STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, LXII, 2, 2017 (p. 97-106)
doi:10.24193/subbbiol.2017.2.09

Plant growth response and nitrate reductase activities of roots of *Chromolaena odorata* in a model spent lubricating oil-polluted soil

Geoffrey O. Anoliefo[1], Beckley Ikhajiagbe[1,✉], Ehimwenma S. Omoregie[2], Raphael A. Aimiebenomor[2] and Pascal C. Okoye[1]

SUMMARY. The ability of *Chromolaena odorata* propagated by stem cuttings and grown for 50 days in the soil containing five (5) different concentrations of spent lubricating oil (SLO) in soil (0, 1, 3, 6, 9 and 12 % SLO), was investigated. The experiments were watered daily at 70% moisture field capacity. Parameters such as number leaves per plant, shoot length, plant height as well as nitrate reductase activities were measured. Shoot length as well as leaf number were significantly ($p < 0.05$) reduced, compared to the control treatment. Results also showed that nitrate reductase activities increased slightly with time. However, beyond 40 days, nitrate reductase activity was not detected in 3% - 12% w/w oil-in-soil treatments, respectively. Pollution indices such as Contamination factor and Hazard Quotient, used in the present study indicated significant reduction in contamination values upon sowing of *C. Odorata*. Values obtained from Bioaccumulation Quotients also indicated that the plant was able to significantly bioaccumulate elements such as Fe, Cu and Ni present in the SLO-polluted soil.

Keywords: bioaccumulation, *Chromolaena odorata*, enzymes, nitrate assimilation, nitrate reductase, phytoremediation.

Introduction

In a farming ecosystem, the soil is the furthermost prized component. However, environmental sustainability is principally subject to appropriate soil maintenance. Sustainable usage of this natural resource is unquestionably obligatory for better agricultural productivity. Soil pollution by crude oil, petroleum products,

[1] *Environmental Biotechnology and Sustainability Research Group, Dept of Plant Biology & Biotechnology, University of Benin, Benin City, Nigeria.*
[2] *Malaria Research, Molecular Biology and Toxicology Unit, Dept. of Biochemistry, University of Benin, Benin City, Nigeria.*
✉ ***Corresponding author: Beckley Ikhajiagbe**, Environmental Biotechnology and Sustainability Research Group, Dept of Plant Biology & Biotechnology,University of Benin, Benin City, Nigeria, E-mail: beckley.ikhajiagbe@uniben.edu*

or other waste petroleum materials is a major ecological concern in many third world countries, including Nigeria. The disposal of spent engine oil in the big cities has been persistently problematic since many automobile mechanics dispose these oils indiscriminately either in gutters or open lands. Nearby farms are affected directly or indirectly from run-offs during rainfall, when these oil materials are deposited therein. This practice adversely affects plants, soil microbes and other soil fauna (Adenipekun *et al.*, 2008).

Oil pollution in whatever form is toxic to plants and soil microorganisms (Adedokun and Ataga, 2007). In an era when most people clamour for organic foods, restoration of oil-polluted soils for purposes of farming may not necessarily be possible through the use of conventional methods like application chemical treatments or even the use of physical methods which may not remove the oil completely. A more cost-effective but slow method, known as phytoremediation, is currently making waves in environmental biorestoration science.

It is however pertinent to note that the selection of phytoremediation methods is usually preceded by a knowledge of the plant's physiology or growth response as well as phytoaccumulation capacities when exposed to the contaminant. One of such physiological response is its nitrate assimilatory capacity; a very important factor considering the role nitrogen plays in plant's vegetative development. Nitrate reductase activity is one of several enzymatic systems that have significant correlation with nitrate assimilation.

The nitrate assimilatory pathway is facilitated by two enzymes, nitrate reductase (EC 1.6.6.2) and nitrite reductase (EC 1.7.7.1). These enzymes catalyze the stepwise conversion of nitrate to nitrite (nitrate reductase), and nitrite to ammonia (nitrite reductase). Before they can be incorporated into amino acids, nitrate absorbed by plants must first be reduced to ammonium (Fan *et al.,* 2002). The first step in the conversion of nitrate is catalyzed by nitrate reductase (NR). Lexa *et al.* (2002) described this substrate-inducible plant enzyme as essential in nitrogen assimilation. Activity of this enzyme is considered to be a limiting factor for growth and protein production in plants (Jackson *et al.*, 2008).

