Effect of Kawrgosk Oil Refinery on Some Physicochemical Characteristics, Microbial Population and Biochemical Properties of Surface Soils

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1. INTRODUCTION

Soil is a dynamic system in which continuous interaction takes place between soil minerals, organic matter and organisms. Each of these three major soil components influences the physicochemical and biological properties of terrestrial system (Anjaneyulu et al., 2011). Soil is a favourable habitat for microorganisms; spatially fertile soil is inhabited by tremendous number of microorganisms. All kinds of organic matters deposited on soil can be decomposed by soil microorganisms, releasing different kinds of enzymes, responsible for various oxidation-reduction reactions to release the nutrients (Nath and Samanta, 2012). Enzyme activities have been used as indicators of soil quality and changes in biogeochemical function. Since enzymes catalyse all biochemical transformations, measurements of soil enzyme activities are useful indicators of biological activity as well as to understand how...
human activity is changing biogeochemical cycles in ecosystems (Verchot and Borelli, 2005). Soil itself has no any enzyme activity for solubilisation as well as mobilization of minerals. But the huge number of microorganisms present in soil makes it possible to recycle the nutrients from both organic and inorganic substances (Nath and Samanta, 2012). Technologies, industrial and economic progress leads to emission of pollutants into natural environment including oil compounds and heavy metals. Soil pollution with crude oil has become an important problem of our days (Lipinska et al., 2013). The growing threat to the natural soil environment is growing which is caused by oil products due to leakage from tanks and pipes, truck tanks, during distribution process as well as by car and railway transport and petrol station in addition to the direct emission of these pollutants, dusts of burning gases along with oil have managed to add toxic and harmful substances to the soils (Marinescu et al., 2010). Toxicity of crude oil or petroleum products varies widely, depending on their composition, concentration, and environmental factors and on the biological state of the organisms at the time of the contamination (Eze et al., 2014). Soil pollution with heavy metals and trace elements has been reported to have toxic effects on soil biology and biochemical processes. The sources of these contaminants can come from various industrial activities among them deposition from air pollutants as a result of different fuels burning (Utobo and Tewari, 2015). Development of biological indicators to assess changes in soil quality is an increasingly important research area in the world. Assessment of soil enzymatic activities is of particular importance due to high and rapid development in the oil industry at the last century in Kurdistan Region of Iraq, and occurring of many different problems among them environmental pollution which began to raise serious questions that need rapid solutions. Soil pollution is one of these environmental problems due to such oil industrial activities because of using huge amount of consumable fuel in the power plants and oil refinery and releasing high rate of fume, solid particulates and toxic gases more than other industries. The existence of these industries such as Kawrgosk Oil Refinery, west of Erbil city is more hazardous effects to the environment of the Erbil city, urban area and the agricultural terrains. Therefore, it has been found an importance to carry out this study to evaluate effects of oil refinery on environment of some related areas with particular reference to some soil characteristics.

2. MATERIALS AND METHODS

2.1. Study area and sample collection

Erbil Refinery is located in Khabat district, at Kawrgosk village, 40 km west of Erbil city, and it occupies a land of 2.5 Km² to the left of upper Zab River. The refinery is composed at this stage of three production lines for crude Oil refining, production, storage, distribution and supply of petroleum products as per applied standards, which represent the first plant for crude oil refining in Kurdistan region. Construction of this refinery started in 2005. The refinery produces the following oil products: naphtha, kerosene, gasoil (desel), fuel oil, gasoline and liquid gas (after operating the second production line), these products are stored and distributed in storage tanks then transported through loading stations by tankers, or may be pumped through a pipe to Erbil Depot according to the request (KAR website, 2015). For the present study, twelve sites were studied; Kawrgosk Oil Refinery and ten different sites located at different distances from the centre of the Refinery and a control soil was selected in the Greenhouse of College of Science faraway from pollution sources (Figure 1).
During October 2014, triple surface soil sample (0-15 cm depth) from each site was collected into polyethylene bags using plastic garden trowel. The samples were analysed as soon as possible. Soils were air-dried, crushed and sieved through 2-mm stainless sieve to remove debris (Pansu and Gautheyrou, 2006).

