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Impact of Combined Industrial Effluent on Metal Accumulation, Nitrate Reductase Activity and Yield of Two Cultivars of *Vigna unguiculata* (L.) Walp

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Abstract: Combined industrial effluent from Ikeja Central Treatment Plant, Lagos, was used to irrigate *Vigna unguiculata* L. Walp (cowpea), cultivars IT89KD-349 (white) and IT84E-124 (red). The effluent was alkaline (pH 9.8) and had a significantly higher concentration of Ca (11.53 mg L^{-1}), NO_3 (83.20 mg L^{-1}), SO_4 (22.73 mg L^{-1}), Cl (15.45 mg L^{-1}) and Cd (2.16 mg L^{-1}) than the experimental soil. Nitrate reductase activity was enhanced almost throughout the period of growth of both treated cultivars but for the peak at 35 DAP shown by control white. There was a corresponding increase in the net assimilation rate and a significant increase ($p \le 0.05$) in the biomass of leaves and pods of treated red cowpea but only the pods of treated white cowpea. Heavy metal uptake by seeds of treated plants was negligible and this may be attributed to the high accumulation of Ca by these seeds. Undiluted combined industrial effluent has good agro potential in the cultivation of red cowpea.

Key words: Nitrate, calcium, heavy metal uptake, net assimilation, growth, yield

INTRODUCTION

The application of municipal sewage sludge to croplands is aimed at recycling nutrients and carbon. Sewage effluent has been shown to have agro potential but continuous loading of soil with the effluent may increase the concentration of trace elements in plants grown in these lands, potentially affecting the growth and safe consumption of the plants (Berti and Jacobs, 1996). Untreated textile effluents have been shown to inhibit the germination and growth of winter vegetable crops, while treated forms enhanced the parameters (Rehman *et al*., 2008). Kannan and Upreti (2008) also reported similar effects on germination and growth by treated and untreated distillery effluents. Sugar factory effluent has been shown to stimulate physiological and biochemical parameters of *Gossypium hirsutum* L. (Muthusamy and Jayabalan, 2001) but inhibited the growth of plants such as *Zea mays*, *Vigna radiata* and aquatic plants such as *Eichhornia crassipes* and *Pistia stratiotes* (Ayyasamy *et al*., 2008). Undiluted minta effluents also caused marked inhibitory effects on the growth of *Vigna unguiculata* (Umebese and Onasanya, 2007).

Nitrate reductase is the major enzyme that catalyzes the reduction of nitrates to nitrite in plants (Crawford, 1995). This enzyme is found mostly in the cytoplasm of epidermal and cortical cells of root and shoot mesophyll cells (Berezi and Moller, 2000). Elements presented

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in effluents either anions or cations have been shown to affect Nitrate Reductase Activity (NRA) and invariably the dry matter yield of plants. The cations: Ca, Fe, Zn and the anions: SO_4, NO_3, HPO_4, HCO_3 stimulate NRA by values ranging from 12 to 62% (Mengel and Pilbeam, 1992).

This study investigates the possibility of using combined industrial effluent from Ikeja Central Treatment Plant for irrigating crops by studying the effects of the effluent on the nitrate reductase activity, net assimilation rate, plant biomass, yield and metal accumulation of two cultivars of *Vigna unguiculata* (cowpea), a legume grown for its leaves, fruits and mature seeds.

MATERIALS AND METHODS

Experiments Were Conducted Between 2001 and 2002

Collection of Materials

Seeds of *Vigna unguiculata* L. Walps, cultivars IT89KD-349 (white) and IT84E-124 (red) were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The combined effluent was collected from the Ikeja Central Treatment Plant, Lagos.

Effluent and Soil Analysis

Soil samples were collected from the experimental site. The pH of the soil and effluent was measured using a pH meter (Jenway model). The concentrations of 3 nutrient cations: iron (Fe), zinc (Zn) and calcium (Ca), 3 nutrient anions: nitrate (NO_3), sulphate (SO_4) and chloride (Cl) and 5 heavy metals: nickel (Ni), cadmium (Cd), manganese (Mn), mercury (Hg) and lead (Pb) in the soil and effluent were determined according to procedures outlined by Allen *et al.* (1974). The soil was first passed through an alkaline fusion at 950°C for 15 min in a furnace for metals while the effluent was digested using the nitric acid-perchloric acid digestion and analyzed using an atomic absorption spectrophotometer (Alpha 4 model).

