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Research Article

Assessment of Energy Generating Enzyme Activities in Seedlings Grown in Hydrocarbons-Treated Soil

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Abstract: The damaging potency of hydrocarbons on natural and cultivated crops in petroleum-producing areas of the world is voraciously documented. This research investigated the noxiousness of hydrocarbons on aerobic and anaerobic enzymes using seedlings of cowpea and maize as a model. Viable seeds of these food crops were planted in soil treated with different concentrations of various hydrocarbons. Each group was prepared five times and six groups were constituted. The setup was watered daily. After growth periods, the activities of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in the seedlings were determined using standard methods at four days interval up to twelve days after planting. The data produced was subjected to analysis of variance for comparisons. The presence of the various hydrocarbons in soil significantly ($P<0.01$) and reciprocally altered the activities of the respiratory enzymes in the leaves of the seedlings: decreased succinate dehydrogenase activity but increased activity of lactate dehydrogenase. Kerosene polluted soil was more potent than the other hydrocarbons investigated. It is evident from the study that hydrocarbons in soil predispose plants to anaerobic energy production, as a way of energy generation, to enable them to survive hydrocarbon toxicity.

Keywords: Cowpea, Maize, Enzymes, Hydrocarbons, Soil.

1. INTRODUCTION

Petroleum compounds can enter the cropped lands through natural, mechanical, and human activities [1]. This can occur either accidentally or by various intentional acts and eventually damages the biota along with inherent organisms. The first impact of petroleum contamination of soil is the reduction in available water and air [2]. This culminates in an upset in a microbial consortium of the affected soil that translates to limiting the availability of oxygen to plants cultivated on such soil with great impacts on them [3] Petroleum exposed plants were reported to cause a decrease in seed germination, retard plant growth, and cause oxidative stress in exposed plants [4, 5]. The harmful potentials disturb metabolic activity of plants [6, 7, 8, 9]. The exposure of organisms to a polluted environment causes physiological stress in a living organism that predisposes it to adjust energy demand which will trigger a shift in energy metabolizing enzymes [10,11,12,13].

One of the important enzymes in energy production in living organisms is succinate dehydrogenase. It is an active participant in the running of the Krebs cycle [14]. The activity of succinate dehydrogenase is an indication of the level of the tricarboxylic acid cycle (TCA) activity as well as one of the efficient methods of measuring the vigor of living organisms[15, 16]. Another important enzyme involved in anaerobic energy generation is lactate dehydrogenase [17]. Therefore, the energy metabolic status of any organism experiencing poisonous insult can best be examined through measuring changes in the activities of enzymes involved in aerobic and anaerobic respiration. Thus, this research investigated the noxiousness of hydrocarbons, as it relates to energy metabolism, vis-a-vis on aerobic and anaerobic enzymes using seedlings of cowpea and maize as a model.

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2. MATERIALS AND METHODS

2.1 Materials

Maize was obtained from Delta Agricultural Development Project (DTADP), Ibusa, Delta State, Nigeria The International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria supplied the cowpea seeds. The petroleum hydrocarbons were obtained from Warri Refining and Petrochemical Company, Nigeria. Reagents of analytical grade were utilized during the experiment. The soil sample was obtained from Delta State University, Abraka in site 2 vacant agricultural plot. The physicochemical properties of the soil had been reported [18].

2.2 Treatment of soil and planting of seedlings

The soil was dried and was sieved using 2 mm-mesh in an open space in the laboratory of the Department of Biochemistry, Delta State University, Abraka, Nigeria. Polythene bags were filled with the soil sample (1600 g). They were distributed into six groups. Every group consisted of five bags. The concentrations of hydrocarbons used varied between 0.1%, to 2.0% (v/w) while the untreated soil was used as control. These applications were prepared for each of the hydrocarbons.

2.3 Determination of damaged seeds

Floatation method was used to determine presumed seed viability which is predicated on the principle that seeds floated on the water were damaged and were discarded and those that did not float on the water were presumed viable and used for the experiment.

2.4 Planting of seeds

The planting involved three seeds per bag which were sown to a depth of about 2cm and placed under shade. The treated soil and control were kept moist daily by applying water (80 ml). The entire set up was observed daily for up to twelve days for germination and any seed that failed to germinate was assumed dead.

