Responses of *Amaranthus hybridus* to effluent from an aluminum plating industry in Lagos, Nigeria

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Abstract The study investigated the responses of *Amaranthus hybridus* to effluent from an aluminum plating plant in Lagos, Nigeria. Seedlings were raised from seeds and were grouped into three categories, each representing a treatment. The first group which is the control received 250 ml of water via the roots every 3 days, the second category received half-strength effluent via the roots every 3 days, while the third group received full-strength effluent via the roots every 3 days throughout the study period. Physiological and biochemical, as well as metabolic, parameters representative of oxidative damage and antioxidant activity were evaluated after the treatments. The physicochemical analysis of the effluent showed that total suspended solids, Al and Zn levels were higher than the recommended standards. The biomass and the protein content of the control were 18.01 g and 0.835 mg/g dry wt, respectively, as against 12.04 g and 0.368 mg/g dry wt observed in the treated plants. In contrast, effluent treatment caused a significant increase in malondialdehyde level as well as activities of antioxidant enzymes. The present study revealed that the effluent adversely affected growth and development of *A. hybridus*. Continual discharge of these industrial effluents could have a negative impact on the environment especially to fragile ecosystems.

Keywords *Amaranthus hybridus* · Environment · Growth inhibition · Industrial effluent · Toxicity

1 Introduction

Environmental pollution caused by effluents emanating from industrial firms is now a growing concern in many countries of the world due to a number of harmful substances contained in them and their potential health effects. These industries generate wastewater of diverse chemical composition, which when discharged could lead to drastic changes in both biological and chemical parameters of the receiving ecosystem (Gomez et al. 2008).

To minimize impacts on ecosystems, it is important to treat effluents before discharge into the environment. Although authorities of different countries have extant laws that mandate every industry to treat their wastewaters before discharge into the environment, studies have shown that analysis of effluent indicates the presence of chemical substances capable of causing harm to the living organisms (Radic et al. 2010). In some cases, the treated effluent does not exceed the discharge limits but the results of toxicity test shows potential toxicity (Lin et al. 1994).

Industrial effluents are generally decontaminated by physicochemical treatment, where pollutants are insolubilized, thereby generating two phases: water and sludge which are subsequently separated by a physical process (Charles et al. 2011). It is now known that chemical precipitation does not remove the whole pollutant load, and their impacts on the natural environment are difficult to forecast (Kumari and Tripathi 2015). The presence of toxic pollutants can be detected by the changes caused in an organism with the use of bioassays (Valerio et al. 2007; Di Salvatore et al. 2008). In particular, the phytotoxicity tests using plants present several advantages. First, these tests are simple, quick and reliable. Second,

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they are inexpensive and do not require sophisticated equipment. Third, plants can be more sensitive to environmental stress than other organisms (Gopalan, 1999; Valerio et al. 2007).

Higher plant toxicity assays, including *Allium cepa*, *Vicia faba*, *Arabidopsis thaliana*, *Hordeum vulgare*, *Triticum aestivum*, *Zea mays*, *Lactuca sativa*, have been calibrated with a broad range of chemicals (Yi and Meng 2003; Ma et al. 2005). Since higher plant systems have unique advantages for in vitro monitoring and screening (Ma et al. 2005), it is recommended that higher plant systems should be accepted by regulatory authorities as first-tier assay system for detecting the possible biotoxicity associated with environmental stress (Han et al. 2011).

In Nigeria, environmental regulations on pollution control of industrial discharges and other pollutants are enforced by the National Environmental Standards and Regulations Enforcement Agency (NESREA) which relies on conventional physicochemical procedures. Lagos is a highly populated metropolitan city with quite a number of industries, a development that has put the city under serious pressure in terms of environmental safety. Consequently, the city and its environs have witnessed environmental degradation leading to decreased biodiversity and extinction of many sensitive organisms. Pollution of the environment with effluents from various industries has become a serious problem in Lagos, as industrial growth and development has been on the increase in the last 10 years. Pharmaceutical, agro-allied, aluminum plating, plastic, chemical and food industries, etc., are located in different parts of the city. Due to the nature of their operations which requires large volume of water, most of the effluents generated are discharged into the environment, thereby altering its natural state in terms of quality.