Planting Procedure

Seeds (35 seeds per plot) of the two cultivars were grown in loamy soil at the Botanic garden of the University of Lagos, using completely randomized block design with three replicates. The growth area had a light intensity range of 1546 to 9100Lux and temperature range of 29.0 to 32.5°C. Irrigation was done every day with the combined effluents and another batch of plants from the two cultivars was watered with tap water at the same time.

Nitrate Reductase Activity

Samples of expanded third and fourth leaves from the apex, representative of the entire canopy were taken from plants at 14, 28, 35, 42 and 49 Days After Planting (DAP) and assayed for nitrate reductase activity as outlined by Umebese and Motajo (2008). Three-hundred milligrams of leaves from each replicate were cut into small pieces and put in a test tube to which 5 mL of incubating medium comprising 100 mL phosphate buffer (pH 7.5), 150 mL sodium nitrate and 4% propan-l-ol was added. Incubation was done for 1 h. Thereafter, 1 mL of 1% sulphanilic acid in 2 N HCl followed by 1 mL of 0.01% Naphthylethylene diamine dichloride were added to terminate the reaction. The optical density was read on spectrophotometer at 540 nm. The blank comprised all the above chemicals without the leaves. The standard curve was prepared using 0-0.8 mol mL^{-1} nitrite.

Determination of Net Assimilation Rate

A plant was randomly selected from each of the plots at weekly intervals from the 14-49th DAP. The plant was separated into leaves, stems, roots and pods. Leaf area was measured by the proportionate method of weighing cut outs of traced areas with standard paper of known weight to area ratio. Plant part biomass was measured after air drying for a week. Net assimilation rate (NAR) was calculated using the following equation (Noggle and Fritz, 1976):

$$NAR = \frac{(W_2 - W_1)\ 2.303\ (Log_{10}\ A_2 - Log_{10}\ A_1)}{(t_2 - t_1)\ (A_2 - A_1)}$$

where, W_1, A_1, W_2, A_2 represent biomass (shoot and root) and leaf area at the time of harvest t_1 and t_2.

Plant Heavy Metal Uptake

Three replicates each of seeds of polluted and control plants were air dried for 96 h and digested as outlined by Jones (1984). The concentrations of nutrient elements (Fe, Zn and Ca) and heavy metals (Ni, Cd, Mn, Hg and Pb) in the seeds were determined as outlined by Allen *et al*. (1974), using atomic absorption spectrophotometry (Alpha 4 model).

Statistical Analysis

All analysis were carried out in three replicates (n = 3) and results recorded in averages with standard error. Using a one-way classification, Analysis of Variance (ANOVA) of the treated and control plants were done to test the level of significance between the plants at $p \leq 0.05$.

RESULTS AND DISCUSSION

The combined effluent from the Ikeja treatment plant was alkaline (pH 9.8) and had significantly high ($p \leq 0.01$) levels of some nutrients (Ca, NO_3, SO_4 and Cl) and the heavy metal Cd, than the experimental soil (Table 1). The application of the effluent to two cultivars of *Vigna unguiculata* IT 89 KD-349 (white cowpea) and IT 84E-124 (red cowpea) showed some beneficial effects. Nitrate Reductase Activity (NRA) of treated plants showed a steady increase from early vegetative to pod maturity stage and was generally higher than that of

Table 1: Mineral elements in effluent and experimental soil

	Concentration of minerals (mg L^{-1})	
Mineral elements	Effluent	Soil
Zn	0.06±0.05	1.60±0.02**
Fe	9.98±1.85	283.30±0.73**
Ni	0.02±0.03	0.09±0.04**
Cd	2.16±0.48**	0.02±0.04
Mn	0.21±0.06	3.65±0.14**
Hg	0.83±0.03	0.83±0.03
Pb	4.05±0.66	5.05±0.23*
Ca	11.53±0.99**	2.34±0.24
NO_3	83.20±0.40**	27.60±0.03
SO_4	22.73±5.41**	5.73±1.70
Cl	15.45±2.84**	5.83±0.61
pH	9.80	5.90

