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## Pharmaceutical effluent pollution in *Amaranthus Hybridus*

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Experiments were carried out to study the ability of *Amaranthus hybridus* to accumulate heavy metals and active pharmaceutical ingredients (APIs) from two pharmaceutical effluent samples: Paracetamol (P) and Amoxicillin (A). The experiment involved the use of unsterilized soil, poultry manure, compost made from *Delonix regia* leaves, access to natural sunlight, air and rainwater. Results from analysis of the effluent samples showed they contained cadmium ( $3.002 \text{ mg L}^{-1}$ ), zinc ( $3.11 \text{ mg L}^{-1}$ ), iron ( $4.02 \text{ mg L}^{-1}$ ) and Paracetamol ( $25.24 \text{ } \mu\text{g ml}^{-1}$ ) for effluent P, and Cd ( $2.30 \text{ mg L}^{-1}$ ), Zn ( $2.12 \text{ mg L}^{-1}$ ), Fe ( $1.21 \text{ mg L}^{-1}$ ) and Amoxicillin ( $60.82 \text{ } \mu\text{g ml}^{-1}$ ) for effluent A. *Amaranthus hybridus* accumulated up to  $5.53 \text{ mg kg}^{-1}$  of Zn in the leaves and  $5.49 \text{ mg kg}^{-1}$  in its roots. Only a minute quantity of Cd was recorded in the plant parts. The highest accumulated Paracetamol ( $1.60 \text{ } \mu\text{g ml}^{-1}$ ) in the plant part was recorded in the leaf of T<sub>5</sub> (Soil + Compost + effluent P) while the highest accumulated Amoxicillin ( $5.93 \text{ } \mu\text{g ml}^{-1}$ ) was recorded in T<sub>3</sub> stem (Soil + Poultry manure + effluent A). Therefore, *A. hybridus* was able to withstand pharmaceutical pollution effects better when it was exposed to a natural environment. The accumulation of heavy metals suggest caution in the use of pharmaceutical effluents for irrigation in agriculture.

**Keywords:** Effluent, Pollution, *Amaranthus*, Paracetamol, Amoxicillin, Heavy metals.

### INTRODUCTION

Effluents may contain heavy metals, pharmaceutical and personal care products (PPCPs) and POCs even after treatment. According to (Cui et al. 2004) and (Islam et al. 2006), accumulated POCs and heavy metals in plants grown with effluents can be transferred to organisms along the food chain, after long-term application and consumption. Antimalarial drugs such as artemether and lumefantrine that are widely used in Africa, as well as amoxicillin were detected at concentrations ranging from 3 to  $32 \text{ } \mu\text{g L}^{-1}$  in Tanzania (Miraji et al. 2016). Pharmaceuticals such as Erythromycin, Chloramphenicol, Nalidixic acid, Tetracycline, Sulfamethoxazole, Acetaminophen, Diclofenac, and Ibuprofen have been detected in Umgeni

surface water and in a dam along the Umgeni River used for water supply in KwaZulu-Natal South Africa (Agunbiade and Moodley, 2014). Varying concentrations of Diclofenac, Chloroquine, Paracetamol and Ciprofloxacin were  $17 \text{ } \mu\text{g L}^{-1}$ ,  $5 \text{ } \mu\text{g L}^{-1}$ ,  $3 \text{ } \mu\text{g L}^{-1}$  and  $1 \text{ } \mu\text{g L}^{-1}$ , respectively were also detected in surface and groundwater, irrigation canals and several wells in the pharmaceutical industrial area of Sango Ota, Ogun State, Nigeria (Olaitan et al. 2014). PPCPs and POCs are not easily degradable either through biodegradation or photodegradation, and they can contaminate groundwater; however, photodegradation products of naproxen have been reported to have more toxic effects than the parent compound on algae, rotifers, and microcrustaceans (Isidori et al. 2005). Metabolite

concentrations may also be more significant than that of some parent PPCP compounds; e.g. N4-acetylsulfapyridine was found to be more toxic in algae than the parent compound, sulfapyridine (Garcia-Galan, et al. 2012).

Commercial cultivation of various *Amaranthus* species is common in West Africa due to its potentials as a vegetable (the edible leaves), cereal from the ground seeds and its vigorousness of growth (Farrukh et al. 2003). As a vegetable, *Amaranthus* species are good sources of flavour, vitamins, dietary minerals and medicine. The root juice reduces blood pressure and cholesterol level (Farrukh et al. 2003; Atayese et al. 2009). Due to its high recommendation in diet, it is cultivated using various effluents or processing liquids to quicken high productivity. No member of this genus is known to be poisonous, but when grown inorganically on nitrogen-rich soils or effluent-irrigated soils, they are known to increase concentrations of nitrates, persistent organic contaminants (POCs) and heavy metals (Cui et al. 2004; Islam et al. 2006; and Atayese et al. 2009).

