T_7 did not differ significantly in inflorescence.

Table 4: Effects of natural attenuation and remediation on the fresh weight of *Amaranthus hybridus* polluted with pharmaceutical effluents at weeks 7 and 10.

Treatments At week 7	Leaf (g)	Stem (g)	Root (g)	Inflorescence (g)
T_1	21.42 ± 0.73^{a}	21.57 ± 1.20^{b}	7.10 ± 0.36^{a}	6.26 ± 0.47^{a}
T_2	13.89 ± 0.59^{b}	17.67 ± 0.62^{c}	5.25 ± 0.28^{b}	3.32 ± 0.15^{b}
T_3	23.41 ± 1.06^{a}	27.01 ± 0.95^{a}	7.41 ± 0.40^{a}	3.82 ± 0.36^{b}
T_4	10.96 ± 0.76^{c}	12.97 ± 1.32^{d}	4.09 ± 0.16^{c}	2.19 ± 0.44^{c}
T_5	10.23 ± 0.40^{c}	13.15 ± 0.64^{d}	3.98 ± 0.22^{c}	0.24 ± 0.22^{d}
T_6	10.09 ± 0.48^{c}	12.02 ± 0.90^{d}	4.12 ± 0.20^{c}	0.17 ± 0.15^{d}
T_7	5.03 ± 0.57^{d}	5.45 ± 0.58^{e}	1.85 ± 0.20^{d}	0.18 ± 0.16^{d}
At week 10				
T_1	26.37 ± 2.03^{b}	35.66 ± 3.11^{c}	9.69 ± 1.19^{b}	14.59 ± 1.26^{a}
T_2	32.72 ± 1.03^{a}	55.56 ± 1.82^{a}	11.77 ± 0.78^{a}	10.44 ± 0.69^{b}
T_3	32.13 ± 0.55^{a}	46.18 ± 0.55^{b}	$10.32 \pm 0.49^{a, b}$	11.76 ± 0.63^{b}
T_4	20.90 ± 0.85^{c}	22.21 ± 0.83^{e}	$7.01 \pm 0.33^{c, d}$	6.35 ± 0.44^{c}
T_5	14.35 ± 0.57^{d}	18.50 ± 0.57^{e}	5.44 ± 0.31^{d}	4.11 ± 0.18^{d}
T_6	24.22 ± 1.00^{b}	27.68 ± 0.99^{d}	7.49 ± 0.42^{c}	8.22 ± 0.44^{c}
T_7	7.91 ± 0.59^{e}	7.98 ± 0.21^{f}	2.45 ± 0.18^{e}	2.65 ± 0.23^{d}

Values represent means ± standard error. Means with different alphabets in the same column are significantly different using DMRT ($p \leq 0.05$). **Key:** $\mathbf{T_1}$ = Soil + Poultry manure + Water; $\mathbf{T_2}$ = Soil + Poultry manure + Effluent P; $\mathbf{T_3}$ = = Soil + Poultry manure + Effluent B; $\mathbf{T_4}$ = Soil + *Delonix regia* leaves + Water; $\mathbf{T_5}$ = Soil + *Delonix regia* leaves + Effluent P; $\mathbf{T_6}$ = Soil + *Delonix regia* leaves + Effluent B; $\mathbf{T_7}$ = Soil + Water (control).

At week 7, T_1 and T_3 recorded significantly ($p< 0.05$) the highest mean dry weight of leaves and root while T_1 recorded the highest mean value in dry weight of inflorescence (0.64 ± 0.05 g); T_3 gave the highest stem weight (2.73 ± 0.11 g). T_7 (control) recorded the lowest mean value in the dry weight of leaves (0.51 ± 0.06 g), stem (0.56 ± 0.06 g) and root (0.19 ± 0.02 g). T_4, T_5 and T_6 had fairly low mean dry weight of leaves, root and stem. T_5, T_6 and T_7 were not different in weight of inflorescence.

