

EFFECT OF PETROLEUM PRODUCTS TREATMENT OF SOIL ON SUCCINATE DEHYDOGENASE AND LACTATE DEHYDOGENASE ACTIVITIES IN COWPEA (*Vigna unguiculata*) AND MAIZE (*Zea mays*) SEEDLINGS

ABSTRACT

The effects of petroleum products (kerosene, diesel, engine oil and petrol) treatment of soil at various sublethal concentrations (0.0%, 0.1%, 0.25%, 0.5%, 1.0%, 1.5% and 2.0%) on succinate dehydrogenase and lactate dehydrogenase activities in cowpea and maize seedlings were studied. The results indicated that petroleum products treatment of soil resulted in decrease in succinate dehydrogenase activity in both cowpea and maize seedlings as well as a corresponding decrease in lactate dehydrogenase activity in the two seedlings. The toxic effect of kerosene was more severe than the other petroleum products studied. Similarly, Cowpea seedlings were affected more than maize seedlings. Generally, the data suggest that seedlings exposed to petroleum products treated soil tend to move towards anaerobic respiration in a bid to survive under petroleum stress.

Key words: cowpea seedlings, maize seedlings, succinate dehydrogenase activity, lactate dehydrogenase activity

INTRODUCTION

Petroleum compounds have multifarious industrial and domestic uses. They are extensively employed in solvents, dry cleaning fluids quick-products, automobile fuel, household solvents, and lubrication of machines, cosmetics, water proofing agents, cleaning agents, and specialty chemicals (Kenny *et al.*, 2002). These anthropogenic activities have led to the widespread contamination of the environment. Oil spill is the major cause for the high influx of petroleum to the biosphere (Achi, 2003; Tolutope, 2004). However, other points of soil pollution with refinery products are petrol stations, garages servicing car and tractors, seaports areas (Michalcewicz, 1995). Other areas of concern are mining and distribution of petroleum-based products (Song and Barter 1990; Amadi *et al.*, 1996; Jorsgensen *et al.*, 2000). Beside, heavier use of machinery in agriculture leads to higher consumption of diesel. Certain negligence when transporting, collecting or storing refinery products together with unsatisfactory care while disposing of old or used petroleum products leads to considerable pollution of the natural environment (Odjegba and Sadiq 2002).

Various chemicals entering the ecosystem through human activities, either accidentally or by design may cause adverse effects on the biota, including deleterious changes which disrupt metabolic activity at the biochemical levels (Chourpagar and Kulkarni, 2009). When any organism is exposed to polluted medium, a sudden stress is developed for which the organism should meet more energy demand to overcome the toxic stress.

Succinate dehydrogenase is the component of complex II of the respiratory chain that catalyses the oxidation of succinate to fumarate in the Krebs cycle. Flavin adenine

dinucleotide (FAD) is also part of the succinate dehydrogenase active enzyme complex. The oxidation of succinate to fumarate is the only Krebs reaction that takes place in the inner membrane itself as opposed to the other reactions that are catalysed by soluble enzymes. Succinate is the most efficient energy source, so the succinate dehydrogenase activity is the most efficient method of measuring the vitality of living organisms (Kregiel *et al.*, 2008) and can be taken as an indication of the level of operation of the tricarboxylic acid cycle (TCA) (Bhagyalakshmi *et al.*, 1984).

Lactate dehydrogenase catalyses the conversion of pyruvic acid to lactic acid in anaerobic condition (Valarmathi and Azariah, 2003). To study the strategy of energy production adopted by cowpea and maize seedlings exposed to petroleum products, it is necessary to consider the changes in the activities of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH).

MATERIALS AND METHODS

Refined petroleum products and planting materials

The refined petroleum products of known physical properties were obtained from Warri refining and petrochemical company, Warri, Nigeria. Improved varieties of maize (*Zea mays*) were obtained as single batch from Delta Agricultural Development Project (DTADP) Ibusa Delta State, Nigeria. While improved varieties of *Vigna unguiculata* (L) walp was obtained from IITA, Ibadan, Nigeria. The soil (sand 84%, silt 5.0%, clay 0.4% and organic matter 0.6%, pH 6.1) was obtained from a fallow land in Delta State University, Abraka.

Soil treatment and planting of seeds

One thousand six hundred grams of soil was added to each hundred small size planting bags (1178.3cm^3 , 15 cm deep) and divided into six groups of five replicates. Groups 1 to 5 contained 0.1%, 0.25%, 0.5%, 1.0% and 2.0% (v/w) respectively of each of the petroleum products while group six served as control (0.0%). To the first bag, 1.6 ml of kerosene, corresponding to 0.1%, was added. The petroleum product treated soil samples were mixed vigorously with the hand to obtain homogeneity of the mixture. The procedure was repeated for 0.25%, 0.5%, 1.0%, 1.5% and 2.0%. This same procedure was applied to diesel, engine oil and petrol. Each treatment including control was replicated five times and consisted of three sown seeds per polythene bag. The treatments were watered every day in order to keep the soil moist. The design of the experiment was completely randomised one.