As many scientists use a plant's capacity to assimilate nitrate nitrogen as one of several indicators of changes in the environment, NR activity measurements becomes a much more reliable choice (Mahan *et al.,* 1998; Ghoulam *et al.,* 2002). These authors have previously reported NR activity inhibition due to plant's exposure to metals such as copper and salinity. Ghoulam *et al.* (2002), Mahan *et al.,* 1998) also reported significant decreases in NR activity due to drought. In the present study therefore, phytoremediative capacities of the test plant, *Chromolaena odorata* is measured against NR activity for selected periods. The test plant was chosen because of previous reports of its capacity to survive oil contamination (Anoliefo *et al*., 2003, 2006).

Materials and methods

Garden soil was collected from an area measuring 10 m x 10 m marked on a land. Care was taken to ensure that soil was obtained from an area that had never been impacted with petroleum oil spill. Soil was analysed for selected chemical parameters before use (Table 1). Thereafter, 10 kg soil each was placed in large buckets. Spent lubricating oil (SLO) was obtained as pooled sample from an auto-mechanic workshop.

The emphasis on the study was the test plant's capacity for heavy metal remediation in a polymetallic soil. The polymetallic soil environment in this study was created by contaminating soil with spent lubrication oil. The choice is based on the report of Whisman *et al.* (1974); Ikhajiagbe and Anoliefo (2012) that most heavy metals such as V, Pb, Mn, Cd, Cr, Ni, and Fe were present in high quantities in spent lube. The soils in each bucket were then mixed thoroughly with SLO in 5 different levels: 0, 1, 3, 6, 9 and 12 % w/w. The 100 g SLO measured 135.2 ml. The control was not contaminated with SLO. Treatments and control existed in replicates of 5. The entire set up was left in a well-ventilated screen house for 1 month to attenuate, without mechanically disturbing the soil.

Afterwards, fresh stem cuttings of *Chromolaena odorata* (girth, 1.89±0.42 cm; length 30.00±0.00 cm) were obtained for the study from a fallow area. These stem cuttings were obtained from midway through the plant axil. Care was taken to ensure that the stem was devoid of injury prior to use. Single stem-cuttings were sown per bucket at a depth of 10cm. the set up was observed for phytoremediative capabilities for 50 days as well as nitrate reductase activity. Total plant accumulated figures for HM were determined by atomic absorption spectrophotometry (model, Buck Scientific 210 VGP), according to the methods of SSSA (1971) and AOAC (2005). Nitrate reductase activity (NRA) of root sample was determined according to Stewart *et al.,* 1972) with slight modifications from Ajakaiye (1987) and Cerqueira *et al.* (2009). Means were separated by using the Least Significant Difference $p \leq 0.05$ significant level. The Superior Performance Software System (SPSS) (version 16.0 for windows) package was used for statistical analyses.

In order to compare remediative success of the test plant, residual metal contents were compared with standard benchmark (Efroymson *et al*., 1997a,b; Cal-EPA, 2005).

Contamination Factor (CF)

CF expresses the ratio of the eventual concentrations of pollutant and its pre-industrial concentration (Ikhajiagbe, 2010).

$$CF = \frac{\text{Concentration of pollutant at the specific date of concern}}{\text{Background/Pre-industrial Concentration, before pollution}}$$

If CF > 1, the implication is that the inherent contamination due to that particular pollutant is as a result of the amendment by the researcher.

Bioaccumulation Quotient (BQ)

BQ expresses the possibility of the contaminant being significantly accumulated in plant parts, thereby posing health threats (Ikhajiagbe and Anoliefo, 2012). The Bioaccumulation Quotient is expressed

$$BQ = \frac{\text{Concentration of accumulated pollutant in the accumulant}}{\text{Concentration of accumulated pollutant in Soil (Source)}}$$

When BQ > 1= Significant accumulation in of the pollutant is implied.
When BQ < 1= Bioaccumulation is not of significant effect.

Results and discussion

The chemical parameters of the garden soil used for the study has been presented on Table 1. As provided, when CF > 1, the implication is that the inherent contamination due to that particular pollutant is as a result of the amendment by the researcher. Contamination factor after 50 days of plant exposure to polymetallic oil-polluted soil showed higher than unit values for Mn and Cu in soils due to SLO pollution. The meaning is that remediation may not have been entire after 50 days exposure (Table 2). Heavy metal accumulation in whole *C. odorata* plant at 50 days after sowing has been presented on Table 3. Accumulations in SLO-polluted soils significantly differed from those in the control soil thus indicating the capacity of *C. odorata* to accumulate metals in polymetallic soils. Accumulated Mn ranged from 1.2 – 5.8 mg kg^{-1} per dry wt. of whole plant. Accumulation of Cd in *Chromolaena* plants was below detection in both polluted and control soils. Results showed significant accumulation of Cu in plant parts of *Chromolaena odorata* (Table 4). Significant bioaccumulation quotient values were also recorded for Fe and Ni in polluted soils. Significant accumulation in of the pollutant is implied when BQ > 1.