2.2. Determination of soil physicochemical properties

Hydrometer method was used for particle size distribution and determination of soil textural classes using ISSS triangle. The pH and EC of the soils were determined in 1:5 (soil: water suspension) using a calibrated pH-meter (JENWAY 3505) and an electrical conductivity meter (JENWAY 4510) according to the method given in (Ryan et al., 2001). Gravimetric method was used for soil moisture content determination as described by (Jaiswal, 2003). Walkly-Black procedure 1934 was followed for determination of soil organic matter as given by (Pansu and Gautheyrou, 2006).

2.3. Determination of soil oil content

Oil content in the soil samples was determined by toluene extraction method.
according to (Amadi et al., 1996). For 10 g of soil, 200 ml of toluene was added and shake vigorously for 30 minutes by shaker; the liquid phase was extracted and measured spectrophotometrically at 420 nm. A standard curve of the absorbance of different known concentration of petroleum hydrocarbons in the extractants was derived using fresh crude oil, appropriately diluted with the solvent. Petroleum hydrocarbons concentration in soil was then calculated and expressed in µg.Kg\(^{-1}\).

### 2.4. Determination of soil heavy metals

Soil heavy metal contents were determined by using X-Ray fluorescence method using a portable XRF instrument model (CIT 3000). Soil samples were dried in air, sieved through 2 mm sieve to remove non-soil particles and then XRF measured (Ulmanu et al., 2011). The X-ray fluorescence method is a non-destructive analytical technique, allowing both qualitative and quantitative analysis of heavy metals in soils (Derzi and Naji, 2014) based on the phenomenon of the emission of x-rays by the atoms of a sample when excited by an external source of radiation. This technique can be used for screening the metal contamination in soil with significant accuracy and reduced overall costs. Results expressed in g.Kg\(^{-1}\).

### 2.5. Counting of soil microorganisms

Soil microbial populations including bacterial and fungal populations were counted by standard plate method using serial dilution technique. One gram of each soil sample was serially diluted (10\(^{-3}\)-10\(^{-7}\)) and one ml was poured into petri plates of sterile nutrient agar (pH 7.2) and incubation period of 24 hours at 30 °C for total bacterial count as given by (Harley and Prescott, 1996). For total fungal count, 0.1 ml from the diluted sample was spread with a sterile spreader on potato dextrose agar containing 0.002% Chloramphimicol for 7 days at 25 °C (Aneja, 2003). After incubation period, colonies formed on the surface of growth media were counted by colony counter. Results of soil total bacterial population expressed as cfu.g\(^{-1}\) dry soil multiplied by 10\(^7\), and soil total fungal population expressed as cfu.g\(^{-1}\) dry soil multiplied by 10\(^3\).

#### 2.6. Estimation of soil enzyme activity

##### 2.6.1. Estimation of dehydrogenase

The dehydrogenase activity was determined by the modified procedure of Casida 1977 given by (Anjaneyulu et al., 2011). For 5 g of soil in a test tube, 2.5 ml of sterile distilled water and 1ml of 3% aqueous solution of triphenyl tetrazolium chloride (TTC) was added and incubated at 30 °C for 24 hours. The triphenyl tetrazolium formazone end product was measured at 485 nm. The results expressed as µg TPF.g\(^{-1}\) dry soil.24h\(^{-1}\).

##### 2.6.2. Estimation of urease

Urease activity was determined by the modified method of Hoffmann and Teicher 1961 described by (Uzun and Uyanoz, 2011). For 1 g of soil, 0.25 ml toluene, 0.75 ml citrate buffer (pH 6.7) and 1 ml of 10% urea substrate solution were added and incubated for 3 hours at 37 °C. Formation of ammonium was found out spectrophotometrically at 636 nm (Bashour and Sayegh, 2007). Results expressed as µg N-NH\(_4\).g\(^{-1}\) dry soil.3h\(^{-1}\).