The ecological and toxicological problems resulting from the discharge of effluents from the aluminum plating industry under review has been the most important water pollution problems in this area. This problem is further aggravated because some residents along the stream axis depend directly on this stream as source of portable water and other domestic purposes. In addition, local farmers, particularly vegetable growers, use the water from this stream directly for irrigation purposes. Consequently, there is likelihood that toxicants from this effluent may affect plants and animals, enter the food chain and affect a larger human population. Therefore, this work aimed to evaluate the toxicity of discharged effluent from an aluminum plating industry using *A. hybridus* as a model plant. The plant is a popular leafy vegetable cultivated in domestic gardens and consumed largely by the people in Lagos, Nigeria.

2 Materials and methods

2.1 Plant Growth and Treatment

Effluent was collected from the discharge point of the industry (06°36'25"N, 05°18'39.8"E and 06°36'43"N, 03°18'66"E) that flows into a drainage canal that connects to a river. The physicochemical parameters of the collected sample were determined in its crude natural state within 6 h of collection.

Seeds of *A. hybridus* were germinated and nursed on a nursery bed (3 m × 1.2 m) for 3 weeks. Thereafter, healthy seedlings of 15 cm in height were transplanted into polythene bags filled with loamy soil to achieve plant stand of one seedling per bag. They were arranged in rows in a screen house at the Botanical Garden, University of Lagos (06°31'4.8"N, 03°24'2.18"E and 06°31.08'N, 03°24.04'E). Seedlings were kept moist by watering when necessary and were allowed to acclimatize for 5 days before they were subjected to industrial effluent treatment for 3 weeks. The plants were grouped into 3 categories, each representing a treatment and replicated 12 times. Category 1 served as the control and received 300 ml of water every 3 days throughout the experiment period, category 2 received 300 ml of half-strength effluent every 3 days, while the third category was subjected to 300 ml of full-strength effluent every 3 days. Plants were harvested 14 and 21 days after the initial treatment for dry weight analysis to generate data for the relative growth rate (RGR) determination and 21 days for every other analysis.

2.2 Dry weight determination

Plants were uprooted carefully and washed thoroughly in a running tap water to remove soil particles. After rinsing with distilled water, they were placed in labeled paper bags and oven-dried at 65 °C for 72 h. The dried samples were weighed using a digital top loading weighing balance (Mettler AE 100) to determine the dry weight (Guo et al. 2010).

2.3 Relative growth rate (RGR)

The RGR of the plants was determined according to method described by Causton (1994) using the relationship:

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad \text{g g}^{-1} \text{wk}^{-1}$$

2.4 Relative water content (RWC)

The fourth leaf from the apex of plants representing each treatment was harvested and weighed to determine their

fresh weight (Fr. wt). The leaves were then submerged separately in distilled water for 24 h in the dark. They were removed from the water, mopped dry using an absorbent and weighed to determine their saturated weight (SW). The leaves were then placed in labeled envelopes and dried in an oven at 65 °C for 3 days to determine the dry weight. The relative water content was calculated according to Turner (1981) using the formula: $RWC = [(fresh\ weight - dry\ weight) / (saturated\ weight - dry\ weight)] \times 100$.

2.5 Total chlorophyll determination

Plant leaves (0.3 g) were ground in 10 ml 80 % acetone in the dark. After centrifugation at 18 °C, 4000×g for 3 min, the absorbance of the supernatant was read at 645 and 663 nm (Arnon, 1949). The total chlorophyll content was calculated according to Machlachlan and Zalik (1963).

2.6 Lipid peroxidation assay

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following the procedure described by Wang and Jin (2005).

2.7 Protein determination

The protein level was determined by using micro-Kjeldahl method for the estimation of total organic nitrogen in the dried plant samples as described by Piorreck et al. (1984). The total nitrogen content was multiplied by 6.25 to obtain the protein concentration.