The toxic impact of spent lubricating oil on soil fauna and flora relies basically on its hydrocarbon and heavy metal composition. Although most heavy metals may not be necessarily present in unused lubricating oils, however after they have been used in motor vehicles and other heavy machines, under very high temperatures, heavy metals such as V, Pb, Al, Ni, and Fe, which were below detection in unused engine oil, eventually give high mg kg^{-1} values in used oil (Whisman *et al.,* 1974).

The occurrence of these heavy metals in soil, especially in high concentrations is very distressing, particularly in their relationships with plant growth. Not only these metals, but hydrocarbons which have, in previous studies shown significant phytotoxic effects, in most cases, leading to plant death (Ikhajiagbe, 2010). The bioavailability of these contaminants to plants in the polluted soils is the starting point to a resultant phytoxic impact on the growing plant population inn affected soil. Metals, whether absorbed in very low quantities or not, are capable of replacement of essential metals in pigments or enzymes, thereby unsettling their

function (Henry, 2000). However, plant resistance to these environmental pollutants has been demonstrated in previous studies (Wong and Chu, 1985; Vwioko and Fashemi, 2005). Plant tolerance to these contaminants is majorly a factor of its capability for selective absorption from soil solution by its roots. Contaminants, like metals may be bound to exterior exchange sites on the root and not actually taken up (Efroymson *et al.,* 1997a,b). In some other cases, when absorbed by plants, they are stored in bio-unavailable forms in harvestable plant parts (phytoextraction).

Table 1.

Chemical parameters of garden soil used in the study

Parameters	**Soil (per Dry wt.)**
Ph	5.62
Electric conductivity (μs cm^{-1})	237.24
Total organic carbon (%)	0.49
Total Nitrogen (%)	0.11
Exchangeable acidity (meq.$100^{-1}g^{-1}$)	0.23
Fe (mg kg^{-1})	212.32
Cu (mg kg^{-1})	2.90
Cd (mg kg^{-1})	ND
Mn (mg kg^{-1})	12.76
V (mg kg^{-1})	0.08
Pb (mg kg^{-1})	0.03
Ni (mg kg^{-1})	1.02
Total hydrocarbon content (mg kg^{-1})	191.02

Table 2.

Contamination factor for heavy metal composition of oil polluted soil 50 days after sowing *C. odorata.*

Treatments	Mn	Fe	Cu	Pb	V	Ni	Cd
	Background concentration (mg kg^{-1})*						
	12.76	212.32	2.90	0.03	0.08	1.02	ND
	Contamination factors (units)						
0%	0.92^{b}	0.96^{a}	0.74^{c}	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{c}
1%	1.42^{b}**	0.06^{b}	0.47^{c}	0.14^{bc}	0.13^{cd}	0.06^{cd}	0.00^{c}
3%	1.90^{ab}**	0.10^{b}	0.78^{c}	0.22^{bc}	0.28^{bc}	0.07^{bcd}	0.00^{c}
6%	2.01^{a}**	0.12^{b}	1.42^{bc}**	0.39^{ab}	0.34^{b}	0.09^{abc}	0.05^{b}
9%	2.28^{a}**	0.14^{b}	3.30^{ab}**	0.53^{ab}	0.53^{a}	0.14^{abc}	0.08^{a} ^{b}
12%	2.81^{a}**	0.17^{b}	4.28^{a}**	0.66^{a}	0.63^{a}	0.16^{a}	0.12^{a}
LSD (0.05)	1.06	0.26	1.13	0.38	0.18	0.08	0.04

**Background concentration here refers to the natural concentration of the elements in the soil prior to contamination with SLO. (See Table 1)*

***If CF > 1, Contamination is due SLO application to soil.*

Means of the same column with similar alphabetic superscripts do not differ from each other (p>0.05)

Nitrate reductase activity (NRA) in developing *C. odorata* plants was reported in 20, 30, 40 and 50 days (Table 5). After 20 days, NRA was 0.311 $\mu Mhr^{-1}g^{-1}$ in the control, compared to 0.157 – 0.287 $\mu Mhr^{-1}g^{-1}$ in pants exposed to oil-polluted soil. This decrease in NRA is in accordance with increasing oil concentration in the soil. This was similar for the 30th, 40th and 50th days respectively. NRA increased with the age of plants. However, NRA was undetected for plants in 12% oil-in-soil treatments on the 50th day. This was probably because plants did not survive at 50 days.