Seed viability was determined by floatation. All seeds that floated on water were discarded and others that remained at bottom of water were deemed potentially viable. Three seeds were planted in each test bag to an approximate depth of 2cm and under partial shade. During the experiment 80cm^3 of water was supplied to the set up as at when needed to keep the soil moist. Germination [which is indicated by the appearance of epicotyls (for cowpea) and hypocotyls (for maize) above the soil level] records were taken at 4 days interval up to 12 days. Seeds, which failed to sprout after 12 days were regarded as not germinable.

Preparation of extract and determination of succinate dehydrogenase activity in the leaves of cowpea and maize seedlings.

The leaves (1.0g) of cowpea seedlings after four days of germination was taken into mortar containing 0.5g of acid washed sand. This was followed by the addition of 5.0ml of 0.05M potassium phosphate buffer, pH 7.4 and ground with a pestle. The homogenate was filtered through cheesecloth. The filtrate was centrifuged at 1000g for 5 minutes at 4⁰C to sediment the nuclear fraction. The supernatant was further centrifuged at 10,000g for 30 minutes at 4⁰C. The supernatant was discarded and pellet resuspended in 80ml of 0.05 M potassium phosphate buffer, pH 7.4. The resuspended pellet is kept in ice bath for use as source of enzyme. The extraction was repeated for cowpea seedlings after eight and twelve days of germination. This same procedure was adopted for the preparation of extract from maize leaves after four, eight and twelve days of germination in petroleum products treated soil.

SDH catalyzes the oxidation of succinate to fumarate in the presence of FAD, which is subsequently reduced to FADH₂. In the presence of cyanide the electron from FADH₂ are picked by 2, 6 dichlorophenolindophenol (DCIP). The reduction of DCIP can be monitored at 600 nm and can be used to follow the reaction over time.

The activity of succinate dehydrogenase (SDH) was determined by adding 2.5ml of 0.05ml of potassium cyanide and 0.5ml of succinate into preincubated cuvette. The tube was covered with paraffin and mixed thoroughly. The change in absorbance was monitored for 30 minutes at 600 nm with an Sp 1800 UV/VIS Spectrophptometer after the addition of 0.5ml of enzyme extract. The blank was prepared by replacing the enzyme extract with distilled water (Sajan et al., 1995). Succinate dehydrogenate activity was

expressed as units and one unit is defined as one mole of dichlorophenolindophenol reduced /minute (Sajan, et al; 1995).

Preparation of extracts and determination of lactate dehydrogenase activities in the leaves of cowpea and maize seedlings.

The leaves (2.0g) of cowpea seedlings after four days of germination was homogenized in 10ml of Tris-HCl buffer (0.1M) pH 8.8 containing EDTA using mortar and pestle. The homogenate was filtered through several layers of cheese cloth and the extract centrifuged for 10 minute at 1,000g. The supernatant was raised to 20% saturation with solid $(\text{NH}_4)_2\text{SO}_4$ with constant stirring and left for 20 minutes at 4°C . The mixture was centrifuged as before and the precipitate which contained NADH oxidase was discarded. The crude supernatant obtained at this point was used as the enzyme source. The extraction process was repeated using the leaves of cowpea seedlings after eight days and twelve days. This pocedure was adopted for the preparation of extracts from maize seedlings after four, eight and twelve days of germination.

LDH catalyzes the oxidation of lactate to pyruvate in the presence of NAD^+ , which is subsequently reduced to NADH. The rate of NADH formation at 340 nm is directly proportional to LDH activity. The method of Kaiglova et al (2001) was adopted. The assay mixture which was equilibrated at 37°C contained 1ml of 60mM Tris-HCl buffer, pH 8.8, 0.5ml of 2.0 mM lactic acid, 0.5ml of 6 mM NAD^+ and 0.1ml of enzyme extract. The rate of $\text{NADH} + \text{H}^+$ formation was monitored at 340 nm. The enzyme activity was assayed with an Sp 1800 UV/VIS Spectrophptometer

RESULTS AND DISCUSSION

Earlier reports had indicated that petroleum in soil caused displacement of air in the pore spaces (Achuba, 2006; Anigboro and Tonukari, 2008) which culminates in oxygen tension cum hypoxia in exposed plants (Anoliefo and Okoloko, 2000; Eriyamremu et al., 1999). This may result in changes in energy metabolism, vis-à-vis an alteration in the activity of respiratory enzymes in which lactate dehydrogenase and succinate dehydrogenase (Valarmathi and Azariah, 2003) form a part.