*Amaranthus hybridus* has been used in phytoremediation studies on crude oil polluted sites (Odjegba and Sadiq, 2002; Omosun et al. 2008), on heavy metal polluted roadside (Atayese et al. 2009) and on effluent polluted river banks (Orwa, 2019). The authors reported uptake of heavy metals mainly in the plant leaves. There is dearth of information on the effects of pharmaceutical effluents on the mineral composition of *Amaranthus* species. Hence, this study investigated the effect of heavy metal uptake by *Amaranthus hybridus* polluted with pharmaceutical effluents.

## MATERIALS AND METHODS

Two effluent samples: Amoxicillin (A) and Paracetamol (P) were collected from the two pharmaceutical production plants at the point of discharge from the factory, just before they empty into the drainage channels. The effluent samples were subjected to chemical, heavy metal and active pharmaceutical ingredients (APIs) analyses.

Seeds of *A. hybridus* were sown in a nursery made in a wide basket filled with garden soil, and irrigated with tap water. Three-week-old seedlings were transplanted from this nursery into planting bags containing seven different planting media, one plant per bag. The seven different planting media with their different irrigation treatments were as follows: 2 kg of garden soil mixed with

poultry manure in a ratio of 4 kg: 1 kg and irrigated with water (T<sub>1</sub>), with effluent P (T<sub>2</sub>) and with effluent A (T<sub>3</sub>); 2 kg of garden soil mixed with fresh leaves of *Delonix regia* in a ratio of 8 kg: 1 kg and irrigated with water (T<sub>4</sub>), with effluent P (T<sub>5</sub>) and with effluent A (T<sub>6</sub>), and 2 kg of plain garden soil irrigated with water (T<sub>7</sub>, control). The experiment was replicated three times. The plants were watered with 100 ml of the respective effluent, every two days for the duration of the experiment. After 10 weeks, four plants from each treatment were harvested randomly, separated into plant parts (leaves, stem, root and seeds) and then, analysed for heavy metals and APIs.

In the chemical analyses, pH of the effluent samples, plant parts and soil samples were measured with a pH meter. The electrode of the pH meter was inserted into the effluent samples and the values read off the screen. For determination of the pH of the plant parts and soil samples, 5 g of soil sample or ground and sieved plant part was digested with 20 ml of 20% H<sub>2</sub>SO<sub>4</sub> and filtered. The mixture was made up to 50 ml using distilled water. The electrode of the pH meter was inserted into the digested plant sample and the values read off the screen.

Determination of heavy metals in the effluents, soil and plant parts was conducted using Varian AA240 Atomic Absorption Spectrophotometer (AAS). Two ml of effluent sample was digested with 20 ml of 20% H<sub>2</sub>SO<sub>4</sub>, while soil and plant part samples were prepared by digesting 0.5 g of sieved soil or dry, ground and sieved plant material or with 20 ml of 20% H<sub>2</sub>SO<sub>4</sub>. Each mixture was filtered and made up to 50 ml using distilled water. A portion of each mixture was poured into a cuvette and passed through the AAS. These were measured against the corresponding wavelength for each metal.

Estimation of paracetamol concentration in pharmaceutical effluent, harvested plant parts and soil sample was determined using modified spectrophotometric method of (Sivasubramanian et al. 2010). This method involved preparation of stock solution by dissolving 100 mg of Paracetamol (or effluent, soil or dry, ground and sieved plant material as the case may be) in 10 ml of 0.1N NaOH in a 100 ml volumetric flask. This was followed by sonication for 10 minutes; the volume was made up to 100 ml with 0.1N NaOH. Preparation of calibration curve was developed by measuring the absorbance of the dilutions at 257 nm using UV spectrophotometer (Shimadzu model 1800). Paracetamol in the stock solution was estimated by pipetting 10 ml of each stock

solution into a 100 ml volumetric flask and made up to 100 ml volume. This was shaken for 10 minutes and filtered. The absorbance was measured at 257 nm. The readings were extrapolated in the calibration curve to obtain the concentration of Paracetamol in the effluent (as well as soil and plant parts).

Estimation of Amoxicillin concentrations in pharmaceutical effluent, harvested plant parts and soil sample was carried out using modified spectrophotometric method of (Vidyadhara et al. 2015). This method includes preparation of stock solution by dissolving 100 mg of Amoxicillin (or effluent, soil or dry, ground and sieved plant material, as the case may be) using 10 ml of 0.1N HCl in a 100 ml volumetric flask, followed by sonication for 10 minutes before the volume was further diluted to 100 ml with 0.1N HCl. Each mixture was shaken for 10 minutes, filtered and absorbance was measured at 229 nm with UV spectrophotometer (Shimadzu model 1800). The readings were extrapolated in the calibration curve to obtain concentration of Amoxicillin in the samples.