Table 3.

Heavy metal accumulation in whole *C. odorata* plant at 50 days after sowing

Heavy metals	1%	3%	6%	9%	12%	Control	LSD (0.05)
	Metal conc. in soil (mg kg^{-1})						
Mn^{2+}	3.8^{a}	4.2^{a}	4.7^{a}	5.3^{a}	5.8^{a}	1.2^{b}	2.3
Fe^{3+}	164.2^{de}	191.4^{cd}	253.1^{bc}	299.0^{ab}	341.3^{a}	100.2^{e}	86.3
Pb^{2+}	ND^{c}	ND^{c}	0.049^{bc}	0.103^{ab}	0.185^{a}	ND^{c}	0.094
Cu^{2+}	1.54^{ab}	1.93^{ab}	2.30^{a}	2.78^{a}	2.24^{a}	0.63^{b}	1.38
V^{2+}	ND^{c}	0.06^{c}	0.14^{bc}	0.29^{ab}	0.40^{a}	ND^{c}	0.22
Ni^{2+}	ND^{c}	0.27^{c}	1.43^{b}	1.95^{ab}	2.65^{a}	ND^{c}	0.98
Cd^{2+}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	0.001

Means of the same row with similar alphabetic superscripts do not differ from each other (p>0.05). NA not available. Means of the same column with similar alphabetic superscripts do not differ from each other (p>0.05). ND not detected (<0.0001 mg kg^{-1})

Table 4.

Bioaccumulation quotient for heavy metals accumulated in whole *C. odorata* plant at 50 days after sowing

Heavy metals	Cu	Pb	Cd	Fe	Ni
	Benchmark* (mg kg^{-1})				
	0.20	5.00	0.01	5.00	0.20
	Bioaccumulation quotient				
0%	3.15^{b}**	0.00^{b}	0.00^{a}	4.80^{d}**	0.00^{c}
1%	7.70^{ab}**	0.00^{b}	0.00^{a}	32.80^{c}**	0.00^{c}
3%	9.65^{ab}**	0.00^{b}	0.00^{a}	38.20^{bc}**	1.35^{c}**
6%	11.50^{a}**	0.01^{ab}	0.00^{a}	50.80^{ab}**	7.15^{b}**
9%	11.20^{a}**	0.02^{ab}	0.00^{a}	59.80^{ab}**	9.75^{ab}**
12%	13.90^{a}**	0.04^{a}	0.00^{a}	68.20^{a}**	13.25^{a}**
LSD (0.05)	6.92	0.03	0.001	22.64	5.35

**Benchmark given is FAO recommended Maximum concentration of trace elements for crops (FAO, 1985).*

*** Significant accumulation in of the pollutant is implied.*

Means of the same column with similar alphabetic superscripts do not differ from each other (p>0.05)

Plant survival under conditions of stress relies greatly on its nitrogen assimilation capacities. One of numerous biochemical explanations on improved nitrogen utilization by plants is dependent on an understanding of its nitrate reductase (NR) activities. NRs, which are molybdoenzymes, reduce nitrate to nitrite (Solomonson *et al.,* 1990). This reduction reaction is critical for the assembly of protein in most plants, as nitrate is the predominant source of nitrogen in fertilized soils (Marschner and Petra, 2012). The nitrate uptake system in plants must be versatile and robust because plants have to transport sufficient nitrate to satisfy total demand for nitrogen in the face of environmental stress. This is very important for metabolic processes including chlorophyll synthesis, photosynthesis, as well as antioxidative defences.

NR is found largely in the cytosols of root epidermal and cortical cells as well as in shoot mesophyll cells (Rufty *et al*., 1986; Vaughn and Campbell, 1988; Fedorova *et al.,* 1994). Incidentally, these are also sites of heavy metal bioconcentration. The possibility of direct impact on NR activity therefore suffices. This study showed that waste oil contamination (heavy metals and hydrocarbons) negatively affected NR activity of the test plant. Increased time of exposure as well as concentration were two important factors to recon in their phytotoxic effects.

Table 5.