2.8 Antioxidant enzyme assays

Fresh leaves (0.3 g) were ground in 2 ml ice-cold extraction buffer containing 50 mM Tris-HCl (pH 7.8), 1 mM EDTA and 2 % (w/w) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000×g for 8 min, and the supernatant was used as crude extract for catalase (CAT) determination. CAT activity was determined by measuring the consumption of H₂O₂ in 2 ml reaction mixture (50 mM potassium phosphate buffer, 1 % H₂O₂ and 50 µl crude extract). H₂O₂ consumption was monitored for 1.5 min as the decrease in absorbance at 240 nm according to Aebi (1984). For ascorbate peroxidase (APX), fresh leaves (0.3 g) were homogenized in 2 ml ice-cold extraction buffer containing 50 mM phosphate buffer solution (pH 7.8), 2 mM ascorbic acid and 5 mM EDTA. After centrifugation, the supernatant was used as the crude extract for assay of APX activities following the method described by Nakano et al. (2000).

2.9 Statistical analysis

Data were subjected to statistical analysis using SPSS version 16.0 statistical package (SPSS, Chicago, IL). Means of three replicates as well as the standard error (SE) were determined. The test of significance between the treatments was done using a one-way analysis of variance (ANOVA).

3 Results

3.1 Physicochemical characteristics of the effluent

Table 1 depicts the physicochemical variables of the effluent as well as the recommended limits by the regulatory authority in Nigeria. The effluent's TSS and Zn levels were significantly ($p = 0.05$) higher than the reference standards, while the Al concentration in the effluent was higher than the recommended standard but not statistically significant. The concentrations of TSS and Zn in the effluent were 42 and 9.44 mg/l, respectively, as against 40 mg/l and <1 standard limits, respectively. The concentration of Al in the effluent was 3.29 mg/l, a value that is above the recommended discharge limit of 3.00 mg/l.

Table 1 Physicochemical characterization of the effluent from aluminum plating industry in Lagos, Nigeria

S/N	Parameters	Values detected	NESREA standard limits
1.	Ph	8.77	6–9
2.	Conductivity (µs cm ⁻¹)	562	7000
3.	TDS	375	2000
4.	TSS	42	40
5.	Turbidity (FTU)	68	–
6.	Cl ⁻	24	600
7.	CN ⁻	0.05	0.1
8.	NO ₃ ⁻	5.48	20
9.	SO ₄ ²⁻	8.0	500
10.	NH ₄ ⁺	0.16	0.6
11.	COD	45	200
12.	BOD ₅ ²⁰	18	30
13.	Al	3.29	3
14.	Cr	0.03	<1
15.	Cu	0.56	<1
16.	Ni	0.02	<1
17.	Pb	0.3	<1
18.	Zn	9.44	<1

All units are in mg/L, except for pH and where noted otherwise

3.2 Effect of effluent on plant growth and biochemical characteristics

The biomass of *A. hybridus* decreased significantly with increase in effluent concentration. After the treatment period, the control plants had a mean dry weight of 18.01 g, while plants treated with 50 and 100 % effluent had 16.2 and 12.04 g, respectively (Fig. 1). The RGR of the plant was significantly reduced as the concentration increased. Plants treated with 50 and 100 % effluent had mean RGR values of 1.21 ± 0.08 and 1.09 ± 0.04 g g⁻¹ wk⁻¹, respectively, as against 1.42 ± 0.06 g g⁻¹ wk⁻¹ observed for control (Fig. 2).

Relative water content (RWC) of leaves was higher in untreated plants, which decreased significantly with increase in effluent concentration. It was observed that the RWC for the control plants was 84.26 % as against 75.25 and 58.29 % in plants treated with 50 and 100 % effluent, respectively (Fig. 3).