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  $p \leq 0.05$  levels of significance.

## RESULTS

Chemical analysis shows that both pharmaceutical effluents were highly acidic (pH of effluent P and A were 4.49 and 4.24 respectively) and were beyond FAO (2006) limits for effluent pH (5 – 9).

Analyses of the plant parts showed that there

was high significant difference in pH and heavy metal accumulation in the plant parts, across the treatments at  $p \leq 0.05$ . The leaves of T<sub>1</sub> were significantly ( $p \leq 0.05$ ) the highest and the most alkaline while T<sub>7</sub> leaves were neutral, and had the lowest recorded mean. Significantly ( $p \leq 0.05$ ) highest mean concentration of zinc was recorded in T<sub>3</sub> while iron was significantly ( $p \leq 0.05$ ) highest in T<sub>5</sub>; cadmium was detected in T<sub>5</sub> leaves but not detected in T<sub>3</sub>, T<sub>4</sub> and T<sub>7</sub> at all.

In the stem, results showed that the significantly ( $p \leq 0.05$ ) highest mean pH was recorded in T<sub>4</sub>. T<sub>6</sub> recorded the highest mean concentration for zinc, T<sub>3</sub> was highest in iron while Cadmium was only detected in T<sub>2</sub> and in T<sub>6</sub>. The root gave significantly ( $p \leq 0.05$ ) highest mean pH in T<sub>2</sub>; highest mean concentration of zinc was recorded in T<sub>3</sub> while T<sub>5</sub> had the highest concentration of iron. Cadmium was highest in T<sub>6</sub> but not detected in T<sub>3</sub>, T<sub>4</sub> and T<sub>7</sub>.

In the seeds, T<sub>1</sub> had the highest mean pH; the highest mean concentration of zinc was recorded in T<sub>6</sub>, the concentration of iron was highest in T<sub>3</sub> while Cadmium was only detected T<sub>2</sub> (0.0015 mg kg<sup>-1</sup>) and in T<sub>6</sub> (0.0010 mg kg<sup>-1</sup>). In the soil, results showed the highest mean pH in T<sub>4</sub>; the highest mean concentration of zinc was recorded in T<sub>6</sub>; iron was highest in T<sub>3</sub> and cadmium was highest in T<sub>6</sub> but was not detected in T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>7</sub>.

Result of the uptake of active pharmaceutical ingredients in *A. hybridus* polluted with pharmaceutical effluents is shown in Table 5. It shows that the highest concentration of Paracetamol occurred in T<sub>5</sub> soil while there was fairly high accumulation in the root and leaves of T<sub>5</sub>. The highest concentrations of Amoxicillin occurred in the stem of T<sub>3</sub> and in the root of T<sub>6</sub>.

**Table 1: Chemical analysis of Paracetamol and Amoxicillin effluents.**

TEST	P	A	FAO limit*
pH	4.49	4.24	5 – 9
BOD (mg L <sup>-1</sup> )	0.42	0.63	40
COD (mg L <sup>-1</sup> )	227.41	205.82	120
TOC (mg L <sup>-1</sup> )	124.44	36.35	NA
Zinc (mg L <sup>-1</sup> )	3.11	2.12	2.0
Iron (mg L <sup>-1</sup> )	4.07	1.21	5.0
Cadmium (mg L <sup>-1</sup> )	3.00	2.30	0.01
Paracetamol concentration (µg ml <sup>-1</sup> )	25.24	ND	NA
Amoxicillin concentration (µg ml <sup>-1</sup> )	ND	60.82	NA

\*Source: Food and Agriculture Organisation (2006) standards for wastewater and effluents. A = Effluent containing Amoxicillin; P = Effluent containing Paracetamol; ND = Not detected; NA = Not applicable.

**Table 2: pH and concentration of heavy metals in the leaf of 10-week-old *Amaranthus hybridus* polluted with pharmaceutical effluents.**

Treatment	pH	Cd (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
<b>LEAF</b>				
T <sub>1</sub>	9.22 ±0.31 <sup>a</sup>	0.0003 ± 0.0001 <sup>b</sup>	1.87 ±0.17 <sup>e</sup>	4.02 ±0.07 <sup>e</sup>
T <sub>2</sub>	8.66 ±0.12 <sup>b</sup>	0.0043 ± 0.0004 <sup>a</sup>	4.08 ±0.10 <sup>c</sup>	5.49 ±0.06 <sup>c</sup>
T <sub>3</sub>	8.55 ±0.10 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>b</sup>	5.53 ±0.12 <sup>a</sup>	5.97 ±0.18 <sup>b</sup>
T <sub>4</sub>	7.18 ±0.05 <sup>d, e</sup>	0.0000 ± 0.0000 <sup>b</sup>	1.67 ±0.07 <sup>e</sup>	3.24 ±0.04 <sup>f</sup>
T <sub>5</sub>	7.60 ±0.20 <sup>d</sup>	0.0050 ± 0.0004 <sup>a</sup>	3.59 ±0.10 <sup>d</sup>	6.48 ±0.02 <sup>a</sup>
T <sub>6</sub>	8.09 ±0.06 <sup>c</sup>	0.0047 ± 0.0002 <sup>a</sup>	4.73 ±0.04 <sup>b</sup>	5.09 ±0.10 <sup>d</sup>
T <sub>7</sub>	7.00 ±0.07 <sup>e</sup>	0.0000 ± 0.0000 <sup>b</sup>	1.62 ±0.08 <sup>e</sup>	3.19 ±0.07 <sup>f</sup>