Nitrate reductase activity in developing plants

Nitrate reductase activity ($\mu M\ hr^{-1}\ g^{-1}$)	Number of days after sowing			
	20	30	40	50
Control	0.311^{a}	0.434^{a}	0.587^{a}	2.086^{a}
1 %	0.287^{a}	0.415^{ab}	0.531^{ab}	0.957^{b}
3 %	0.283^{a}	0.323^{bc}	0.385^{abcd}	0.142^{c}
6 %	0.198^{b}	0.304^{c}	0.312^{bcd}	0.103^{c}
9 %	0.146^{c}	0.196^{d}	0.229^{cd}	0.093^{c}
12%	0.157^{c}	0.178^{d}	0.174^{d}	ND^{c}
LSD (0.05)	0.032	0.103	0.209	0.242

ND= Not Detected (below value of 0.0001). *Means of the same column with similar alphabetic superscripts do not differ from each other (p>0.05)*

As earlier reported, the inhibitory effect of the used oil could partly be attributed to the toxic nature of some of its constituents on this enzyme. It has been reported that polycyclic aromatic hydrocarbons (PAH) is a toxic and recalcitrant portion of used engine oil (Wang *et al.,* 2000). Heavy metals affect the activities of a wide range of enzymes. A number of metals, like Pb have been reported to interfere with the free –SH groups of plant enzymes, others block the –COOH group. Burzynski (1987) reported significant decreases in nitrogen assimilation and enzyme activity due to heavy metal contamination.

NR activity in the study was only investigated in the first 50 days of the plant life. During this period, it was observed that NR activity increased steadily. This however does not provide the adequate information required for ascertaining impact of plant age on NR activity.

Conclusions

The capability for bioconcentration of heavy metals *Chromolaena odorata* has been reported in this study. Although the test plant may have been reported in earlier studies to be resilient to used oil contamination; the study showed that this depends on the concentration of the contaminant. Having survived in concentrations of as much as 12%, it is suggested that more phytotoxicity studies be conducted on the plant upon exposure to oil concentrations above 12% so as to be able to place a peg on possible benchmark concentration for survival.

Acknowledgements. The researchers appreciate the efforts of Mr. Osazuwa Omoregbee and other postgraduate students of the Environmental Biotechnology and Sustainability Research Group Lab., Dept of Plant Biology & Biotechnology, University of Benin, Benin City, Nigeria. The study was funded by the researchers.

R E F E R E N C E S

Adedokun, O.M., Ataga, A.E. (2007) Effects of amendments and bioaugumentation of soil polluted with crude oil, automobile gasoline oil, and spent engine oil on the growth of cowpea *Vigna unguiculata, Sci. Res. Essay* **25**: 147-149

Adenipekun, C.O., Oyetunji, O.J., Kassim, L.Q. (2008) Effect of spent engine oil on the growth parameters, chlorophyll content of *Corchorus olitorius* Linn*., Environmentalist* **28**: 446-450

Ajakaiye, C.O. (1987) Leaf nitrate reductase activity, crude protein in *Corchorus olitorius* L., *Ceratoteca sesamoides* esond, *Nig. J. Botany* **1**: 36-42

Anoliefo, G.O, Vwioko, D.E., Mpamah, P. (2003) Regenerations of *Chromolaena odorata* L.K. & R. in crude oil polluted soil, a possible phytoremediation agent, *Benin Science Digest* **1:** 9-16

Anoliefo, G.O., Isikhuemhen, O.S., Eghwrudjakpor, K.O., Iredia, H.O. (2006) Growth of *Chromolaena odorata* in crude oil polluted soil; an enhancement on the natural biodegradation process, *Nigerian Journal of Botany* **19(2):** *360-376*

AOAC (2005) Methods of Analysis. Association of Official Analytical Chemists, Washington D. C. Pp.326

Burzynski, M. (1987) The influence of lead, cadmium on the absorption, distribution of potassium, calcium, magnesium, iron in cucumber seedlings, *Acta Physiol. Plant.* **9**: 229-238

Cal-EPA (2005) Air Toxics Hot Spots Program Risk Assessment Guidelines, Part II Technical Support Document for Describing Available Cancer Potency Factors, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, May (2005)

Cerqueira, N.M., Gonzalez, P.J., Brondino, C.D., Romão, M.J., Romão, C.C., Moura, I., Moura, J.J. (2009) The effect of the sixth sulfur ligand in the catalytic mechanism of periplasmic nitrate reductase, *J. Comput. Chem.* **30(15)**: 2466-2484

