

Activity and Thermodynamic Parameters of Urease Enzymes in Soils Treated with Some Heavy Metals Under Different Temperatures and Moisture Levels

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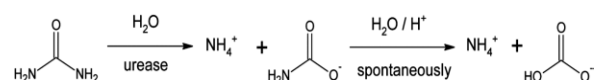
Abstract : Incubation studies were conducted to reveal effected heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) add at critical concentrations to soils with different texture on urease activity and thermodynamic parameters (Ea and Q₁₀) incubated under different temperatures (10, 20, 30, 40, 50, 60 and 70) °C for 14 days under field capacity and waterlogged moisture levels. Urease activity was measured and thermodynamic parameters were calculated. Results showed that the urease activity increased with increasing temperature of incubation from 10 to 50°C then the activity decreased as temperature increased above 50°C at both moisture levels and at all heavy metals treatments. Increasing moisture level from field capacity to water-logged significantly (P=0.05) decreased urease activity, while increased Ea value, at all heavy metals treatments. The soil texture significantly affected urease activity and thermodynamic parameters (Ea and Q₁₀). Results also indicated that effect of heavy metals on urease and thermodynamic parameters differed according to the soil temperature and the moisture level.

Key words: Urease, Ea, Q₁₀, Heavy metals, Moisture levels soil.

Introduction

Determination of enzyme activity is essential to determine the biological activity of the soil. Soil enzymes stimulate the biochemical processes of the decomposition of organic matter in soils (Hang *et al.*, 2013). Source of urease enzyme in the soil is living and dead microorganisms such as bacteria, fungi, algae, invertebrates, plant residues, and plant roots and it can be found in soil in as of external enzymes like most soil enzymes (Follmer, 2008). Urease stimulates the hydrolysis of

urea to ammonia and carbon dioxide with high efficiency about 10¹⁴ times higher than non-enzymatic reaction (Maroney & Ciurli, 2013; Cordero *et al.*, 2019).



Oliveira & Pampulha (2006) showed that the activity of urease enzyme in the soil is influenced by several factors, including organic matter content, soil depth, soil management, soil pH values, the concentration of subject matter, soil moisture, temperature, and heavy metals.

Contamination of heavy metals seriously adversely affects the natural environment, including reduce enzyme activity in the soil, and the activity of urease enzyme is negatively associated with heavy metals in the soil (Meng *et al.*, 2018). Krajewska (2009) reported that heavy metals inhibit urease activity by forming bonds with sulfhydryl groups in the active site of the enzyme and have taken the following sequence in inhibiting urease activity: $Ag^+ = Hg^{2+} > Cu^{2+} > Ni^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+} > Fe^{3+} > Pb^{2+} > Mn^{2+}$. Enzymatic activity decreases immediately after the introduction of heavy metals into the soil and over time the activity returns to its original level (Ciarkowska & Gambus´ 2004). Soil enzymatic activity is a sensitive indicator of natural and human changes in the ecosystem, which is used to assess the impact of various pollutants including heavy metals in the soil in a long and/or short period time (Ciarkowska *et al.*, 2014). Yang *et al.* (2006) reported that the activity of urease enzyme increases with increasing temperature. Kumari & Rao (2017) reported that the activity of urease enzyme increased by increasing the temperature from 20 to 70°C and then decreased dramatically as the temperature increased to 90°C. Machuca *et al.* (2015) and Al-Ansari (2000) stated that Urease activity increases with increasing temperature from 10 to 50°C. Fraser *et al.* (2013) explained as well the significant correlation between urease activity and temperature with activation energy (Ea) of 73.4 kJmol⁻¹. Al-Jabri (2010) found that increasing the temperature from 10 to 60 °C increased urease activity in seven soils with different properties, and Q₁₀ value decreased as temperature increased. Al-Ansari *et al.*

(2019) also reported that Q₁₀ of urease enzymes increased as temperature increased. Dick & Tabatabai (1999) noted that the optimal water content of enzyme activity in soils is at the field capacity. Zhang *et al.* (2016) revealed that Water-logging reduced soil enzyme activity and soil microbial biomass. Gu *et al.* (2019) reported that the urease activity in a soil in immersed for 3, 6 or 9 days decreased as compared to that of the aerated soil. Moreover, the results indicated as immersing period increased, the negative effect on the urease activity increased. Little information is available on the effect of soil temperature and moisture on the role of heavy metals in the activity and thermodynamic parameters of the urease enzymes in the soil of the southern region of Iraq, hence this study was conducted.

Materials & Methods

Soil samples from three locations differ in their agricultural status located at southern part of Iraqi, Basrah province (table1) were collected from depth of 0-30 cm. These soils were silty clay classified (fine silty, mixed, active, calcareous, hyperthermic, typic torrifluven), silty clay loam classified (fine clayey, mixed, active, calcareous, hyperthermic, typic torrifluven) and loamy sand classified (sandy, mixed, active, calcareous, hyperthermic, typic torripasmments). Collected samples were kept in a refrigerator (4°C) for urease enzymes measure. Sub-samples were air dried, grounded and passed through 2mm sieve. Some physical and chemical properties of the soil were determined following standard procedures described by page *et al.* (1982) and presented in table (1).

Table (1): Some chemical, physical and biological properties of studied soils.

Soil	Silty clay	Silty clay loam	Loamy Sand
pH	7.83	7.59	7.82
ECe (dS m ⁻¹)	11.86	8.67	4.96
Organic C (gm kg ⁻¹)	2.04	1.93	1.50
Organic matter (gm kg ⁻¹)	3.46	3.28	2.55
CaCO ₃ (gm kg ⁻¹)	396	342	279
Total N (gm kg ⁻¹)	4.22	3.64	1.21
Urease activity μg N-NH ₄ ⁺ gm ⁻¹ soil 2 hrs ⁻¹	112	80.5	56

100 g (on air dry bases) of each soil was placed in containers and treated with critical

concentration of Cr, Cd, Pb, Mn, