

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

abstract should include the
rationale of study i.e. why petroleum
contaminated soil is of importance.

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**

25 INTRODUCTION

26 Petroleum compounds have multifarious industrial and domestic uses. They are
 27 extensively employed in solvents, dry cleaning fluids quick-products, automobile fuel,
 28 household solvents, and lubrication of machines, cosmetics, water proofing agents,
 29 cleaning agents, and specialty chemicals (Kenny *et al.*, 2002). These anthropogenic
 30 activities have led to the widespread contamination of the environment. Oil spill is the
 31 major cause for the high influx of petroleum to the biosphere (Achi, 2003; Tolutope,
 32 2004). However, other points of soil pollution with refinery products are petrol stations,
 33 garages servicing car and tractors, ^{and} seaports areas (Michalcewicz, 1995). Other areas of
 34 concern are mining and ^{industry} (distribution of petroleum-based products) (Song and Barter 1990;
 35 Amadi *et al.*, 1996; Jorsgensen *et al.*, 2000). ^{rewrite} [Beside, heavier use of machinery in
 36 agriculture leads to higher consumption of diesel. ^{need to discontinue on} Certain negligence when transporting, ^{transport it}
 37 collecting or storing refinery products together with unsatisfactory care while disposing ^{distributing to}
 38 of old or used petroleum products leads to considerable pollution of the ^{storage.} natural ^{This seemed}
 39 environment (Odjegba and Sadiq 2002). ^{to be out of place.}

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

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

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

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

60 MATERIALS AND METHODS

61 Refined petroleum products and planting materials

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

70

71 **Soil treatment and planting of seeds**

72 One thousand six hundred grams of soil was added to each hundred small size
73 planting bags (1178.3cm^3 , 15 cm deep) and divided into six groups of five replicates.

74 Groups 1 to 5 contained 0.1%, 0.25%, 0.5%, 1.0% and 2.0% (v/w) respectively of each of
75 the petroleum products while group six served as control (0.0%). To the first bag, 1.6 ml

76 of kerosene, corresponding to 0.1%, was added. The petroleum product treated soil

77 samples was mixed vigorously with the hand to obtain homogeneity of the mixture. The

78 procedure was repeated for 0.25%, 0.5%, 1.0%, 1.5% and 2.0%. This same procedure

79 was applied to diesel, engine oil and petrol. Each treatment including control was

80 replicated five times and consisted of three sown seed per polythene bag. The treatments

81 were watered every day in order to keep the soil moist. The design of the experiment was

82 completely randomised one.

83 Seed viability was determined by floatation. All seeds that floated on water are
84 discarded and others that remained at bottom of water deemed were potentially viable.

85 Three seeds were planted in each test bag to an approximate depth of 2cm and under

86 partial shade. During the experiment 80cm^3 of water was supplied to the set up as at

87 when needed to keep the soil moist. Germination [which is indicated by the appearance

88 of epicotyls (for cowpea) and hypocotyls (for maize) above the soil level] records were

89 taken at 4 days interval up to 12 days. Seeds, which failed to sprout after 12 days were

90 regarded as not germinable.

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

96 The leaves (1.0g) of cowpea seedlings after four days of germination was taken
 97 into mortar containing 0.5g of acid washed sand. This was followed by the addition of
 98 5.0ml of 0.05M potassium phosphate buffer (pH 7.4) and ground with a pestle. The
 99 homogenate was filtered through cheesecloth. The filtrate was centrifuged at 1000g for 5
 100 minutes at 4°C to sediment the nuclear fraction. The supernatant was further centrifuged
 101 at 10,000g for 30 minutes at 4°C. The supernatant was discarded and pellet resuspended
 102 in 80ml of 0.05 M potassium phosphate buffer (pH 7.4). The resuspended pellet is kept in
 103 ice bath for use as source of enzyme. The extraction was repeated for cowpea seedlings
 104 after eight and twelve days of germination. This same procedure was adopted for the
 105 preparation of extract from maize leaves after four, eight and twelve days of germination
 106 in petroleum products-treated soil.

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

111 The activity of succinate dehydrogenase (SDH) was determined by adding 2.5ml of
 112 0.05ml of potassium cyanide (conc?) and 0.5ml of succinate (conc?) into preincubated cuvette. The tube
 113 was covered with paraffin and mixed thoroughly. The change in absorbance was
 114 monitored for 30 minutes at 600 nm with an Sp 1800 UV/VIS Spectrophotometer after
 115 the addition of 0.5ml of enzyme extract. The blank was prepared by replacing the enzyme
 116 extract with distilled water (Sajan et al., 1995). Succinate dehydrogenate activity was
 H₂O₂

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

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

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

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

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* Statistics analysed by ?
* - parameters for stats?