Efroymson, R.A., Will, M.E., Suter, G.W. (1997a) Toxicological Benchmarks for Screening Contaminants of Potential Concern for Effects on Soil, Litter Invertebrates, Heterotrophic Process, 1997 Revision. Oak Ridge National Laboratory, Oak Ridge, TN. ES/ER/TM-126/R2 http,//www.hsrd. ornl.gov/ecorisk/reports.ht@

Efroymson, R.A., Will, M.E., Suter, G.W., Wooten, A, C. (1997b) Toxicological Benchmarks for Contaminants of Potential Concern for Effects on Terrestrial Plants, 1997 Retiision. Oak Ridge National Laboratory, Oak Ridge, TN, ES/ER/TM-85/R3 http,//www.hsrd. ornl.gov/ecoriskheports. html

Fan, X.H., Tang, C., Rengel, Z. (2002) Nitrate uptake, nitrate reductase distribution, their relation toproton release in five nodulated grain legumes, *Ann. Bot.* **90**:315-323

Fedorova, E., Greenwood, J.S., Oaks, A. (1994) *In-situ* localization of nitrate reductase in maize roots, *Planta* **194**: 279-286

Ghoulam, C., Foursy, A., Fares, K. (2002) Effects of salt stress on growth, inorganic ions, proline accumulation in relation to osmotic adjustment Amaranthus hybridus, *Bio. Sci. Res. Comm* **14**:101-105

Henry, J.R. (2000) An Overview of Phytoremediation of Lead, Mercury. –NNEMS Report. Washington, D.C. pp. 3-9

Ikhajiagbe, B. (2010) Synergism in Bioremediation, Phytoassessment of Waste Engine Oil Polluted Soils after Amendment, Bioaugmentation, LAP Lambert Academic Publishing, Köln, Germany, pp.276

Ikhajiagbe, B., Anoliefo, G.O. (2012) Comparative phytotoxicity study of a waste engine oil-polluted soil exposed to different intervals of natural attenuation, *Asian Journal of Experimental Biological Sciences* **32***:* 335-343

Jackson, L.E., Burger, M.. Cavagnaro, T. R. (2008) Roots, nitrogen transformations, ecosystem services, *Annu. Rev. Plant Biol*. **59**: 341-63

Lexa, M., Genkov, T., Brzobohaty, B. (2002) Inhibitory effects of elevated endogenous cytokinins on nitrate reductase in expressing tobacco are eliminated by short-term exposure to benzyladenine, *Physiol Plantarum* **115**: 284-290

Mahan, J.R., Oliver, M.J., Sherman, T.D. (1998) Nitrate reductase activity during desiccation, rehydration of the desiccation-tolerant moss *Tortula ruralis*, *Environ. Exp. Bot.* **39**: 67-76

Marschner, P. (2012) *Marschner's mineral nutrition of higher plants,* 3rd ed., Amsterdam, Elsevier/Academic Press, pp. 135

Rufty, T.W., Thomas, J.E., Remmler, J.L., Campbell, W.H., Volk, R.J. (1986) lntracellular localization of nitrate reductase in roots, *Plant Physiol*. **82**: 675-680

Solomonson, L.P., Barber, M.J. (1990) Assimilatory nitrate reductase: functional properties and regulation, *Annu. Rev. Plant Physiol*. **41**: 225-253

SSSA (1971) Instrumental Methods for Analysis of Soil, Plant Tissue. Soil Science Society of America, Corporated, Wisconsin, U.S.A, pp. 27-32

Stewart, G.R., Lee, J.A., Orebamjo, T.O. (1972) Nitrogen metabolism of halophyte, Nitrate reductase activity, utilization, *New Phytol.* **72**: 539-546

Vaughn, K.C., Campbell, W.H. (1988) lmmunogold localization of nitrate reductase in maize leaves, *Plant Physiol*. **88**: 1354-1357

Vwioko, D., Fashemi, S.D. (2005) Growth response of *Ricinus communis* L. Castor oil in spent lubricating oil polluted soil, *J. Appl. Sci. Environ*. *Mgt.* **92**: 73-79

Wang, J., Jia, C.R., Wong, C.K., Wong, P.K. (2000) Characterization of polycyclic aromatic hydrocarbons created in lubricating oils, *Water, Air,, Soil Poll*. **120**: 381-396

Whisman, M.L., Geotzinger, J.W., Cotton, F.O. (1974) Waste lubricating oil research, In: *An Investigation of several Re-refining Methods*. Bureau of Mines. Bartlesville, Energy Research Center, pp. 352