2.5 Preparation of succinate dehydrogenase extracts

Extracts for the determination of succinate dehydrogenase were prepared following a slightly modified method of Price and Thimann [19]. In each case, the leaves (1.0 g) were collected separately and homogenized in a mixture containing, acid-washed sand (0.5 g) and phosphate buffer (5.0 ml of 0.5M at) pH 7.4). The homogenate produced was filtered with cheesecloth and subjected to centrifugation in the cold (4^0C) for 5 mins at 10,000g to remove the nuclear fraction. The supernatant produced was subjected to further centrifugation at 10,000g for 30 minutes at 4^0C to generate another supernatant that was discarded and the pellet produced suspended in 80ml of 0.05 M potassium phosphate buffer, pH 7.4 and kept in the ice bath for use as a source of enzyme.

2.6 Determination of succinate dehydrogenase activity

The enzyme activity was based on the conversion of succinate to fumarate in an assay containing 2, 6 dichlorophenolindophenol (DCIP), cyanide, and FAD that acts as the electron acceptor. In a medium containing cyanide, the electron from $FADH_2$ is picked by 2, 6 dichlorophenolindophenol (DCIP). The reduction of DCIP gives a chromophore that can be measured at 600 nm. To determine succinate dehydrogenase activity, 2.5ml of 0.05M of potassium cyanide and 0.5ml of 0.05M succinate was added into the preincubated cuvette, mixed thorough and covered with paraffin. The change in absorbance at 600 nm was measured at an interval of 30 mins with an SP 1800 UV/VIS Spectrophotometer after the addition of 0.5ml of enzyme extract. The blank was performed with distilled water in place of the enzyme extract [17]. The enzyme activity was expressed in units and one unit is defined as one mole of dichlorophenolindophenol reduced /minute [17].

2.7 Preparation of lactate dehydrogenase extracts

Homogenate for lactate dehydrogenase was prepared using the modified extraction procedure of Rivoal et al. [20]. The leaves (2.0g) in each case of cowpea and maize seedlings respectively were homogenized in a mixture containing Tris-HCl buffer (10 ml of 0.1Mat pH 8.8) and a small quantity of EDTA with mortar and pestle. The homogenate produced was filtered with cheesecloth and the filtrate was subjected to centrifugation at 1,000 g for 10 mins. The supernatant obtained was raised to 20% saturation with solid $(NH_4)_2SO_4$, stirred and left to stand for 20 mins at 40^0C. The mixture was

subjected to centrifugation as above to produce a precipitate which contained NADH oxidase and was discarded. The crude supernatant obtained here was used as the enzyme source.

2.8 Determination of lactate dehydrogenase activity

Lactate is oxidized to pyruvate by lactate dehydrogenase in the presence of NAD+, which is reduced to NADH. The rate of NADH formation at 340 nm is synonymous with LDH activity. The method of Kaiglova et al [11] was adopted. The assay mixture 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 and was equilibrated at 37oC. The rate of NADH + H+ was determined at 340 with an SP 1800 UV/VIS Spectrophotometer.

2.9 Statistical Analysis

Experimental data were compared via a two-way analysis of variance (ANOVA) with the Graph Pad Prism, version 5.3. Significant differences were set at $p<0.01$. All final results were expressed as mean + SE.

3. RESULTS

The existence of all the organic solvents in soil altered succinate dehydrogenase activity in the leaves of cowpea seedlings when measured at four days interval (Fig. 1). The result is inconsistent and varied, but generally, at higher concentrations of the organic solvents to the soil, a significant ($P<0.01$) decrease in succinate dehydrogenase activity was observed across all the organic solvents applied. The effects stimulated by kerosene were found to be more pronounced than those relative to the other experimental hydrocarbons.