The total chlorophyll content declined gradually with increase in effluent concentration. At the end of the treatment period, the control plants had a mean value of 0.824 ± 0.022 mg g⁻¹ fr wt. This value was significantly higher than 0.734 ± 0.026 and 0.638 ± 0.02 mg g⁻¹ fr wt

observed for plants treated with 50 % and 100 % effluents, respectively (Fig. 4).

To assess the effects of the effluent on lipid peroxidation, malondialdehyde (MDA) content in the leaves of the plants was measured. Treatment of plants with 50 and 100 % effluent, respectively, led to more than 16 and 70 % increase in MDA content when compared to the control plants (Fig. 5).

Figure 6 depicts the effect of the industrial effluent on the protein content of *A. hybridus*. It was observed that the effluent significantly decreased the protein content in the treated plants. The protein content of the control plants was significantly ($p = 0.05$) higher than the values observed for plants exposed to the effluent. The control plants had a mean value of 0.835 ± 0.02 mg g⁻¹ dry wt, compared to 0.579 ± 0.04 and 0.368 ± 0.04 mg g⁻¹ dry wt observed for plants treated with 50 and 100 % effluent, respectively.

It was observed that the effluent induced a strong antioxidative response in *A. hybridus* as evident in the assessment of CAT and APX activities in both the treated and the untreated plants (Figs. 7, 8). Plants treated with 100 % effluent had mean values of 28.49 ± 1.30 μ mol H₂O₂ g⁻¹ fr wt min⁻¹ and 0.081 ± 0.002 μ mol ascorbate g⁻¹ fr wt min⁻¹ as CAT and APX activities, respectively. These

Fig. 1 Whole plant dry weight of *A. hybridus* treated with effluents for an aluminum plating industry

Fig. 2 Effects of effluents from aluminum plating industry on the relative growth rate (RGR) of *A. hybridus*

Fig. 3 Relative water content of *A. hybridus* leaves as affected by effluent treatments

Fig. 4 Total chlorophyll content of *A. hybridus* after exposure to effluent from aluminum plating industry

Fig. 5 Malondialdehyde content of *A. hybridus* as affected by effluents from aluminum plating industry

Fig. 7 Impact of effluents from aluminum plating industry on catalase activities in *A. hybridus*

Fig. 6 Total protein content of *A. hybridus* after exposure to effluents from aluminum plating industry

Fig. 8 Ascorbate peroxidase activities in *A. hybridus* after treatments with effluents from aluminum plating industry

values were significantly higher than $17.62 \pm 1.12 \mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{ fr wt min}^{-1}$ and $0.037 \pm 0.02 \mu\text{mol ascorbate g}^{-1} \text{ fr wt min}^{-1}$ that were, respectively, observed as CAT and APX activities in the control plants.

4 Discussion

This study indicates that effluent from the aluminum planting industry induced oxidative stress and significantly reduced the growth, biomass and nutrients in *A. hybridus* seedlings. The results imply that the effluent might cause biotoxicity on living organisms. The physicochemical analysis of the effluent showed that TSS, Al and Zn exceeded the permissible limits, an indication that the conventional technology used in wastewater treatment appears to be insufficient for a complete removal of all the chemical compounds present in the effluent (Ternes 1998).

The significant decrease in whole plant biomass as well as inhibition of RGR observed for plants treated with effluent suggests bioaccumulation of pollutants, possibly heavy metals such as Cu, Cr, Ni and Pb present in the

effluent. Application of effluent to soil over a period could lead to accumulation of toxic metals as well as a change in the soil environment, such as pH, redox potential and organic matter contents (Hossain et al. 2015). The toxicity of heavy metals appears to arise from complex interactions among different essential and non-essential elements, which may inhibit metabolic enzymes activities that could negatively affect the growth of the plant (Latif et al. 2008). The significance of different aluminum species in different environments as well as their toxicity effects has been documented (Gardner et al. 2002; Guibad and Gauthier 2003). Apart from the free Al^{3+} species, the highly reactive hydroxo-complexes, for instance $\text{Al}(\text{OH})_2^+$ and AlOH^{2+} , are quite toxic and they predominate at pH range 6–9 (Sacan and Balcioglu 2006).

The significant decrease in leaf relative water content (RWC) observed in plants treated with effluent could be due to increased ionic content of soil water as a result of effluent application which subsequently resulted in low water potential of the solution that consequently affected water absorption by plant roots. This observation is in conformity with the findings of Ashraf et al. (2002).

The decline in total chlorophyll content observed in effluent-treated plants can be regarded as general responses associated with metal toxicity. The loss of photosynthetic pigment content as a result of industrial effluent treatments has been reported (Sacan and Balcioglu 2006; Radic et al. 2010; Odjegba and Bamgbose 2012). Generally, the destruction of chlorophyll by heavy metals could be due to disruption of electron transport chain, substitution of Mg^{2+} of the tetra-pyrrole ring of the chlorophyll molecules, inhibition of enzymes associated with chlorophyll biosynthesis or peroxidation of chloroplast membrane lipids by ROS (Van Assche and Clijsters 1990; Sandalio et al. 2001).

An important feature of metal-induced environmental stress is the activation of a common mechanism involving the production of ROS in cells of the affected organism (Shi and Zhu 2008; Wang et al. 2004). Since membrane lipid is one of the preferred targets of ROS in plant under environmental stress, it is considered to be a reliable indicator of controlled modulation of ROS levels and oxidative stress (Halliwell and Gutteridge 1999). We investigated the levels of lipid peroxidation by measuring MDA in the leaves. *A. hybridus* showed high level of MDA under effluent treatment. It was an indication that pollutants contained in the effluent were absorbed by the plants and induced the generation of excessive ROS; thus, the plant experienced substantial oxidative stress. Similar results were reported previously by Verma and Dubey (2003), Zhang et al. (2007) and Radic et al. (2010).

There was a significant decrease in protein content of the effluent-treated plants compared to the control. Similar results were reported by past investigators when plants were subjected to various abiotic stress factors (Osama and Floyd 1984; Caplan et al. 1990; Deepika and Dhingra 2014). The decrease in protein content in the treated plants could be related partly to the inhibition of glutamine synthetase (GS) and glutamate synthase (GOGAT) activities (Nemat Alla et al. 2008). The failure in GS-GOGAT system function would cause accumulation of unassimilated ammonia and, consequently, diminution in amino acid formation with a subsequent drop in protein synthesis.

Plants possess complex antioxidative defense system comprising of non-enzymatic and enzymatic components to scavenge ROS. Under normal conditions, potentially toxic oxygen radicals are generated at a low level and are adequately detoxified by different antioxidant enzymes present in the plant cells. The balance between production and quenching of ROS may be perturbed by a number of adverse environmental factors, giving rise to rapid increase in intracellular ROS levels (Sharma et al. 2010). In order to avoid the oxidative damage, higher plants raise the level of endogenous antioxidant defense and such response reflects an adaptation of a plant to its environment (Yordanova et al. 2004). In the present study, it was observed that the activities

of catalase and ascorbate peroxidase increased significantly when *A. hybridus* was treated with effluent from aluminum plating industry. The increase in activity of these antioxidant enzymes as well as MDA content underscored the fact that the effluent caused oxidative stress in the plant.

5 Conclusion

The present investigation confirmed that the effluent from aluminum plating industry affected the growth, dry biomass, relative water content, photosynthetic pigment and protein content of *A. hybridus*. Hence, ecological and toxicological problems may arise from continuous discharge of effluents from the aluminum plating industry into the environment in its present form. The physicochemical analysis of the effluent showed that TSS, Al and Zn levels were higher than the recommended standards. These data indicate that effluent from this aluminum plating industry could cause toxic effects on organisms and that the treatment carried out on the effluent before discharge into the environment is not as efficient as it ought to be in removing the hazardous contaminants present in it. To this end, it is suggested that effluent from aluminum plating industries should be used with caution especially by vegetable growers for irrigation of agricultural lands.

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