Succinate dehydrogenase is component of complex II of the respiratory chain that catalyzes the oxidation of succinate to fumarate in the Krebs cycle (Soliola et al., 2004). It is the most efficient energy source in the cell (Samolchvalov et al., 2004; Kregiel et al., 2008). Therefore, inhibition of succinate dehydrogenase activity may portend a decrease in rate of energy generation by the cell. Previous report indicates that heavy metals induced a decrease in succinate dehydrogenase activity (Valarmathi and Azariah, 2003). This is in consonance with the present investigation in which refined petroleum products caused a decrease in succinate dehydrogenase activity after four, eight and twelve days of germination (Fig.1). Moreover, the toxic effect of kerosene was more pronounced compared to the effect of other refined petroleum products. The high degree of toxicity of kerosene had been documented (Wemedo et al., 2002). Similarly, the vulnerability of cowpea seedlings to petroleum hydrocarbon compared to maize seedlings was apparent (Fig.1). The higher toxic effect of refined petroleum product on cowpea compared to maize had been attributed to the fact that cowpea seedlings have the ability to absorb hydrocarbon from the soil more than maize seedlings (Ayotamuno and Kogbara, 2007; Aboaba et al., 2007).

163 The findings of the present study indicate that the time-dependent effect of
 164 petroleum products on LDH activity of tissues of cowpea and maize seedlings was varied
 165 or inconsistent (Fig. 2). However, the observed increase in LDH activity is consistent
 166 with similar toxicant mediated increase in lactate dehydrogenase activity which has been
 167 reported earlier (Valarmathi and Azariah, 2003). The increase in lactate dehydrogenase
 168 activity may reflect an increase dependence on anaerobic carbohydrate metabolism by the
 169 leaves of the respective seedlings. Thus, the non availability of oxygen, the inhibition of
 170 succinate dehydrogenase activity and simultaneous elevation of lactate dehydrogenase
 171 activity may suggest a bias toward the anaerobic glycolytic pathway. The reciprocal
 172 relationship between succinate dehydrogenase activity and lactate dehydrogenase activity
 173 had been reported previously (Valarmathi and Azariah 2002, 2003). Similar to succinate
 174 dehydrogenase activity, kerosene seems to affect both cowpea and maize seedlings more
 175 than the other refined products, though not significant. In addition, the response of maize
 176 seedlings was significantly ($P<0.01$) higher relative to cowpea seedlings (Fig. 2). This
 177 may be predicted on the backdrop of starchy seeds ability to undergo fermentative
 178 respiration (Guglielminetti et al., 1995). Generally, it is obvious that refined petroleum
 179 product, most especially kerosene could impose anaerobiosis in exposed plant.

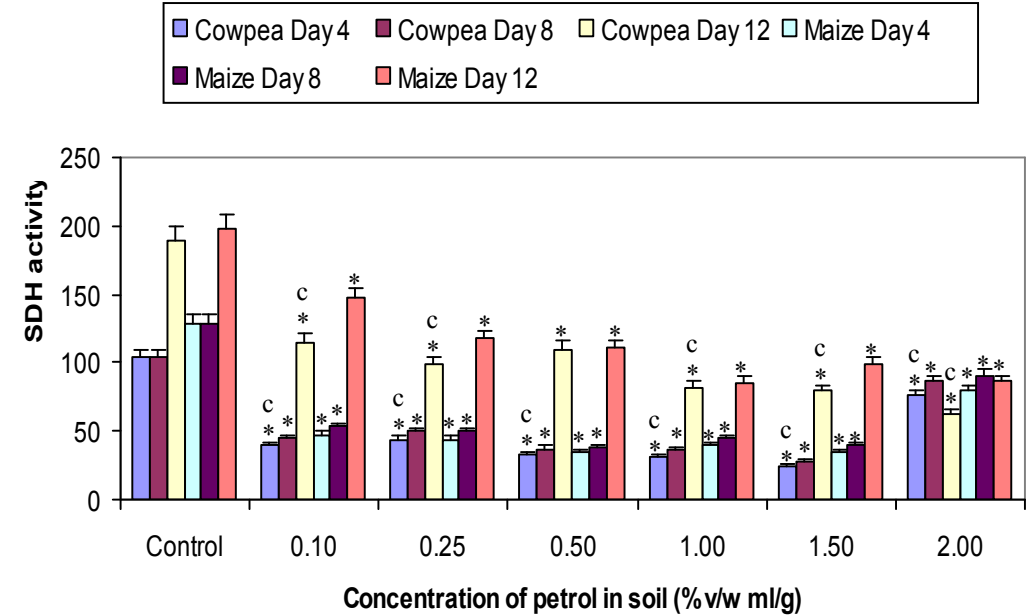
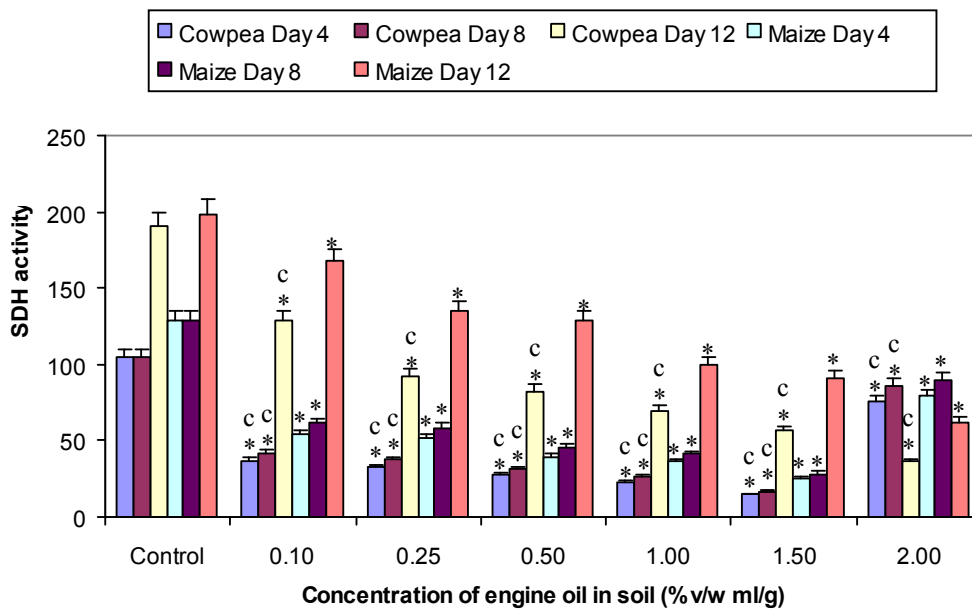
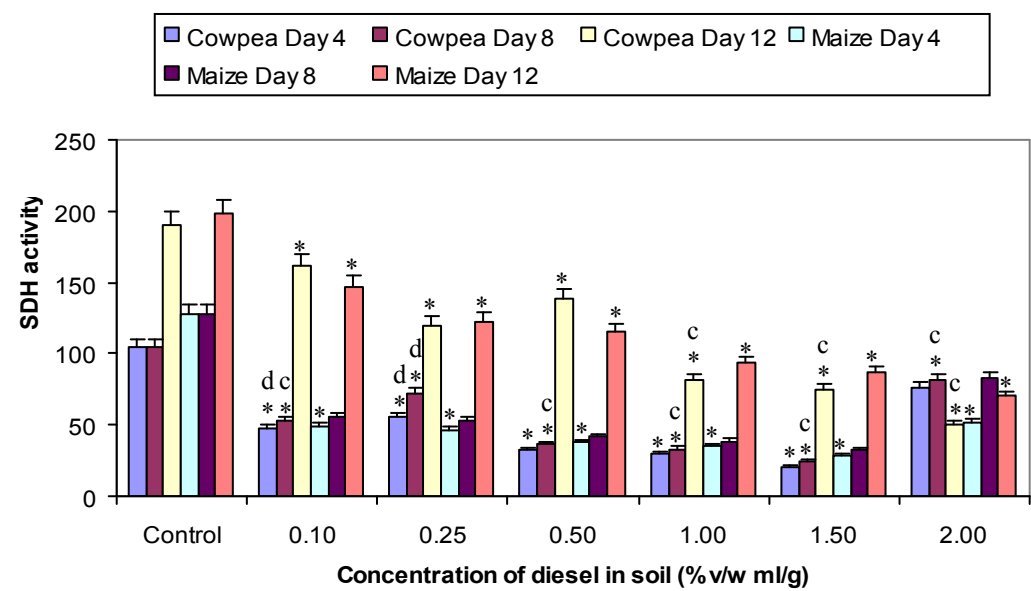
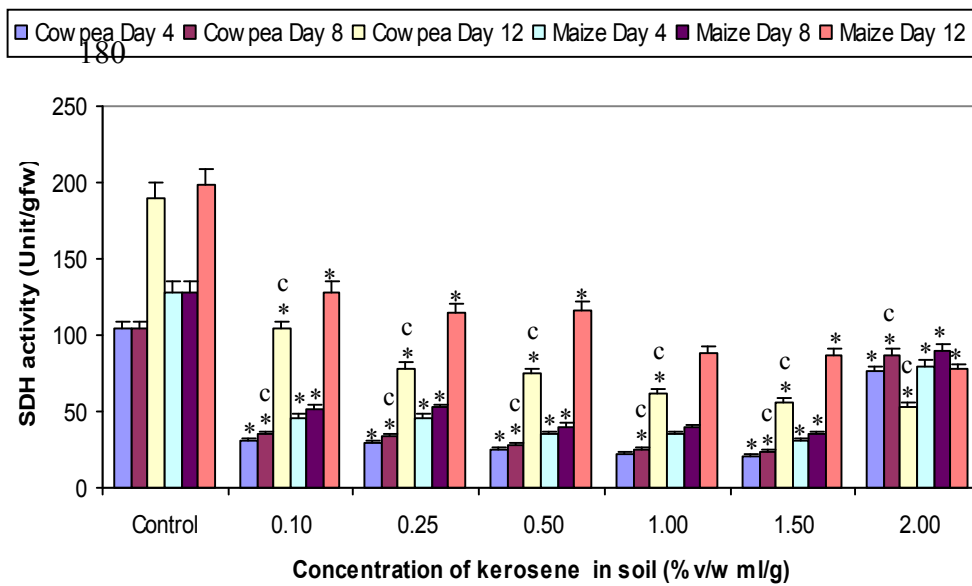


Fig. 4.4.1: Effect of concentration of petroleum products on SDH activities in leaves of cowpea and maize after four, eight and twelve days of germination. *Significantly lower as compared to control; +Significantly lower as compared to engine oil; ++Significantly lower as compared to kerosene; °Significantly higher relative to control; ^aSignificantly lower relative to other petroleum products; ^bSignificantly higher relative to other petroleum products; ^cSignificantly lower in cowpea relative to maize seedlings; ^dSignificantly higher in cowpea relative to maize seedlings

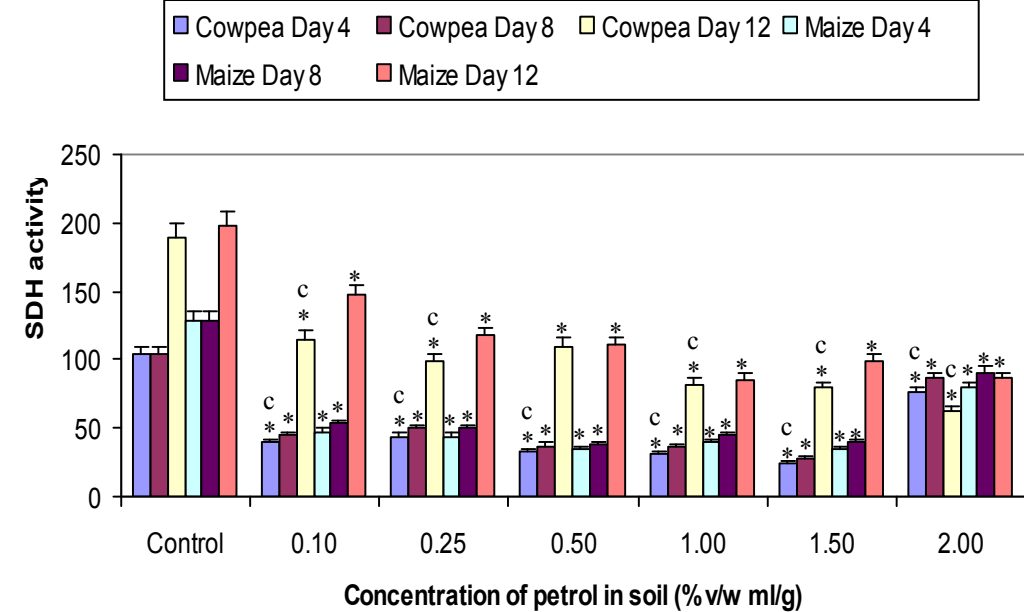
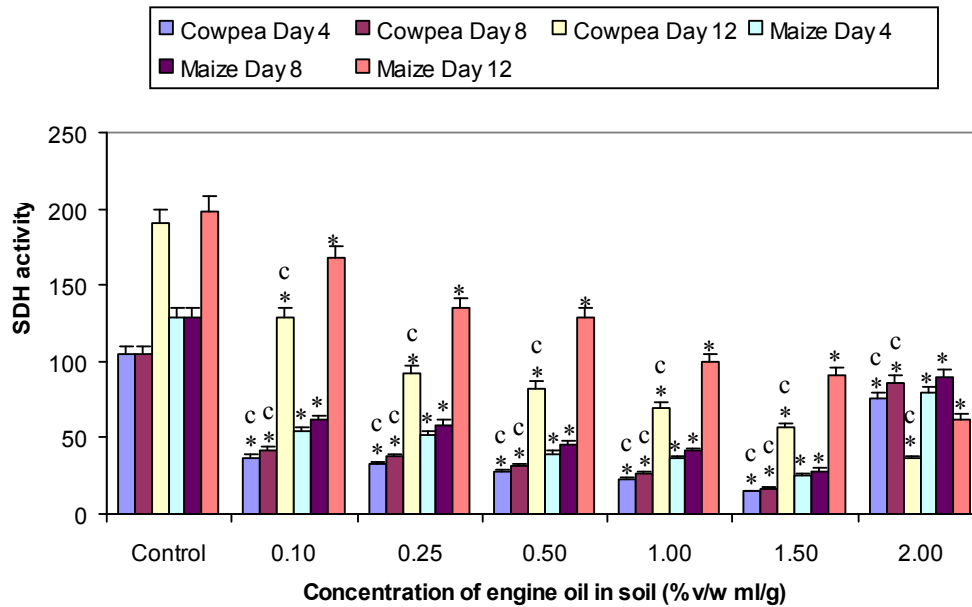
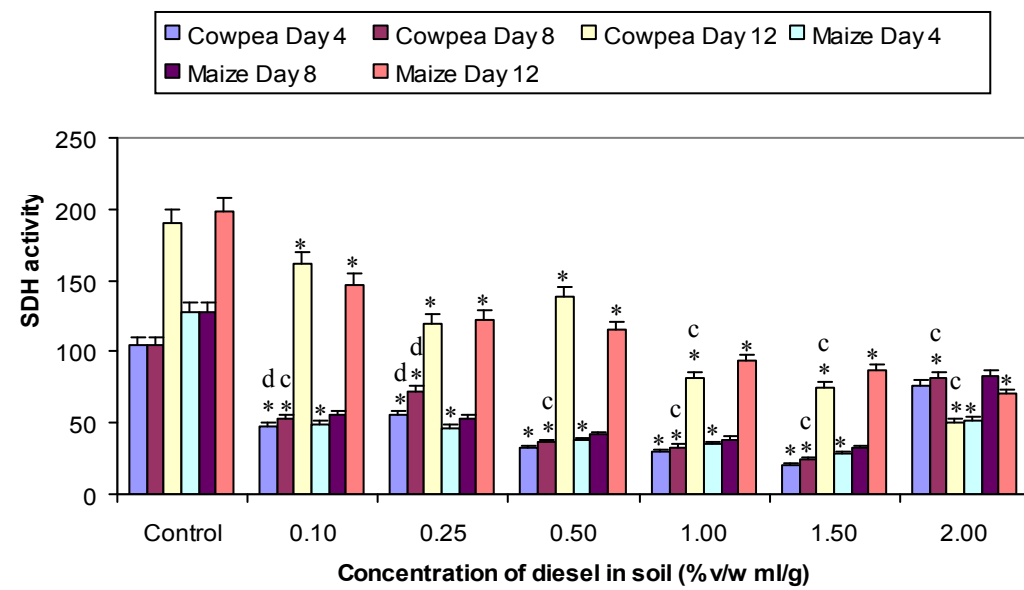
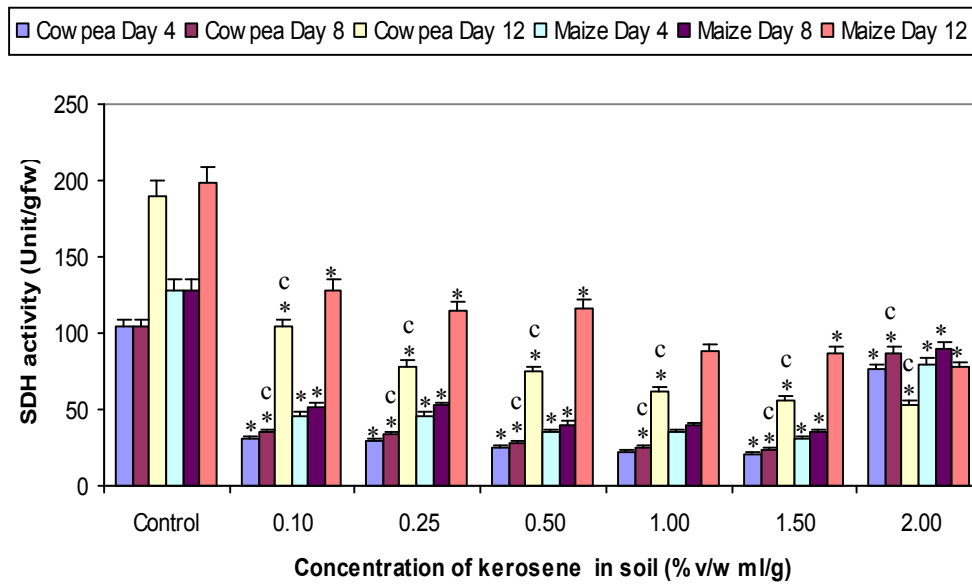


Fig. 1: Effect of concentration of petroleum products on SDH activities in leaves of cowpea and maize after four, eight and twelve days of germination. *Significantly lower as compared to control; +Significantly lower as compared to engine oil; ++Significantly lower as compared to kerosene; °Significantly higher relative to control; °Significantly lower relative to other petroleum products; °Significantly higher relative to other petroleum products; °Significantly lower in cowpea relative to maize seedlings; °Significantly higher in cowpea relative to maize seedlings

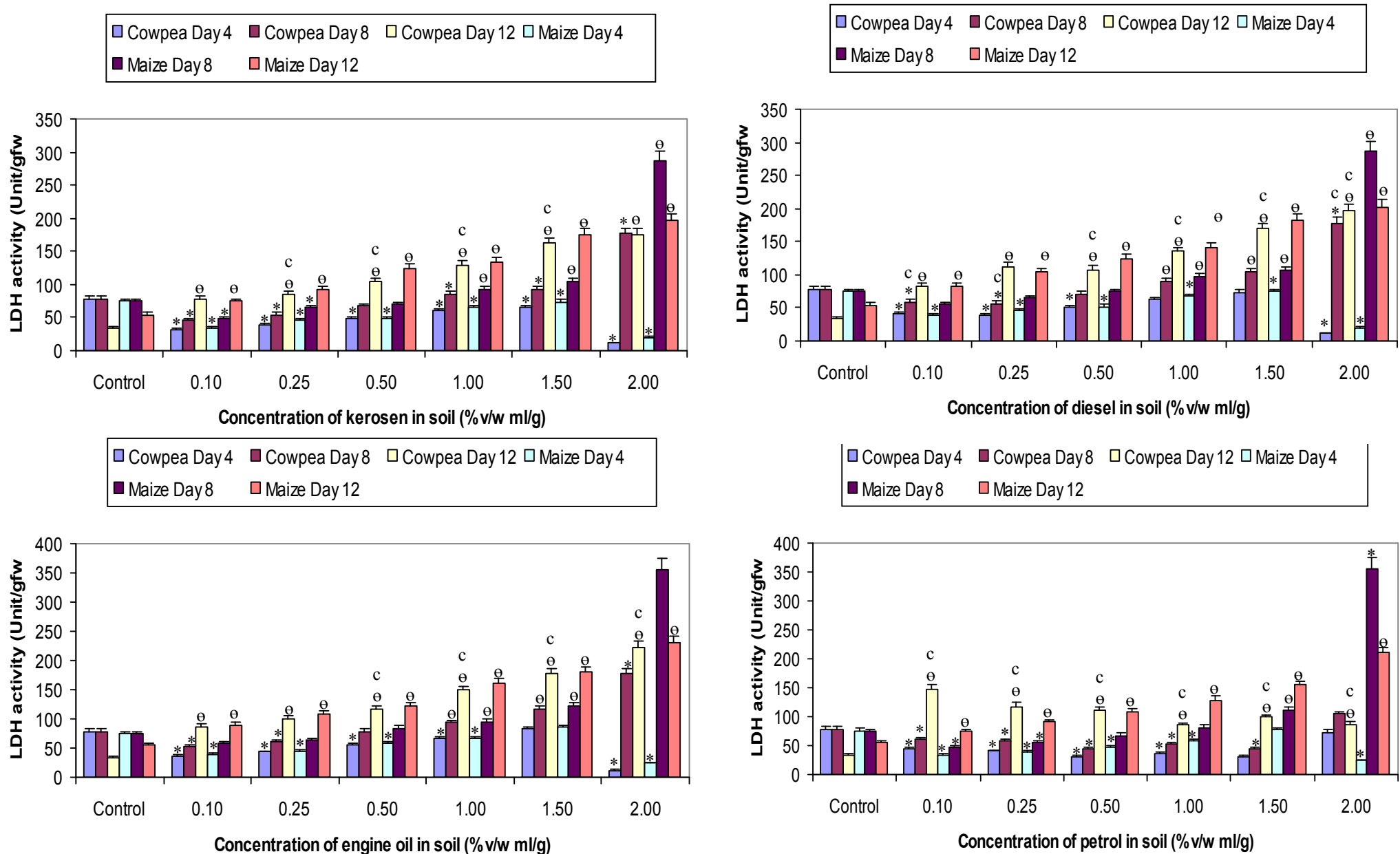


Fig. 2: Effect of concentration of petroleum products on LDH activities in leaves of cowpea and maize after four, eight and twelve days of germination. *Significantly lower as compared to control; ⁺Significantly lower as compared to engine oil; ⁺⁺Significantly lower as compared to kerosene; ^oSignificantly higher relative to control; ^aSignificantly lower relative to other petroleum products; ^bSignificantly higher relative to other petroleum products; ^cSignificantly lower in cowpea relative to maize seedlings; ^dSignificantly higher in cowpea relative to maize seedlings

196 REFERENCE

197

198 Aboaba, O.A., Aboaba, O.O., Nwachuku, N.C., Chukwu, E.E., and Nwachukwu, S.C.U. (2007).

199 Evaluation of bioremediation of agricultural soils polluted with crude oil by planting beans

200 seeds, phaseolus vulgaris. *Nature and Science*, 5(4)53-60

201 Achi, C. (2003). Hydrocarbon exploration, environmental degradation and poverty. The Nigeria

202 Delta experience. Diffuse pollution conference.

203 Achuba, F. I. (2006). The effects of sublethal concentrations of crude oil on the growth and

204 metabolism of cowpea (*Vigna unguiculata*) seedlings. *The Environmentalist*. 26:17-20.

205 Amadi, A., Abbey, S.D. and Nma, A. (1996). Chronic effect of oil spil on soil properties and

206 michroflora of rain forest ecosystem in Nigeria. *Water, air and soil pollution*. 86:1-11.

207 Anigboro, A.A. and Tonukari, J.N. (2008). Effect of crude oil on invertase and amylase activities

208 in cassava Leaf extract and germinating cowpea seedlings. *Asian J. Biol. Sci.* 1(1): 56-60

209 Anoliefo, G.O. and Okoloko, G.E. (2000). Comparative toxicity of forcados blends crude oil and

210 its water soluble fraction on seedlings of cucumeropsis manni naudin. *Nig. J. Appl. Sci.* 18:

211 39-49.

212 Ayotarmuno, J.M. and Kogbara, R.B. (2007). Determining the tolerance level of zea mays

213 (Maize) to crude oil polluted agricultural Biol. African Journal of Biotechnology. 6(11):

214 1332-1337

215 Bhagyalakshmi, A.P., Reddy, P.S., and Ramamurthy, R. 1984. Subacute stress induced by

216 sumithion on certain bio-chemical parameters in Oziotelphusa senex. The fresh water rice

217 field crab. *Toxicol. Lett.*, 21: 127-134

218 Chourpagar, A. R. and Kulkarni, G.K. (2009). Toxic effect of copper sulphate on lactate

219 dehydrogenase activity in a freshwater crab, barytelphusa cunicularis (Westwood). *World J.*

220 *Zool.* 4(3): 180-183

- 221 Eriyamremu, G.E., Iyasele, J.U., Osubor, C.C., Anoliefo, G.O, Osagie, V.E. and Osa, M.O
- 222 (1999). Bonny light crude oil alters protease and respiratory activities of germinating beans
- 223 (*Vigna unguiculata*) (L) Seeds, *J. Sci Eng. Technol* 6(1): 1589 – 1600.
- 224 Guglielminetti, L Ferela, P and Alpi, A (1995). Effect of anoxia on carbohydrate metabolism in
- 225 rice seedlings. *Plant Physiol.* 108: 735 -741
- 226 Jorgensen, K.S. Purstinen, J., and Sourti A. M. (2000). Bioremediation of petroleum
- 227 hydrocarbon-contaminated by compositing in biophiles. *Environ. Pollut.* 107:245-254
- 228 Kenny, J., Kutcherov, V., Bendeliani, N. and Alekseev, V. (2002). the evolution of multi-
- 229 component system of high pressures: VI. The thermodynamic stability of the hydrogen
- 230 carbon system: the genesis of hydrocarbon and the origin of petroleum *Proc. Nat. Acad. Sci.*
- 231 *99:10976-10981.*
- 232 Kregiel, D., Berlowsk, J. and Ambroziak, W. (2008). Succinate dehydrogenase activity assay in
- 233 situ with blue tetrazolium salt in crabree-positive *saccharomyces cerevisia* strain. *Food*
- 234 *Technol. Biotechnol.* 46(4) 376-380
- 235 Michalcewicz, (W). (1995). The effects of diesel oil on number of bacteria and actionomyces
- 236 and biomas soil micaroorganisms (in Polish). *PzH.* 46(1):91.
- 237 Odjegba, V. J and Sadiq, A. O. (2002). Effects of spent engine oil on the growth parameters,
- 238 chlorophyll and protein level of *Amaranthus hybridus* L. *The Environmentalist.* 22:23-28.
- 239 Samokchvalov, M.V., Ignator, V. and Kondrashava, M. (2004). Inhibition of Krebs cycle and
- 240 activation of glyoxylate cycle in the courses of chronological afing of *saccharomyces*
- 241 *cerevisiae*. *Compenstory role of succinate oxidation, Biochomie,* 86: 39-46
- 242 Song, H. and Bartha R. (1990). Effects of jet fuel spills in the microbisl community of soil. *Appl.*
- 243 *Environ. Microbial. Mar,* 56:646-651.

- 244Tolulope, A.O. (2004). Oil exploration and environmental Degradation. The Nigerian
245 Experience. Environ. *Informatic archive Vol. 2*pp. 387-393
- 246Valarmathi, S. and Azariah, J. (2002). Impact of chlorine on crab sesarma quadratum. J. Nation.
247 *14(1): 21-26.*
- 248Valarmathi, S. and Azariah, J. (2003). Effet of copper chloride on the enzyme activities of the
249 crab sesarma quadratum (Fabricius). *Turk. J. Zool. 27: 253-256.*
- 250Valarmathi, S. and Azariah, J. (2003). Effet of copper chloride on the enzyme activities of the
251 crab sesarma quadratum (Fabricius). *Turk. J. Zool. 27: 253-256.*
- 252Wemedo, S.A., Obire, O. and Ijogubo, O.A. (2002). Myco-Flora of Kerosene polluted soil in
253 Nigeria. *J, Appl. Sci. Environ. Mgt. 6(1): 14-17.*
- 254