Similarly, the presence of all the organic solvents in soil altered succinate dehydrogenase activity ($P<0.01$) in the leaves of maize seedlings when measured at four days interval (Fig 1). In tandem with cowpea seedlings, the result is inconsistent and varied, but generally, at higher amounts of the organic solvents to the soil, a significant ($P<0.01$) decrease in succinate dehydrogenase activity was observed across all the organic solvents applied. Similarly too, the decrease stimulated by kerosene affects the seedlings than the other solvents applied. Besides, treatment of soil with the organic solvents, on the whole, initially (at day 4) a significant

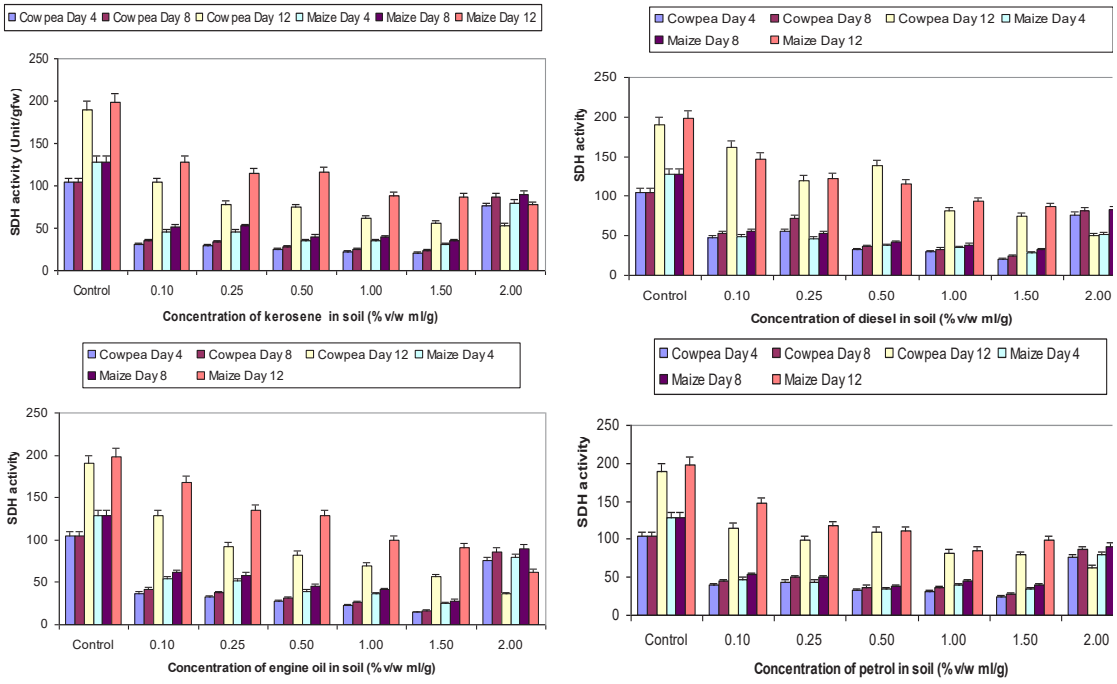

Fig. 1. Effect of concentration of petroleum products on succinate dehydrogenase activities in leaves of cowpea and maize after interval of four days of germination. All values are expressed as Mean ± SEM, values followed by different alphabet superscript in the same graph indicates a significant difference.

(P<0.01) decrease in lactate dehydrogenase activity in the leaves of cowpea seedlings relative to control seedlings but thereafter (eight and twelve days), a significant (P <0.01) increase in the activity of the enzyme was noted at high concentrations of the petroleum solvents (Fig. 1). The increase in enzyme activity was generally significant (P < 0.01) when seedlings raised in kerosene treated- soil were compared with seedlings raised in soil treated with the other solvents.

Furthermore, a significant (P<0.01) decrease in lactate dehydrogenase activity in the leaves of maize seedlings at day 4 relative to control seedlings was recorded. Thereafter (eight and twelve days), a significant (P <0.01) increase in the activity of the enzyme was noted at high concentrations of the petroleum solvents-treated soils (Fig 2). in addition to all the solvents considered, kerosene generally, decreased the enzyme activity more relative to the other petroleum products.

4. DISCUSSION

Crude oil impacted soil suffers from the displacement of air that creates hypoxic conditions in biotic lives in such an environment [21, 22]. This gives rise to oxygen deficiency in plants cultivated in such soil [23]. And as such,z plants try to adapt to the energy crises by either upgrading or downgrading certain respiratory enzymes which may include lactate dehydrogenase and succinate dehydrogenase [24]. In this study, the addition of the various hydrocarbons to the soil inhibited succinate dehydrogenase activity in the leaves of cowpea seedlings and that of maize seedling. This inhibition of succinate dehydrogenase activity in the two seedlings is an indication of a decrease in aerobic energy generation and portends the loss of cell vitality [16, 25], 26. This agrees with toxicant stimulated debility in succinate dehydrogenase activity [20]. Moreover, of the entire hydrocarbon analyzed, kerosene decreased the efficacy of the enzyme more than the other hydrocarbons. The venomous credential of kerosene is in literature [27]. Also, the susceptibility of cowpea seedlings was more relative to maize seedlings. This is due to the ability of cowpea seeds to absorb hydrocarbon from the soil more than maize grains [28, 29].

Hydrocarbons treatment of soil gave varied and inconsistent lactate dehydrogenase activity (LDH) in the tissues of the seedlings of cowpea (Table 3) and that of maize. Moreover, lactate dehydrogenase activities in the two seedlings were time-dependent. The initial decline in the enzyme activity is for

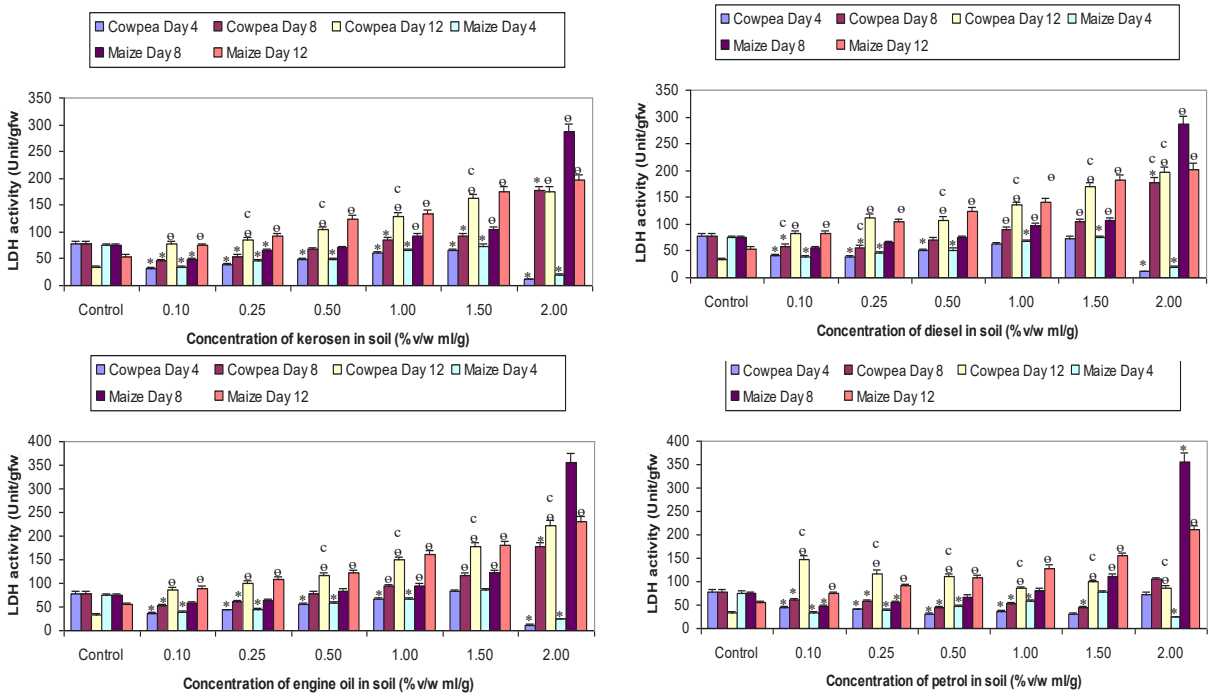

Fig. 2: Effect of concentration of petroleum products on lactate dehydrogenase activities in leaves of cowpea and maize after interval of four days of germination. All values are expressed as Mean ± SEM, values followed by different alphabet superscript in the same graph indicates a significant difference.

the toxic effects of the hydrocarbons at high concentrations; whereas, the increase as the number of days increase may be an adaptive measure to survive in a contaminated environment. A similar response of enzyme to hydrocarbon contaminated soil was reported previously [30, 31]. All the same, the increase in LDH activity, especially on day eight, agrees with the report of Valarmathi and Azariah on the response of the enzyme to the presence of chemical insult [24]. The increase in lactate dehydrogenase activity is a likeness of increased anaerobic carbohydrate metabolism by the leaves of the respective seedlings after sustained exposure to hydrocarbon toxicity. Similarly, kerosene-treated soil increased lactate dehydrogenase activity than the other hydrocarbons-treated soil. Moreover, lactate dehydrogenase activity was more in maize relative to cowpea seedlings. This was attributed to the high degree of fermentative respiration in seeds with high starch content [32].