##### 2.6.3. Estimation of nitrate reductase

Estimation of nitrate reductase was done according to (Nath and Samanta, 2012). Into 150 ml conical flasks, 50 ml of peptone water media amended with 1% KNO\(_3\) were poured and then inoculated with 5 grams of different soil samples. The flasks were all incubated at 30 °C for 3 hours and then 10 ml of each soil suspensions were centrifuged at 5000 rpm for 10 minutes and 1 ml of the supernatants were treated with 1 ml of sulphanilamide. After 20 minutes, 1 ml of N (naphthyl) Ethelene
Diamine Dihydrochloride (NEDD) was added to each sample and left for development of a pink colour. Intensity of the pink colour was measured at 540 nm and un-inoculated media (with sulphanilamide and NEDD) used as blank. Results expressed in µg N-NO₂·g⁻¹ dry soil.3h⁻¹.

2.6.4. Estimation of catalase

The catalase activity was determined by KMnO₄ titration method as described by (Kumar, 2004). Two grams of oven-dried soil was mixed with 40 ml of distilled water and put on rotary shaker. Then 5 ml of 0.3% H₂O₂ was added and the slurry was shaken for 20 minutes at 150 rpm. The remaining peroxide was stabilized by adding 5 ml 3 N H₂SO₄ and 25 ml of filtered aliquots were titrated with 0.1 N KMnO₄. Results were expressed as ml of 0.1 N KMnO₄·g⁻¹ dry soil.20 min⁻¹, equivalent to the peroxide decomposed per gram of oven-dry soil.

2.7. Statistical analysis

Results were analysed using SPSS (version 18) and Microsoft excel 2010. Data is reported as mean ± standard error. One way ANOVA accompanied with Duncan’s test was used for comparing the means. Person’s correlation was done to test the relationship among the studied parameters from the different sites and the results considered statistically significant at (p<0.05) level (Le, 2003).

3. RESULTS AND DISCUSSION

3.1. Soil physicochemical properties

Soil moisture influences both the microbial and enzymatic activity (Aneja, 2003). From the studied soils, moisture content was ranged between 0.84±0.116 and 10.26±0.116 % in both Oil Refinery and control soils respectively (table 1) and same results obtained by Brzezinska et al. (1998) and Barua et al. (2011).

Soil pH considered as a chemical quality indicator of soil (Martinez et al., 2010). It influences a number of factors affecting microbial activity, like solubility and ionization of inorganic and organic soil solution constituents, and these will in turn affect soil enzyme activity (Paul, 2007). As shown in table 1, soil pH of the studied sites was ranged from 7.39±0.003 to 8.42±0.058 in Chaluke Gawra and Shewarash Zab respectively, indicating neutral to slightly alkaline soils, and this finding come in agreement with those obtained by Khudhur and Abdulla (2016). Soil electrical conductivity is used as an overall indicator of the level of macro- and micronutrients in the soil. The range of soil EC was 20±0.577 to 199±2.598 µS.cm⁻¹ in both Jideda Zab and Gaenj soils respectively (table 1) and Khudhur and Abdulla (2016) obtained a range of 14±1.155 to 231±1.155 µS.cm⁻¹ at different distances from Kawrgosk Oil Refinery which may confirm present finding.