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

**Legend:** T<sub>1</sub> = Soil +Poultry manure + Water; T<sub>2</sub> = Soil + Poultry manure + Effluent P; T<sub>3</sub> = Soil + Poultry manure + Effluent A; T<sub>4</sub> = Soil + *Delonix regia* leaves + Water; T<sub>5</sub> = Soil + *Delonix regia* leaves + Effluent P; T<sub>6</sub> = Soil + *Delonix regia* leaves + Effluent A and T<sub>7</sub> = Soil + Water (Control).

**Table 3: pH and concentration of heavy metals in the stem and root of 10-week-old *Amaranthus hybridus* polluted with pharmaceutical effluents.**

Treatment	pH	Cd (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
<b>STEM</b>				
T <sub>1</sub>	7.32 ±0.08 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>b</sup>	1.62 ±0.11 <sup>d</sup>	4.89 ±0.14 <sup>d</sup>
T <sub>2</sub>	7.54 ±0.11 <sup>b</sup>	0.0027 ± 0.0002 <sup>a</sup>	3.69 ±0.18 <sup>c</sup>	5.93 ±0.25 <sup>c</sup>
T <sub>3</sub>	7.22 ±0.15 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>b</sup>	4.18 ±0.02 <sup>b</sup>	8.16 ±0.13 <sup>a</sup>
T <sub>4</sub>	8.24 ±0.06 <sup>a</sup>	0.0000 ± 0.0000 <sup>b</sup>	1.37 ±0.01 <sup>d, e</sup>	3.89 ±0.03 <sup>e</sup>
T <sub>5</sub>	6.70 ±0.15 <sup>d</sup>	0.0000 ± 0.0000 <sup>b</sup>	3.69 ±0.05 <sup>c</sup>	7.69 ±0.15 <sup>b</sup>
T <sub>6</sub>	7.06 ±0.07 <sup>c</sup>	0.0026 ± 0.0005 <sup>a</sup>	4.96 ±0.09 <sup>a</sup>	6.30 ±0.21 <sup>c</sup>
T <sub>7</sub>	6.74 ±0.10 <sup>d</sup>	0.0000 ± 0.0000 <sup>b</sup>	1.27 ±0.05 <sup>e</sup>	4.33 ±0.09 <sup>e</sup>
<b>ROOT</b>				
T <sub>1</sub>	6.84 ±0.21 <sup>c, d</sup>	0.0012 ± 0.0002 <sup>b</sup>	3.29 ±0.15 <sup>d</sup>	3.84 ±0.19 <sup>b</sup>
T <sub>2</sub>	7.66 ±0.16 <sup>a</sup>	0.0010 ± 0.0001 <sup>b</sup>	4.89 ±0.24 <sup>b</sup>	5.64 ±0.27 <sup>a</sup>
T <sub>3</sub>	6.52 ±0.18 <sup>d, e</sup>	0.0000 ± 0.0000 <sup>c</sup>	5.49 ±0.19 <sup>a</sup>	3.19 ±0.02 <sup>c</sup>
T <sub>4</sub>	7.01 ±0.04 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>c</sup>	2.11 ±0.12 <sup>e</sup>	3.12 ±0.10 <sup>c</sup>
T <sub>5</sub>	7.32 ±0.06 <sup>a, b</sup>	0.0008 ± 0.0002 <sup>b</sup>	4.13 ±0.06 <sup>c</sup>	5.84 ±0.10 <sup>a</sup>
T <sub>6</sub>	6.21 ±0.06 <sup>e</sup>	0.0041 ± 0.0004 <sup>a</sup>	3.88 ±0.08 <sup>c</sup>	3.96 ±0.04 <sup>b</sup>
T <sub>7</sub>	6.72 ±0.09 <sup>c, d</sup>	0.0000 ± 0.0000 <sup>c</sup>	1.80 ±0.08 <sup>e</sup>	3.12 ±0.09 <sup>c</sup>