Generally, a reciprocal relationship between succinate dehydrogenase activity and lactate dehydrogenase activity had been established [24, 32]. The inhibition of succinate dehydrogenase activity in addition to the elevation of lactate dehydrogenase activity may predispose the seedlings to anaerobic carbohydrate metabolism amid chemical intoxicated soil.

5. CONCLUSIONS

Hydrocarbons in soil predispose plants to anaerobic energy production, as a way of energy generation, to enable them to survive hydrocarbon toxicity. This is indicated by the hydrocarbon-mediated decrease in succinate dehydrogenase activity and the corresponding decrease in lactate dehydrogenase activity in the plant models.

6. REFERENCES

1. F. I. Achuba and L.O. Iserhienrhien. Effects of soil treatment with abattoir effluent on morphological and biochemical profiles of cowpea seedlings *(V.unguiculata)* grown in gasoline polluted soil. *Ife Journal of Science* 19 (3):051 – 059 (2018).
2. K. C. Onwusiri, C. U. Aguoru and G.F. Akomolafe. Effect of spent engine oil on germination and growth parameters of fluted pumpkin (*Telfaira occidentalis Hook F*.) in Markurdi, Benue State, Nigeria. J*ournal of Research in Forestry, Wildlife and Environment* 9(4): 1-8(2017).
3. A. H. Erhenhi. Growth comparison of four arable on the polluted and unpolluted soils of Bodo City in Ogoni land, Rivers state, Nigeria. *Nigerian Journal of Science & Environment* 15(1):32-36 (2017).
4. F. I. Achuba and P. N. Okoh. An appraisal of the effects of different petroleum chemicals on selected food crop seedlings. *Nigerian Research Journal of Chemical Sciences* 5:75 -81(2018).
5. F. I. Achuba. Spent engine oil mediated oxidative stress in cowpea (*Vigna unguiculata*) seedlings. *Electronic Journal of Environment, Agriculture & Food Chemistry* 9(5): 910-917(2010).
6. A. R. Chourpagar and G. K. Kulkarni. Toxic effect of copper sulphate on lactate dehydrogenase activity in a freshwater crab, *barytelphusa cunicularis* (Westwood). *World Journal of Zoology* 4(3):180-183 (2009).
7. P. Arellano, K.Tansey, H. Balzter and D.S. Boyd. Detecting the effects of hydrocarbon pollution in the Amazon forest using hyperspectral satellite image. *Environmental Pollution* 205: 225-239 (2015).
8. F. I. Achuba and P.O. Erhijivwo. The effect of abattoir waste water on the metabolism of cowpea seedlings grown in diesel contaminated soil. *Nigerian Journal of Science & Environment* 15 (1): 155 – 162 (2017).
9. F.I. Achuba and M. O. Ja-anni. Effect of abattoir waste water on metabolic and antioxidant profiles of cowpea seedlings grown in crude oil contaminated soil. *International Journal of Recycling of Organic Waste in Agriculture* 7(1): 59 -66(2018).
10. G.E. Eriyamremu, J.U. Iyasele, C. C. Osubor, G. O. Anoliefo, V. E. Osagie and M.O. Osa. Bonny light crude oil alters protease and respiratory activities of germinating beans (*Vigna unguiculata*) (L) Seeds. *Journal of Science Engineering& Technology* 6(1):1589–1600 (1999).
11. A. Kaiglova, E. Reichrtova, A. Aclamcakova and L. Wsolora. Lactate dehydrogenase activity in human placenta following exposure to environmental pollutants. *Physiological Research* 50:525-528(2001).
12. F. I. Achuba and P. N. Okoh. Effect of petroleum products on soil catalase and dehydrogenase activities. *Open Journal of Soil Science* 4:399-406 (2014).
13. F I. Achuba and M.N. Oshiokpu. Growth and metabolic activities of cowpea seedlings exposed to artificial pond wastewater-treated soil. *International Journal of Recycling of organic Waste in Agriculture*