Soil organic matter has important effects on microorganism’s activities and soil enzymes has considered as an indicator of soil quality because of its character of nutrient sink and source that can enhance soil physical and chemical properties, also promote biological activity (Fontaine et al., 2003). By the present study, the highest organic matter 77.27±0.07 g.Kg⁻¹ was observed in Agholane Gichka soil, whereas the lowest organic matter was 15.04±0.301 g.Kg⁻¹ in Oil Refinery soil (table 1). Das and Varma (2011) stated that soil enzyme activities are often closely related to soil organic matter and microbial activities, relating to this, Agholane Gichka soil has the highest bacterial population 24.81×10⁷ cfu.g⁻¹ dry soil and dehydrogenase activity 157.651 TPF µg·g⁻¹ dry soil.24h⁻¹ (table 3), and this statement may confirm our finding with regard to the close correlation of organic matter with enzymatic activities including dehydrogenase, urease and catalase (Figure 2). Soil organic...
matter protects soil microorganisms against the effect of hydrocarbons which lead to lower inhibition of microbial biomass. Organic matter and clay content can absorb hydrocarbons and decrease bio-availability during their aqueous phase. Higher organic matter level can provide enough substrate to support higher microbial biomass, hence higher enzyme production (Alrumman et al., 2015).

Table 1: Soil physicochemical properties (mean ± S.E.) of the studied sites.

| Sites               | Moisture % | Clay % | Silt % | Sand % | Texture class | pH       | EC µS.cm⁻¹ | Organic matter g.Kg⁻¹ |
|---------------------|------------|--------|--------|--------|---------------|----------|------------|-----------------------|
| Shewarash Zab       | 3.81±0.029a| 7.75   | 43.94  | 48.31  | L             | 8.42±0.058 | 54±0.577    | 25.53±0.008            |
| Agholane Gawra      | 2.51±0.029b| 0.00   | 36.23  | 63.77  | S L           | 8.04±0.006 | 59±0.577    | 30.70±0.001            |
| Kawrgosk            | 2.30±0.029b| 0.00   | 30.71  | 69.29  | S L           | 7.84±0.173 | 76±0.577    | 41.91±0.292            |
| Gaenj               | 1.32±0.006c| 7.63   | 45.77  | 46.60  | L             | 7.98±0.520 | 199±2.598   | 22.94±0.560            |
| Agholane Gichka     | 3.33±0.038d| 0.00   | 31.75  | 68.25  | S L           | 7.84±0.116 | 65±0.577    | 77.27±0.079            |
| Jidea Zab           | 2.77±0.038b| 39.49  | 42.12  | 18.38  | Si CL         | 7.74±0.116 | 20±0.577    | 47.38±0.397            |
| Girdarasha          | 5.73±0.038c| 42.43  | 39.78  | 17.79  | CL            | 7.75±0.145 | 28±0.289    | 32.43±0.412            |
| Khabat              | 4.25±0.534a| 52.22  | 23.50  | 24.28  | C             | 7.53±0.203 | 75±0.866    | 31.56±0.452            |
| Chaluke Gawra       | 4.75±0.145a| 41.56  | 36.36  | 22.08  | C             | 7.39±0.003 | 27±0.577    | 26.39±0.401            |
| Tobzawa             | 2.24±0.173b| 9.67   | 48.34  | 41.99  | C             | 8.30±0.029 | 39±1.155    | 44.50±0.144            |
| Oil Refinery        | 0.84±0.116c| 40.34  | 32.78  | 26.89  | C             | 8.24±0.173 | 21±0.289    | 15.04±0.301            |
| Control             | 10.26±0.116a| 17.87  | 33.19  | 48.93  | L             | 8.38±0.006 | 66±0.577    | 16.63±0.471            |

Different letters means significant differences between the studied sites.

Figure 2: Correlation between: soil organic matter and enzymatic activities: (A) Dehydrogenase, (B) Urease and (C) Catalase.

3.2. Soil oil content

It is for a long time that oil materials and its derivatives cause soil pollution as a result of transportation or storage. Oil pollution results from rapid population growth and industrialization process vastly around
exploration and refining installations typically via transfer of oil materials (Khakbaz et al., 2012). Results of oil residues of the studied soils were tabulated in Table 2. The highest oil content was 2.6±0.000 µg.Kg\(^{-1}\) in Oil Refinery soil and this come in agreement with a study conducted by Khudhur and Abdulla (2016) who found the highest oil residue 0.0022 mg.Kg\(^{-1}\) five kilometers away from Kawrgosk Oil Refinery and this may refer to the direct emission of some pollutants and dusts of burning gases along with oil into such area (Khakbaz et al., 2012).

### Table 2: Levels of oil residues (µg.kg\(^{-1}\)) and heavy metals: Fe, Ni, As and Cd (g.kg\(^{-1}\)) expressed as (mean ± S.E.) in the studied sites.

| Sites                  | Oil residues | Fe         | Ni         | As          | Cd       |
|------------------------|--------------|------------|------------|-------------|----------|
| Shewarash Zab           | 0.9±0.000    | 26.60±0.173 \(abc\) | 0.108±0.001 \(abc\) | 0.173±0.001 \(abc\) | 0.221±0.001 \(abc\) |
| Agholane Gawra          | 0.8±0.000    | 27.05±0.015 \(bc\) | 0.112±0.001 \(abcde\) | 0.214±0.001 \(c\) | 0.223±0.001 \(bc\) |
| Kawrgosk                | 0.8±0.000    | 26.35±0.245 \(d\) | 0.108±0.001 \(d\) | 0.176±0.001 \(b\) | 0.226±0.002 \(ab\) |
| Gaenj                   | 1.1±0.000    | 22.80±0.520 \(bc\) | 0.117±0.001 \(ab\) | 0.197±0.001 \(f\) | 0.210±0.000 \(cd\) |
| Agholane Gichka         | 1.2±0.000    | 23.00±0.289 \(f\) | \textbf{0.119±0.001} \(a\) | 0.257±0.001 \(c\) | 0.209±0.001 \(cd\) |
| Jieda Zab               | 1.2±0.000    | 21.60±0.173 \(f\) | 0.106±0.002 \(bc\) | \textbf{0.161±0.000} \(a\) | 0.214±0.001 \(bc\) |
| Girdarasha              | 1.3±0.000    | 26.70±0.491 \(d\) | 0.109±0.000 \(d\) | 0.215±0.001 \(e\) | 0.217±0.002 \(d\) |
| Khbat                   | 0.8±0.000    | 23.70±0.491 \(d\) | 0.109±0.001 \(d\) | 0.218±0.001 \(d\) | 0.213±0.001 \(de\) |
| Chaluke Gawra           | 1.1±0.000    | 29.00±0.289 \(bc\) | 0.114±0.001 \(cd\) | 0.262±0.001 \(c\) | 0.226±0.002 \(ab\) |
| Tobzawa                 | 0.7±0.000    | 25.60±0.462 \(c\) | 0.118±0.003 \(b\) | 0.272±0.001 \(b\) | 0.218±0.002 \(b\) |
| Oil Refinery            | \textbf{2.6±0.000} | \textbf{31.50±0.433} | 0.116±0.002 \(bc\) | \textbf{0.300±0.003} \(a\) | \textbf{0.227±0.002} \(ab\) |
| Control                 | 0.2±0.000    | 6.25±0.072 \(b\) | 0.085±0.002 \(bc\) | 0.185±0.001 \(f\) | 0.165±0.001 \(f\) |

* Different letters means significant differences between the studied sites.

#### 3.3. Soil heavy metals

Soil texture represents abiotic factor and one of the most important factors that influences the distribution of organic matter and ultimately play decisive role in retention of heavy metals in soil ecosystem (Sethi and Gupta, 2014). Soil texture classes were: loamy in Shewarash Zab, Gaenj, Tobzawa and Control soils; silty loam in Agholane Gawra, Kawrgosk and Agholane Gichka soils; silty clay loam in Jideda Zab; clayey loam in Girdarasha soil and clayey in Khabat, Chaluke Gawra and Oil Refinery soils (Table 1).

The anthropogenic processes results to soil contamination with heavy metal are numerous among them refining process which releases numerous different metals into the atmosphere and have locally increased the levels of Cd, Co, Cr, Pb, As and Ni in soil up to dangerous levels (Ross, 1994). Consequently, there are substantial air pollution emissions, and a notable odor normally accompanies the presence of a refinery (Sharma and Agrawal, 2005). From the present study, the highest Fe level of 31.50±0.433 g.Kg\(^{-1}\) was obtained in Oil Refinery, while the lowest level of 6.25±0.072 g.Kg\(^{-1}\) was obtained in control (Table 2). Ni was ranged between 0.085±0.002 and 0.119±0.001 g.Kg\(^{-1}\) in the soils of Control and Agholane Gichka respectively. However, As was highest in Oil Refinery soil by a value of 0.300±0.003 g.Kg\(^{-1}\) and lowest in Jieda Zab soil by a value of 0.161±0.000 g.Kg\(^{-1}\), as well as the highest Cd content 0.227±0.002 g.Kg\(^{-1}\) was observed in Oil Refinery while the lowest Cd content 0.165±0.001 g.Kg\(^{-1}\) was in control soil. The anthropogenic sources of soil contamination by arsenic and cadmium can be derived from: metalliferous mining and smelting, industry, atmospheric deposition, agriculture and waste disposal (Rasheed and Saleh, 2016).

#### 3.4. Soil microorganisms

In soil environment, changes in microbial populations can precede detectable changes in the soil’s physicochemical properties. The impact of some chemicals on soil health is dependent on microbial activities. For example,
the concentration of heavy metals in soil will not change over small time periods, but their bioavailability may. In this way, soil enzymes are acting as important indicators of soil (Das and Varma, 2011). Moreover, fungal communities in the soil are an important component because of their participation in regulating microbial activities in polluted soils (Pečiulytė and Volodkienė, 2009). Table 3 shows that the maximum total bacterial population was in Agholane Gichka by a count of 24.81x10^7 cfu.g^-1 dry soil and the minimum population was 0.04x10^7 cfu.g^-1 dry soil in Oil Refinery soil and total fungal population was ranged between 2 – 39 x10^3 cfu.g^-1 dry soil in both Oil Refinery and Kawrgosk soils respectively as the same results observed by Khudhur and Abdulla (2016) and this may refer to the least amount of total organic matter in this area (table 1) which affect fungal population and diversity.

| Sites            | Total bacteria (x10^7 cfu.g^-1 dry soil) | Total fungi (x10^3 cfu.g^-1 dry soil) | Dehydrogenase (TPF µg.g^-1.24h^-1) | Urease (µg N-NH_4^+ g^-1.3h^-1) | Nitrate reductase (µg N-NO_2^- g^-1.3h^-1) | Catalase (ml KMnO_4 g^-1.20min^-1) |
|------------------|----------------------------------------|--------------------------------------|-----------------------------------|---------------------------------|-------------------------------------------|----------------------------------|
| Shewarash Zab    | 1.24                                   | 13                                   | 0.823                             | 293.76                          | 3.660                                     | 0.70                             |
| Agholane Gawra   | 2.59                                   | 12                                   | 45.602                            | 396.96                          | 3.712                                     | 0.80                             |
| Kawrgosk         | 0.72                                   | 39                                   | 85.562                            | 302.64                          | 3.254                                     | 0.95                             |
| Gaenj            | 1.42                                   | 20                                   | 0.422                             | 258.00                          | 3.226                                     | 0.85                             |
| Agholane Gichka  | 24.81                                  | 11                                   | 157.651                           | 356.16                          | 3.126                                     | 0.95                             |
| Jieda Zab        | 2.68                                   | 5                                    | 2.229                             | 162.00                          | 4.658                                     | 0.70                             |
| Girdarasha       | 1.87                                   | 11                                   | 1.787                             | 236.88                          | 3.916                                     | 0.50                             |
| Khabat           | 1.82                                   | 16                                   | 2.189                             | 186.24                          | 3.162                                     | 0.65                             |
| Chaluke Gawra    | 0.58                                   | 24                                   | 1.586                             | 217.44                          | 3.738                                     | 0.80                             |
| Tobzawa          | 2.22                                   | 5                                    | 1.386                             | 216.24                          | 3.688                                     | 1.00                             |
| Oil Refinery     | 0.04                                   | 2                                    | 0.020                             | 179.28                          | 3.623                                     | 0.85                             |
| Control          | 13.96                                  | 15                                   | 124.116                           | 205.44                          | 3.833                                     | 0.90                             |