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

**Legend:** T<sub>1</sub> = Soil +Poultry manure + Water; T<sub>2</sub> = Soil + Poultry manure + Effluent P; T<sub>3</sub> = Soil + Poultry manure + Effluent A; T<sub>4</sub> = Soil + *Delonix regia* leaves + Water; T<sub>5</sub> = Soil + *Delonix regia* leaves + Effluent P; T<sub>6</sub> = Soil + *Delonix regia* leaves + Effluent A and T<sub>7</sub> = Soil + Water (Control)

**Table 4: pH and concentration of heavy metals in the seeds of 10-week-old *Amaranthus hybridus*, and in remnant of the effluent-polluted soil.**

Treatment	pH	Cd (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
<b>SEEDS</b>				
T <sub>1</sub>	8.00 ±0.09 <sup>a</sup>	0.0000 ± 0.0000 <sup>c</sup>	1.54 ±0.11 <sup>b</sup>	5.93 ±0.21 <sup>c</sup>
T <sub>2</sub>	7.97 ±0.17 <sup>a</sup>	0.0015 ± 0.0002 <sup>a</sup>	3.73 ±0.07 <sup>b</sup>	7.35 ±0.18 <sup>b</sup>
T <sub>3</sub>	7.92 ±0.13 <sup>a</sup>	0.0000 ± 0.0000 <sup>c</sup>	4.03 ±0.05 <sup>a</sup>	8.93 ±0.20 <sup>a</sup>
T <sub>4</sub>	7.06 ±0.06 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>c</sup>	0.85 ±0.06 <sup>e</sup>	5.67 ±0.13 <sup>c</sup>
T <sub>5</sub>	7.21 ±0.14 <sup>b, c</sup>	0.0000 ± 0.0000 <sup>c</sup>	3.16 ±0.07 <sup>c</sup>	8.68 ±0.28 <sup>a</sup>
T <sub>6</sub>	7.38 ±0.08 <sup>b</sup>	0.0010 ± 0.0002 <sup>b</sup>	4.19 ±0.19 <sup>a</sup>	5.82 ±1.00 <sup>c</sup>
T <sub>7</sub>	6.95 ±0.08 <sup>c</sup>	0.0000 ± 0.0000 <sup>c</sup>	0.71 ±0.04 <sup>e</sup>	3.88 ±0.21 <sup>d</sup>
<b>SOIL</b>				
T <sub>1</sub>	6.74 ±0.16 <sup>b</sup>	0.0022 ± 0.0005 <sup>b</sup>	3.68 ±0.15 <sup>d</sup>	4.19 ±0.12 <sup>e</sup>
T <sub>2</sub>	6.76 ±0.09 <sup>b</sup>	0.0000 ± 0.0000 <sup>b</sup>	5.98 ±0.10 <sup>b</sup>	6.28 ±0.17 <sup>b</sup>
T <sub>3</sub>	6.29 ±0.05 <sup>c</sup>	0.0000 ± 0.0000 <sup>b</sup>	5.97 ±0.06 <sup>b</sup>	7.47 ±0.13 <sup>a</sup>
T <sub>4</sub>	9.68 ±0.12 <sup>a</sup>	0.0000 ± 0.0000 <sup>b</sup>	2.26 ±0.05 <sup>e</sup>	4.69 ±0.13 <sup>d</sup>
T <sub>5</sub>	6.92 ±0.08 <sup>b</sup>	0.0140 ± 0.0045 <sup>a</sup>	4.87 ±0.25 <sup>c</sup>	7.19 ±0.05 <sup>a</sup>
T <sub>6</sub>	7.00 ±0.05 <sup>b</sup>	0.0033 ± 0.0002 <sup>b</sup>	6.46 ±0.10 <sup>a</sup>	5.50 ±0.21 <sup>c</sup>
T <sub>7</sub>	7.00 ±0.06 <sup>b</sup>	0.0000 ± 0.0000 <sup>b</sup>	2.18 ±0.06 <sup>e</sup>	3.74 ±0.05 <sup>f</sup>

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

**Legend:** T<sub>1</sub> = Soil +Poultry manure + Water; T<sub>2</sub> = Soil + Poultry manure + Effluent P; T<sub>3</sub> = Soil + Poultry manure + Effluent A; T<sub>4</sub> = Soil + *Delonix regia* leaves + Water; T<sub>5</sub> = Soil + *Delonix regia* leaves + Effluent P; T<sub>6</sub> = Soil + *Delonix regia* leaves + Effluent A and T<sub>7</sub> = Soil + Water (Control).