140 RESULTS AND DISCUSSION

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

147 Succinate dehydrogenase is component of complex II of the respiratory chain that
148 catalyzes the oxidation of succinate to fumarate in the Krebs cycle (Soliola et al., 2004).
149 It is the most efficient energy source in the cell (Samolchvalov et al., 2004; Kregiel et al.,
150 2008). Therefore, inhibition of succinate dehydrogenase activity may portend a decrease in the
151 rate of energy generation by the cell. Previous report indicates that heavy metals induced
152 a decrease in succinate dehydrogenase activity (Valarmathi and Azariah, 2003). This is in
153 consonance with the present investigation in which refined petroleum products caused a
154 decrease in succinate dehydrogenase activity after four, eight and twelve days of
155 germination (Fig.1). Moreover, the toxic effect of kerosene was more pronounced
156 compared to the effect of other refined petroleum products. The high degree of toxicity of
157 kerosene had been documented (Wemedo et al., 2002). Similarly, the vulnerability of
158 cowpea seedlings to petroleum hydrocarbon compared to maize seedlings was apparent
159 (Fig.1). The higher toxic effect of refined petroleum product on cowpea compared to
160 maize had been attributed to the fact that cowpea seedlings have the ability to absorb
161 hydrocarbon from the soil more than maize seedlings (Ayotamuno and Kogbara, 2007;
162 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.

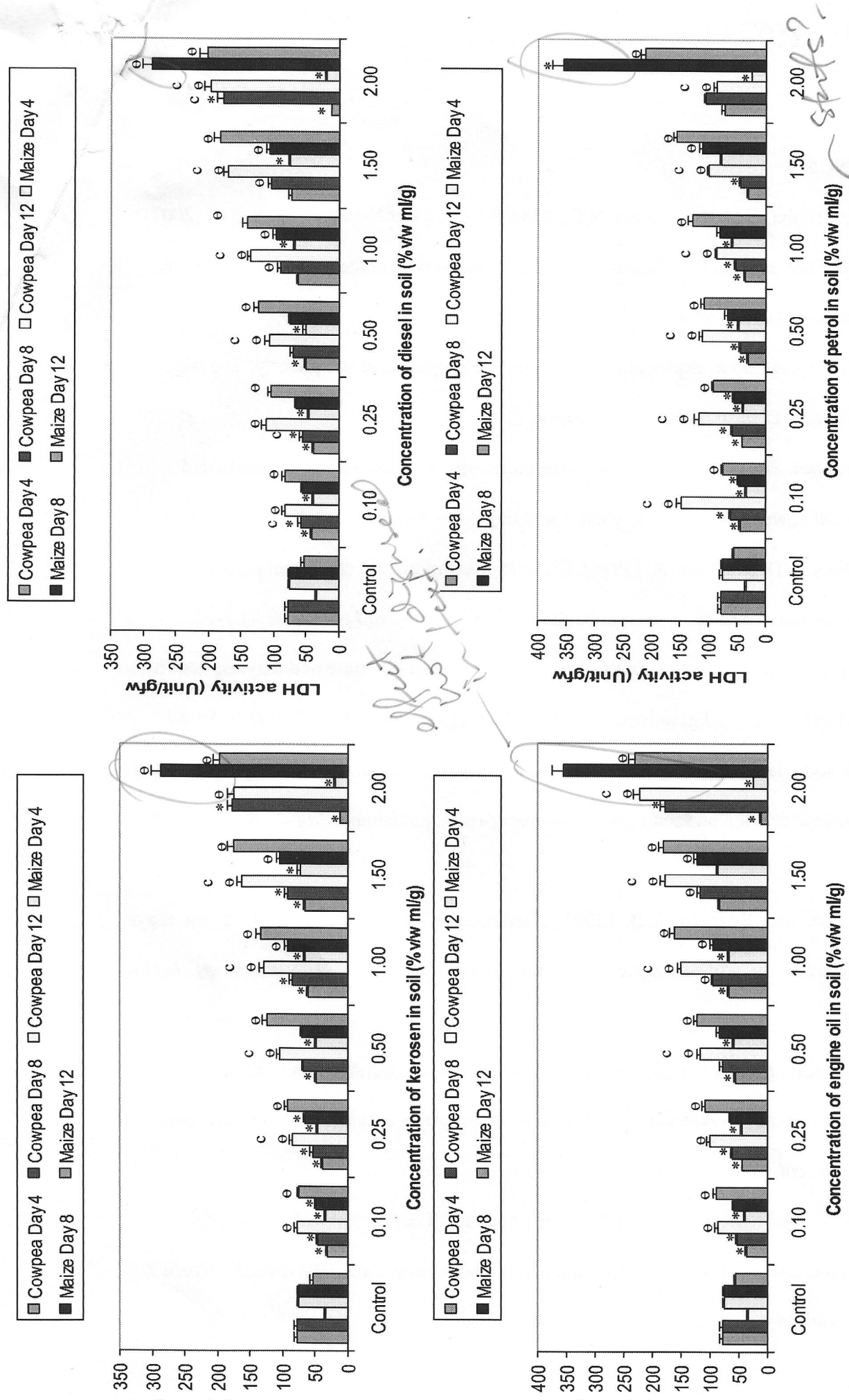


Fig. 2: Effect of concentration of petroleum products on LDH activities in leaves of cowpea and maize after four, eight and twelve days of germination. *Significantly lower as compared to control; ⁺Significantly lower as compared to engine oil; ⁺⁺Significantly lower as compared to kerosene; ^eSignificantly 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