At week 10, T_2 and T_3 recorded the highest mean value in dry weight of leaves and root while T_2 gave the highest value of stem and T_1 recorded the highest mean dry weight in inflorescence (1.49 ± 0.13 g). T_7 produced the lowest mean value in dry weight of leaves (0.80 ± 0.06 g), stem (0.81 ± 0.02 g), root (0.25 ± 0.02 g) and inflorescence (0.28 ± 0.02 g). At week 10, T_2 and T_3 were the same in the dry weight of leaves; root and inflorescence while T_5 and T_7 were the same in the dry weight of inflorescence.

Table 5: Effects of natural attenuation and remediation on the dry weight of *Amaranthus hybridus* polluted with pharmaceutical effluents at weeks 7 and 10.

Treatments	Leaf (g)	Stem (g)	Root (g)	Inflorescence (g)
At week 7				
T_1	2.19 ± 0.07 ^{a}	2.20 ± 0.12 ^{b}	0.72 ± 0.04 ^{a}	0.64 ± 0.05 ^{a}
T_2	1.40 ± 0.06 ^{b}	1.65 ± 0.17 ^{c}	0.54 ± 0.03 ^{b}	0.34 ± 0.15 ^{b}
T_3	2.39 ± 0.12 ^{a}	2.73 ± 0.11 ^{a}	0.75 ± 0.04 ^{a}	0.39 ± 0.04 ^{b}
T_4	1.11 ± 0.07 ^{b,c}	1.35 ± 0.10 ^{c,d}	0.37 ± 0.05 ^{c}	0.23 ± 0.05 ^{c}
T_5	1.05 ± 0.05 ^{c}	1.33 ± 0.06 ^{c,d}	0.41 ± 0.02 ^{c}	0.03 ± 0.02 ^{d}
T_6	1.21 ± 0.21 $^{b, c}$	1.26 ± 0.07 ^{d}	0.43 ± 0.02 ^{c}	0.02 ± 0.02 ^{d}
T_7	0.51 ± 0.06 ^{d}	0.56 ± 0.06^{e}	0.19 ± 0.02 ^{d}	0.02 ± 0.02 ^{d}
At week 10				
T_1	2.67 ± 0.21 ^{b}	3.61 ± 0.31 ^{c}	0.99 ± 0.12 ^{b}	1.49 ± 0.13 ^{a}
T_2	3.28 ± 0.10^{a}	5.57 ± 1.78 ^{a}	1.19 ± 0.08 ^{a}	1.06 ± 0.07 ^{b}
T_3	3.23 ± 0.06 ^{a}	4.65 ± 0.06 ^{b}	1.05 ± 0.05 ^{a,b}	1.20 ± 0.05 ^{b}
T_4	2.11 ± 0.09 ^{c}	2.24 ± 0.09 ^{e}	0.71 ± 0.03 $^{c, d}$	0.65 ± 0.04 ^{c}
T_5	1.45 ± 0.06 ^{d}	1.88 ± 0.07 ^{e}	0.56 ± 0.03 ^{d}	0.42 ± 0.02 ^{d}
T_6	2.44 ± 0.10 ^{b}	2.78 ± 0.10 ^{d}	0.76 ± 0.04 ^{c}	0.83 ± 0.05 ^{c}
T_7	0.80 ± 0.06 ^{e}	0.81 ± 0.02^{f}	0.25 ± 0.02 ^{e}	0.28 ± 0.02 ^{d}

Key: $\mathbf{T_1}$ = Soil + Poultry manure + Water; $\mathbf{T_2}$ = Soil + Poultry manure + Effluent P; $\mathbf{T_3}$ = = Values represent means ± standard error. Means with different alphabets in the same column show significant difference in means by DMRT ($p \leq 0.05$).
Soil + Poultry manure + Effluent B; $\mathbf{T_4}$ = Soil + *Delonix regia* leaves + Water; $\mathbf{T_5}$ = Soil + *Delonix regia* leaves + Effluent P; $\mathbf{T_6}$ = Soil + *Delonix regia* leaves + Effluent B; $\mathbf{T_7}$ = Soil + Water (control).