**Table 5: Concentration of active pharmaceutical ingredients accumulated in *A. hybridus* from pharmaceutical effluents.**

	Treatment	Paracetamol (µg/mg)	Amoxicillin (µg/mg)
<b>Leaf</b>	T <sub>1</sub>	ND	ND
	T <sub>2</sub>	0.87 ±0.07	ND
	T <sub>3</sub>	ND	2.92 ±0.08
	T <sub>4</sub>	ND	ND
	T <sub>5</sub>	1.60 ±0.06	ND
	T <sub>6</sub>	ND	2.31 ±0.12
	T <sub>7</sub>	ND	ND
<b>Stem</b>	T <sub>1</sub>	ND	ND
	T <sub>2</sub>	1.08 ±0.06	ND
	T <sub>3</sub>	ND	5.93 ±0.10
	T <sub>4</sub>	ND	ND
	T <sub>5</sub>	0.43 ±0.03	ND
	T <sub>6</sub>	ND	0.13 ±0.03
	T <sub>7</sub>	ND	ND
<b>Root</b>	T <sub>1</sub>	ND	ND
	T <sub>2</sub>	1.00 ±0.04	ND
	T <sub>3</sub>	ND	1.08 ±0.13
	T <sub>4</sub>	ND	ND
	T <sub>5</sub>	1.26 ±0.16	ND
	T <sub>6</sub>	ND	4.14 ±0.02
	T <sub>7</sub>	ND	ND
<b>Soil</b>	T <sub>1</sub>	ND	ND
	T <sub>2</sub>	1.99 ±0.12	ND
	T <sub>3</sub>	ND	1.68 ±0.18
	T <sub>4</sub>	ND	ND
	T <sub>5</sub>	2.13 ±0.13	ND
	T <sub>6</sub>	ND	2.09 ±0.23
	T <sub>7</sub>	ND	ND

Values represent means ± standard error. ND = Not detected.

**Legend:** T<sub>1</sub> = Soil +Poultry manure + Water; T<sub>2</sub> = Soil + Poultry manure + Effluent P; T<sub>3</sub> = Soil + Poultry manure + Effluent A; T<sub>4</sub> = Soil + *Delonix regia* leaves + Water; T<sub>5</sub> = Soil + *Delonix regia* leaves + Effluent P; T<sub>6</sub> = Soil + *Delonix regia* leaves + Effluent A and T<sub>7</sub> = Soil + Water (Control).

## DISCUSSION

Plant species behave differently regarding heavy metal uptake: some species have high metal contents in their tissues compared to a low concentration in the soil, while the reverse is the case for other species (Maisto et al. 2004). Also, soils with near neutral pH have been found to cause low uptake of heavy metals by vegetables (Akinola et al. 2008). Since the pH of all the soil samples were found in the range of 6.29 to 7 (except for T<sub>4</sub> which recorded a pH of 9), neutral pH may have been responsible for the poor accumulation recorded for cadmium from the two effluents which had tested higher than the limits of FAO standards (Limit= 0.01 mg L<sup>-1</sup>). It does not however, agree with the high accumulation recorded for zinc and iron in this research.

The concentration of zinc in the effluents (3.11 mg L<sup>-1</sup> in P; 2.12 mg L<sup>-1</sup> in A) was higher than the acceptable FAO limits (2.0 mg L<sup>-1</sup>). The recorded accumulation was as high as 5.98 mg kg<sup>-1</sup> in Paracetamol—polluted treatments and 6.68 mg kg<sup>-1</sup> in Amoxicillin—polluted treatments. This is in line with the work of Orwa (2019) which reported that *A. hybridus* grown on highly metal-polluted river banks accumulated up to 17.42 mg kg<sup>-1</sup> from 57.96 mg L<sup>-1</sup> of zinc in water. The plants took zinc up readily because it is required in small quantities by growing plants. High accumulation of zinc in this study also implies that *A. hybridus* was able to use the effluents as alternative sources of nutrients.

The concentration of iron in effluent P (4.07 mg L<sup>-1</sup>) was higher than the acceptable FAO limits (2.0 mg L<sup>-1</sup>) but within the standard in effluent A (1.21 mg L<sup>-1</sup> in A). The recorded accumulation was as high as 7.72 mg kg<sup>-1</sup> in Paracetamol—polluted treatments and 6.73 mg kg<sup>-1</sup> in Amoxicillin—polluted treatments. The plants took iron up readily because it is a mineral nutrient required in small quantities by growing plants. High accumulation of iron in this study also implies that *A. hybridus* was able to use the effluents as alternative sources of nutrients. The speculation is that if *A. hybridus* can accumulate this quantity of metals from low concentrations, then it will be able to withstand higher concentrations and perhaps accumulate greater amounts as was implied by Orwa (2019).