8: 351-359 (2019)
14. D. L Nelson and M. M. Cox. Lehninger Principles of Biochemistry (4th Ed.), WH Freeman &Co, New York (2005)
15. A.P. Bhagyalakshmi, P.S. Reddy and R. Ramamurthy. Subacute stress induced by sumithion on certain biochemical parameters in Oziotelphusasenex, the fresh water rice field crab. *Toxicological Letters* 21:127-134(1984).
16. D. Kregiel, J. Berlowsk and W. Ambroziak. Succinate Dehydrogenase Activity Assay in situ with blue tetrazolium salt in crabtree-positive *Saccharomyces cerevisiae strain*. *Food Technology & Biotechnology* 46(4):376-380(2008)
17. M. P. Sajan, J.G. Satav and R.K. Bhattacharya. Activity of some respiratory enzymes and cytochrome contents in rat hepatic mitochondria following aflatoxin B1 administration. Toxicology 80(1-3):55- 60(1995).
18. F.I. Achuba. Petroleum products in soil mediated oxidative stress in cowpea (*Vigna unguiculata*) and Maize (*Zea mays*) Seedlings. *Open Journal Soil Science* 4: 417-435(2014).
19. C. A. Price and K. V.Thimann. The estimation of dehydrogenases in plant tissue. *Plant Physiology* 29(2):113-124 (1954)
20. J. Rivoal, B. Ricard and A. Pradet. Purification and partial characterization of pyruvate decarboxylase from Oryza sativa L. *European Journal of Biochemistry* 194: 791-797(1990).
21. F. I. Achuba. The effects of sublethal concentrations of crude oil on the growth and metabolism of cowpea (*Vigna unguiculata*) seedlings. Environmentalist. 26:17-20(2006).
22. A .A Anigboro and N. J. Tonukari. Effect of crude oil on invertase and amylase activities in cassava Leaf extract and germinating cowpea seedlings. *Asian Journal Biological Science* 1(1):56-60(2008).
23. G. O. Anoliefo and G.O. Okoloko. Comparative toxicity of forcados blends crude oil and its water soluble fraction on seedlings of *cucumeropsis mannii naud. Nigerian Jounal of Applied Science* 18:39-49 (2000).
24. S. Valarmathi and J. Azariah. Effect of copper chloride on the enzyme activities of the crab *sesarma quadratum* (Fabricius).*Turkish Journal of Zoology* 27:253-256 (2003).
25. D. Voet, J.G. Voet and C.W. Pratt. Fundamentals of Biochemistry.(2nd Edition). John Wiley and Sons Inc., New Jersey. 580-581(2006).
26. M .V. Samokchvalov, V. Ignator and M. Kondrashava. Inhibition of Krebs cycle and activation of glyoxylate cycle in the courses of chronological aging of saccharomyces cerevisiae. Compensatory role of succinate oxidation. *Biochomie* 86:39-46(2004).
27. S. A. Wemedo, O. Obire and O.A. Ijogubo. Myco-Flora of Kerosene polluted soil in Nigeria. *Journal Applied Science & Environmental Management* 6(1):14-17(2002).
28. J. M. Ayotarmuno and R.B. Kogbara. Determining the tolerance level of Zea mays (Maize) to crude oil polluted agricultural soil. *African Journal of Biotechnology* 6(11):1332-133(2007).
29. O. A. Aboaba, O.O. Aboaba, N.C. Nwachukwu, E.E. Chukwu and S. C. U. Nwachukwu. Evaluation of bioremediation of agricultural soils polluted with crude oil by planting beans seeds, *phaseolus vulgaris. Nature and Science* 5(4):53-60 (2007).
30. F. Mohsenzadeh, A. Chehregani Rad and M. Akbari. Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils. *Iranian Journal of Environment Health Science & Engineering* 9(1): 26. (2012).
31. M. N. Ebulue., O. F.C. Nwodo, I.N.E. Onwurah, A.A Uwakwe and M. O. Wegwu. Enzyme–based assay for toxicological evaluation of soil ecosystem polluted with spent engine oil. *International Journal of Innovative Science, Engineering & Technology.* 4(7): 1-13(2017)
32. L.Guglielminetti, P. Ferela and A. Alpi. Effect of anoxia on carbohydrate metabolism in rice seedlings. *Plant Physiology* 108:735-741(1995).