DISCUSSION AND CONCLUSION

The Petri dish and screen house experiments showed similar results in germination and seedling development of *Amaranthus hybridus*. All the treatments in the screen house experiment were planted in sterilized soils with no additional growth medium. In the screen house experiment, seedlings could not grow more than 3 cm in height, and showed stunted development until all the polluted seedlings died off eventually. The yellowing and the stunted development of the seedlings may be attributed to the fact that some of the nutrients present in the effluents are essential but that beyond a particular concentration, they become hazardous (Yasmin *et al.*, 2011). Thus, the poor development of the seedlings may be attributed to inadequate exposure to light and inadequate nutrients in the planting media. Also, the seedlings in effluent treatments might have died off due to chemical stress from Paracetamol and Amoxicilin effluents. This is in line with the work of Makhijani *et al*. (2014) who reported that at high concentrations, tetracycline showed phytotoxic effects on *Cicer arietinum*.

Natural attenuation provided the light and nutrients needed by the plants, unlike the sterilized soil and reduced light penetration experienced in the screen house. Treatments with poultry manure (T_1, T_2, T_3) resulted in the best growth and development of plant parts, evident in the height of the plants, number of leaves, roots circumference, leaf area and the weights of the leaves, stems, roots and inflorescence at both 7^{th} and 10^{th} weeks. Leaf development is the most desired attribute for green leafy vegetables and T_1, T_2 and T_3 enhanced leaf development. There were no visible differences to show which plants were polluted with effluents and which ones were not (control). This means that there were no visible signs of phytotoxicity and there were no visible signs of stress on the plant parts. Also, there were no visible differences to show which ones were polluted with paracetamol effluent and which ones were polluted with Amoxicillin effluent. This agrees with the findings of Wu *et al.* (2013) who reported that four common vegetables (lettuce, spinach, cucumber and pepper) grown for 21 days in a nutrient solution containing mixed pharmaceutical and personal care products (PPCPs) at 0.5 µg/L or 5 µg/L concentrations showed no significant difference in the biomass of plants grown in the PPCP-spiked solution and the control media, indicating an absence of phytotoxicity or other effects from the added PPCPs. Islam *et al.* (2015) also reported that external morphology of vegetables cannot give the guarantee for better quality vegetable. Treatments with *Delonix regia* compost had slightly lower yield than treatments with poultry manure, although they showed healthy development of plant parts with no outward manifestations on the plant parts that indicated accumulation of heavy metals or Active Pharmaceutical Ingredients (APIs). Hence there was no phytotoxicity after treatments with the effluents, after natural attenuation and remediation were carried out. However, phytotoxicity was fully demonstrated in the case of Petri dish and screen house experiments where there was no natural attenuation.

Amaranthus hybridus grown with soil and water alone (T_7 control treatment) passed through the developmental stages using the same length of time as the rest of the treatments and was able to develop up to the reproductive stage, but it had the least yield when compared with the other treatments. *A. hybridus* is known to grow to a height of 50 cm (Farrukh *et al.*, 2003). However, with the introduction of poultry manure, it grew up to 112.24 cm; with *Delonix regia* compost, it grew up to 89.35 cm. This implies that poultry manure and *Delonix regia* compost enhanced growth and development in *A. hybridus* despite pollution with pharmaceutical effluents.

The growing of *A. hybridus* outdoors, with the addition of poultry manure significantly enhanced the productivity of the crop and ameliorated the phytotoxicity of pharmaceutical effluents. Replacement of poultry manure with compost made with *Delonix regia* significantly enhanced this productivity to a comparatively less degree. There is the need to determine whether *A. hybridus* will absorb and phytoaccumulate toxic chemicals that can cause health hazards to human beings.

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