Significant difference occurred between elements in effluent and soil at $^{**}p \leq 0.01$ and $^{*}p \leq 0.05$

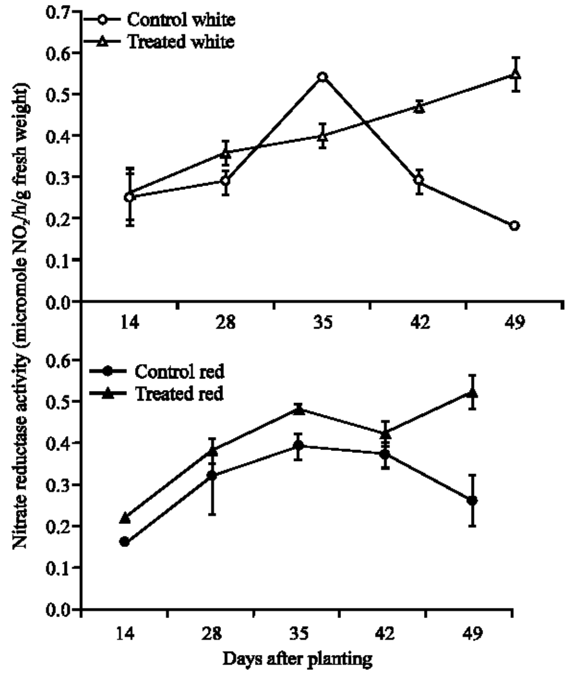

Fig. 1: Nitrate reductase activity of treated white and red cultivars of cowpea during the growth period. Vertical bars show standard deviation of means

the control (Fig. 1). Peak activity of NRA was at the flowering stage (35 DAP) in the control while it was found at the pod maturity stage (49 DAP) in the treated plants. The continuous increase in NRA and subsequent vegetative development of the aerial parts of the plant in the course of growth of both treated cultivars could be attributed to the high nitrate content of the effluent (Bergareche *et al*., 1994). The Ca-rich effluent may have contributed to the increase in NRA even during the flowering and fruiting stages of treated plants as high Ca nutrition have been found to induce nitrate reductase activity (Bergareche *et al*., 1994; Bachmann *et al*., 1995).

The Net Assimilation Rate (NAR) which is a measure of the amount of photosynthetic product going into plant material, increased in the course of growth of treated red cowpea while it decreased in treated white cultivar (Fig. 2). There was a corresponding significant ($p<0.05$) enhancement of the biomass of whole plant, leaves, stems and roots of red cowpea and a reduction in the biomass of the white cultivar (Table 2). The undiluted effluent has a beneficial effect on the growth of red cowpea but is slightly toxic to the white cultivar. Pod yield was significantly enhanced in both treated cultivars. The presence of high levels of Ca, NO_3, SO_4 and Cl in the effluent and the direct influence of Ca and nitrate on enhancing nitrate reductase activity may have contributed to the high dry matter and improved pod yield of treated plants. Osterås (2004) has reported that Ca induces an increase in stem diameter of Norway spruce in the presence of heavy metals. Issa *et al.* (1995) have also reported that calcium-heavy metal interactions cause an increase in growth of *Kirchneriella lunaris* especially where Cd is equally present. However, the difference in the reaction of the two cultivars in this experiment to the effluent irrigation indicates a probability that the influence of calcium-heavy metal interactions is species specific.