3.5. Soil enzyme activity

By the present study, activity of dehydrogenase ranged from high to low levels in the research sites (table 3). Maximum dehydrogenase activity was 157.651 TPF µg.g^-1 dry soil.24h^-1 in Agholane Gichka and the minimum activity was 0.020 TPF µg.g^-1 dry soil.24h^-1 in Tobzawa soil. Since, dehydrogenase is an enzyme that is particularly sensitive to the action of toxic compounds and it can indicate type and significance of pollution in soils (Nwaogu et al., 2012). Therefore, in our studies, the low activity of dehydrogenases in the soils is an indicator of decreased microbiological activity in the environment.

Urease enzyme is responsible for hydrolysis of urea fertilizers applied to soil into NH_3 and CO_2 with the concomitant rise in soil pH. Soil urease originates mainly from plants and microorganisms found as both intra- and extra-cellular enzymes (Uzun and Uyanoz, 2011). As shown in table 3, the least activity of urease was in Jieda Zab by a level of 162 µg N-NH_4^+ g^-1 dry soil.3h^-1, while the highest activity of urease was observed in Agholane Gawra soil which was 396.96 µg N-NH_4^+ g^-1 dry soil.3h^-1 and this result come in agreement with the observation of Płociniak (2009).

Nitrate reductase activity of soil is the product of microbial secretion to its nearest soil particles. It is useful for maintaining nitrogen ratio in atmosphere as well as removal of hazardous nitrate compounds of soil (Nath and Samanta, 2012). Lowest nitrate reductase activity 3.126 µg N-NO_2^- g^-1 dry soil.3h^-1 was detected in Agholane Gichka and the greatest activity was 4.658 µg N-NO_2^- g^-1 dry soil.3h^-1 in Jideda Zab soil and this may refer to the
activity of anaerobic microbial population in this area.

Catalase activities were ranged between 0.5 – 1 ml KMnO₄.g⁻¹.20 min⁻¹ in both Girdarasha and Tobzawa soils respectively. Soil catalase activity shows a significant correlation with organic carbon content, soil microbial biomass, oxygen consumption, carbon dioxide evolution and dehydrogenase activity (Nwaogu et al., 2012).

The observed increase in soil enzymatic activity was connected with the relatively high content of organic matter and microbial population in the soil (tables 2 and 3 and figures 2, 3 and 4). The correlation between total bacterial population and organic matter was significantly positive when r value was 0.582 at (p<0.05) and the correlation between total bacteria and dehydrogenase enzyme activity was highly positive when r value was 0.863 at (p<0.01). In this regard, Płóciniak (2009) demonstrated that the activity level of enzymes in the soil is closely depends on the presence of carbon substrates, as well as dehydrogenase activity has been referred as indirect indicator of the number and activity of soil bacterial and fungal populations (Brzezinska, 2006). Jezierska et al. (2004) reported significant correlations between dehydrogenase activity, organic carbon content and crop yields in light and medium-heavy textured soils.