Green plants have been found to take up APIs and PPCPs when exposed to them. *A. hybridus* was able to take up Paracetamol from pharmaceutical effluent P up to 1.56 µg mg<sup>-1</sup> in the leaves, 1.26 µg mg<sup>-1</sup> in the root and 1.12 µg

mg<sup>-1</sup> in the stem. This is in line with the work carried out by Bartha et al. (2011) who reported that 0.24 µg g<sup>-1</sup> and 0.07 µg g<sup>-1</sup> of Paracetamol were detected in the root and leaf (respectively) of *Typha latifolia* after 72 hours of exposure. Chlortetracycline uptake from soil in cabbage and green onion and their transport to the aerial part has also been reported, while barley and carrots reportedly took up metformin and ciprofloxacin (Lillenberg et al. 2010). The findings of Wu et al. (2013) however disagrees with this accumulation, with their report that at effluent concentration of 0.5 µg L<sup>-1</sup>, most PPCPs were found only in the roots and leaves while at concentration of 5 µg L<sup>-1</sup>, only the roots showed accumulation. Also, according to the findings of Wu et al. (2013), Acetaminophen (Paracetamol) showed negligible uptake into the roots and was not detected in the leaves. This may be because the concentration of Paracetamol in Effluent P (25.24 µg ml<sup>-1</sup>) was greater than the concentration (5 µg L<sup>-1</sup>) in the spiked nutrient media used in their experiment. The highest concentration of Paracetamol adsorbed to the soil (up to 2.13 µg mg<sup>-1</sup>) could remain there for quite some time, since some APIs like tetracycline were found to remain in the soil for years (Boxall et al. 2002). In a study of the uptake of antibiotics in irrigation water by plants in Tianjin, China, most of the target analytes including Sulfamethoxazole, Chloramphenicol, Tetracycline, Chlortetracycline, Ofloxacin were detected in vegetables between 0.1 and 532 µg kg<sup>-1</sup> (Hu et al. 2010). Luo et al. (2011) reported the occurrence and transport of tetracycline, sulfonamide, quinolone and macrolide antibiotics likely originating from veterinary application in swine farms and fish ponds in the Haihe River Basin, China.

The results showed that Amoxicillin accumulated in the stem (up to 5.93 µg mg<sup>-1</sup>) and root (4.14 µg mg<sup>-1</sup>). The concentration left behind in the soil (1.68 µg mg<sup>-1</sup>) was lower than the concentrations detected in the plant parts. This is similar with the findings of Lillenberg et al. (2010) who reported that Ciprofloxacin concentration was 10 µg g<sup>-1</sup> in the soil, while the Ciprofloxacin content of the lettuce planted in it was 44 µg g<sup>-1</sup> – more than four times higher in the plant than in the soil. It was also reported by Lillenberg et al. (2010) that when the vegetation period is longer, antibiotics accumulate in the plant and this occurred in the Amoxicillin treatments in the present investigation. Amoxicillin like some other antibiotics, was found to be a highly degradable compound in Jodeh et al. (2012) and this could be

the reason why the concentration left in the soil was so much lower than those found in the plant parts.

## CONCLUSION

*Amaranthus hybridus* has been found to be able to take up heavy metals and APIs from pharmaceutical effluents and accumulate them in their plant parts, without showing physical manifestations. Results from the experiment showed that polluted plants competed favourably with their non-polluted counterparts, and for this reason, farmers in need of cheap irrigation may become further attracted to the idea of irrigating their farms using other industrial effluents and so, should be warned off. Pharmaceutical and medical personnel should always warn citizens against the indiscriminate use and disposal of pharmaceutical products, especially the edible forms. When plants like *A. hybridus* are able to take up these active pharmaceutical ingredients from soil and water and store them in their harvested plant parts, it becomes pertinent to warn people about the dangers of such irrigation practices. However, further research is needed to determine the effects of these polluted plants on other animals when ingested over time, and to determine if the accumulations are transferable into the next generation of *Amaranthus hybridus*.

## CONFLICT OF INTEREST

The authors declare that the present study was performed in the absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

ACC and AAN designed the experiments and also wrote and reviewed the manuscript. ACC and NCC executed the experiment and collected data and performed data analysis. All authors read and approved the final version of the submission.

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## REFERENCES

- Agunbiade, F. O. and Moodley, B. (2014). Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. *Environ Monit Assess* 186 (11) doi:10.1007/s10661-014-3926-z
- Akinola, M. O., Njoku, K. L. and Ekeifo, B. E. (2008). Determination of lead, cadmium and chromium in the tissues of an economically important plant grown around a textile industry at Ibesh, Ikorodu area of Lagos State, Nigeria. *Adv. Environ. Biol.* 2: 25-30.
- Atayese, M.O., Eigbadon, A.I., Oluwa, K.A. and Adesodun, J.K. (2009). Heavy metal contamination of *Amaranthus* grown along major highways in Lagos, Nigeria. *Afr. Crop Sci. J.* 16(4): 225 – 235.
- Bartha, B., Huber, C. and Schröder, P. (2011). Removal and metabolism of Paracetamol (Acetaminophen) from wastewater – Options for phytotechnologies. *Proceedings of the 12<sup>th</sup> International Conference on Environmental Science and Technology*, 8-10 September 2011. Rhodes, Greece. 12: 166-174.
- Boxall, A. B. A., Fogg, L., Blackwell, P. A., Kay, P. and Pemberton, E. J. (2002). *Review of Veterinary Medicines in the Environment*; R&D Technical Report P6-012/8/TR. pp233.
- Cui, Y. L., Zhai, R. H., Chen, D. Y., Huang, Y. Z. and Qiu, Y. (2004). Transfer of metals from soil to vegetable in an area near a smelter in Nanning, China. *Environ. Pollut.* 30: 785-791.
- Farrukh, H., Syed, S. G., Ijaz, F. and Muffakhira, J. D. (2003). Some Autecological studies on *Amaranthus hybridus*. L. *Pakistan Journal of Weed Science Research* 9(1-2): 117-124.
- Food and Agriculture Organisation (2006). *Environmental management and coordination (water quality) regulations*. FAOLEX, Kenya gazette. pp 23
- Garcia-Galan, M. J., Blanco, S. G., Roldan, R. L., Diaz-Cruz, S. and Barcelo, D. (2012). Ecotoxicity evaluation and removal of sulfonamides and their acetylated metabolites during conventional wastewater

- treatment. *Sci. Total Environ.* 437:403-412
- Hu, X., Zhou, Q. and Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environ. Pollut.* 158: 2992-2998
- Isidori, M., Lavorgna, A., Nardelli, A., Parella, A., Previtera, L. and Rubino, M. (2005). Ecotoxicity of naproxen and its photo-transformation products. *Sci. Total Environ.* 348 (3): 93-101
- Islam, M. O., Khan, M. H. R., Das, A. K., Akhtar, M. S., Oki, Y. and Adachi, T. (2006). Impacts of industrial effluents on plant growth and soil properties. *Soil and Environment* 25(2): 113-118.
- Jodeh, S., Staiti, H., Haddad M., Renno, T., Zaid, A., Jaradat, N. and Kharoaf, M. (2012). The fate of leachate of pharmaceuticals like Amoxicillin, Ibuprofen and Caffeine in the soil using soil columns. *Eur. J. Chem.* 3(4): 480-484
- Lillenberg, M., Litvin, S.V., Nei, L., Roasto, M. and Sepp, K. (2010). Enrofloxacin and Ciprofloxacin uptake by plants from soil. *Agron. Res.* 8 (1): 807-814.
- Luo, Y., Xu, L., Rysz, M., Wang, Y., Zhang, H. and Alvarez, P. J. J. (2011). Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River basin, China. *Environ Sci and Technol.* 45:1827-1833
- Maisto, G., Alfani, A., Baldantoni, D., Marco, A. and Sancto, A. V. (2004). Trace metals in the soil and in *Quercus ilex* L. leaves at anthropic and remote sites of the Campania Region of Italy. *Geoderma* 122: 269-279.
- Miraji, H., Othman, O. C., Ngassapa, F. N. and Mureithi, E. W. (2016). Research trends in emerging contaminants on the aquatic environments of Tanzania. *Scientifica* 6pp doi: 10.1155/2016/3769690.
- Odjegba, V. J. and Sadiq, A. O. (2002). Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. *Environmentalist* 22 (1): 23-28.
- Olaitan, J. O., Anyakora, C., Bamiro, T. and Temitope, T. A. (2014). Determination of pharmaceutical compounds in surface and underground water by solid phase extraction-liquid chromatography. *J. Environ. Chem. Ecotoxicol.* 6:20-26
- Omosun, G., Markson, A. A., and Mbanasor, O. (2008) Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. *American-Eurasian Journal of Scientific Research* 3 (1): 70-74.
- Orwa, T. O. (2019). *Amaranthus hybridus* in Phytoremediation of Heavy Metal Polluted Waters of Nairobi River. *Proceedings of Jomo Kenyatta University of Agriculture and Technology (JKUAT) Annual Scientific, Technological and Industrialization Conference, 14 - 15 November, 2013. Nairobi, Kenya.* 296-304.
- Sivasubramanian, L., Lakshmi, K. S. and Tintu, T. (2010). Simultaneous spectrophotometric estimation of Paracetamol and lornoxicam in tablet dosage form. *Int J Pharm Pharm Sci.* 2 (4): 166-168.
- Vidyadhara, S., Sasidhar, R. L. C., Deepti, B., Wilwin, E. and Sowjanya Lakshmi, B. (2015). Formulation and evaluation of Amoxicillin trihydrate lozenges. *Dhaka Univ. J. Pharm. Sci.* 14 (1): 61-70.
- Wu, X., Ernst, F., Conkle, J. L. and Gan, J. (2013). Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. *Environ Int.* 60 (2013): 15 – 22.