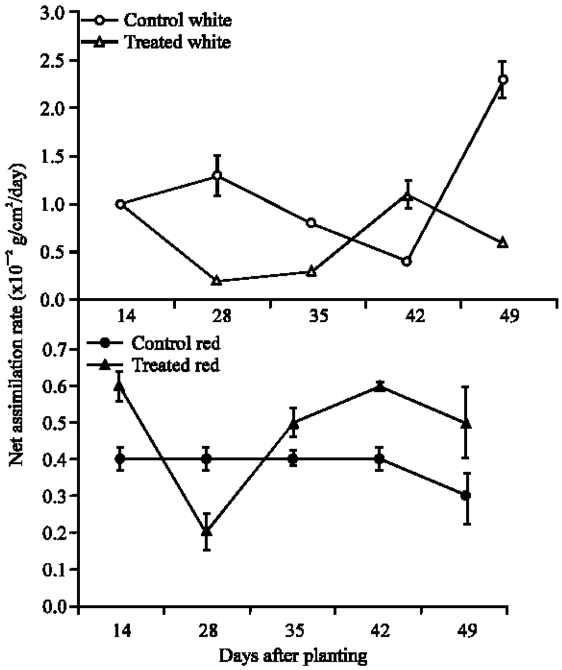

Fig. 2: Net Assimilation Rate (NAR) of treated white and red cultivars of cowpea during the growth period. Vertical bars show standard deviation of means

Table 2: Biomass (g) of leaves, stems, roots and pods of effluent treated cowpea cultivars and the control at various stages of growth

	Days after planting				
Cultivars	14	28	35	42	49
Leaves					
Control white	0.14a	0.43a	0.77a	0.86a	1.798a
Treated white	0.25a	0.45a	0.49b	0.81a	0.81b
Control red	0.08a	0.47a	0.60c	0.95a	1.11c
Treated red	0.12a	0.52a	0.8ad	0.93a	1.45d
Stems					
Control white	0.12a	0.74a	1.00a	1.44a	2.70a
Treated white	0.16b	0.28b	0.71b	1.18a	2.26b
Control red	0.10c	0.51c	0.92a	1.40a	0.29c
Treated red	0.16bd	0.53c	0.99a	1.39a	1.54cd
Roots					
Control white	0.06a	0.08a	0.14a	0.21a	0.57a
Treated white	0.08b	0.14b	0.09b	0.26a	0.41b
Control red	0.05ac	0.08a	0.13ac	0.27a	0.12c
Treated red	0.05ac	0.07a	0.20d	0.54b	0.23d
Pods					
Control white				0.25a	0.70a
Treated white				0.35b	0.86b
Control red				0.05c	0.51c
Treated red				0.13d	0.69ad

Means followed by same letters on the vertical axis are not significantly different ($p \leq 0.05$)

Seeds of treated plants accumulated significantly high ($p \leq 0.01$) level of Ca but the level of all other metals was lower than that of the control (Table 3). The non-accumulation of heavy metals highly present in the effluent by the harvested seeds of both cultivars used in

Table 3: Metal accumulation in seeds of cowpea cultivars IT89KD-349 (White) and IT84E-124 (Red) from plants treated with or without effluent (control)

	Concentration of minerals (mg L^{-1})			
Mineral elements	Treated white	Control white	Treated red	Control red
Zn	4.95	5.50*	4.05	4.65*
Fe	5.7	8.37**	2.73	3.17**
Ni	NEG	NEG	NEG	NEG
Cd	0.001	0.001	0.001	0.001
Mn	2.1	4.0*	8.0	8.0
Hg	NEG	NEG	NEG	NEG
Pb	0.02	0.02	0.02	0.02
Ca	2.2*	1.62	4.2**	3.1

Significant difference occurred between treated and control seeds of each cultivar at $^{**}p<0.01$ and $^{*}p\leq 0.05$. NEG: Negligible

this study could be due to the influence of the high Ca concentration in the effluent. It has been reported that Ca influences the uptake of other elements into the cell and within cells of plants. Osterås (2004) and Osterås and Greger (2003) have shown that Ca limits the uptake of some heavy metals such Cd, Zn and Cu.

CONCLUSION

Direct application of combined effluent on the cultivation of cowpea enhanced the growth and pod yield of the red cultivar while improving only the pod yield of the white cultivar. Enhancement in yield was caused by the high nitrate reductase activity indicating by the plants throughout the growth period as a result of the high nitrate, Ca and SO_4 levels of the effluent as well as the high Ca content directly reduced the heavy metals solubility.

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