### Table 4: Person’s correlation among: physicochemical, microbial population and enzymatic activities in the studied soils.

|                | Total bacteria | Total fungi | Dehydrogenase | Urease | Nitrate reductase | Catalase |
|----------------|----------------|-------------|---------------|--------|------------------|----------|
| Moisture       | 0.374          | 0.081       | 0.382         | -0.177| 0.199            | -0.193   |
| pH             | 0.096          | -0.358      | 0.132         | 0.116  | 0.048            | 0.362    |
| EC             | 0.041          | 0.386       | 0.080         | 0.241  | -0.558           | 0.214    |
| Organic matter | 0.604          | -0.036      | 0.483         | 0.372  | -0.127           | 0.254    |
| Fe             | -0.522         | -0.021      | -0.536        | 0.159  | -0.141           | -0.172   |
| Ni             | -0.154         | -0.130      | -0.321        | 0.251  | -0.347           | 0.128    |
| As             | 0.086          | -0.367      | -0.082        | -0.094| -0.270           | 0.298    |
| Cd             | -0.560         | 0.073       | -0.528        | 0.184  | -0.085           | -0.152   |
| Total bacteria | 1              | -0.129      | 0.864**       | 0.331  | -0.227           | 0.364    |
| Total fungi    | -0.128         | 1           | 0.244         | 0.273  | -0.445           | 0.181    |
| Oil residues   | -0.212         | -0.387      | -0.336        | -0.223| 0.044            | -0.140   |
| Dehydrogenase  | 0.864**        | 0.244       | 1             | 0.478  | -0.313           | 0.515    |
| Urease         | 0.333          | 0.273       | 0.478         | 1      | -0.415           | 0.234    |
| Nitrate reductase | -0.227       | -0.445      | -0.313        | -0.415| 1                | -0.357   |
| Catalase       | 0.364          | 0.181       | 0.515         | 0.234  | -0.357           | 1        |

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).
Figure 3: Correlation between: total bacterial population and enzymatic activities: (A) Dehydrogenase, (B) Urease and (C) Catalase.

Figure 4: Correlation between: total fungal population and enzymatic activities: (A) Dehydrogenase, (B) Urease and (C) Catalase.
Moreover, we found a negative correlation among the studied heavy metals with dehydrogenase and nitrate reductase in the studied area because enzymes are characterized by high susceptibility to unfavorable environmental conditions particularly contents of heavy metals as stated by Płóciniak (2009). More findings in our study are the negative correlations among oil residues with microbial population, dehydrogenase, urease and catalase activities (Figure 5). Hawrot et al. (2005) recorded regardless of pollution rate, higher dehydrogenase activity in sandy soils than loamy soil and this may confirm our finding as shown in tables 1 and 3 regarding to the soils of Agholane Gawra, Kawrgosk and Agholane Gichka which has the higher sand content 63.77%, 69.29% and 68.25% respectively.

Figure 5: Correlation between: oil residue and enzymatic activities: (A) Dehydrogenase, (B) Urease, (C) Nitrate reductase and (D) Catalase.

4. CONCLUSIONS

The following were concluded by this study:

1. Soil content of oil residues and heavy metals Fe, Ni, As and Cd, as well as microbial population and the activity of the examined soil enzymes, showed considerable variability depending on the intensity of exerted anthropogenic pressure.
2. High inactivation of the examined enzymes particularly dehydrogenase in soils exposed to higher anthropogenic influence (area of Oil Refinery) indicates that the contamination of the soil environment by oil residues and heavy metals reached levels that reduce microbial population.
3. Influence of oil residue and heavy metals on microbial population and enzymatic activity is related to soil physicochemical properties.
4. A significant correlation between dehydrogenase activity and total bacteria, and the content of organic matter and total bacteria was confirmed.
5. With the increase of oil residue and heavy metals content of the soils, the enzymatic activity was decreased.

6. Dehydrogenase has proven to be the best indicator for estimating the drop of microbial activity in such soils.

7. Measuring soil enzymatic activities can provide information about the function and structure of soil microbial communities in contaminated soils.

5. RECOMMENDATION

1. Conducting further studies on determining total hydrocarbons and polycyclic aromatic hydrocarbons in different depths of soil in these areas and determining their effects on soil biochemical properties.

2. Determining these pollutants from air and effluents of Oil Refinery.

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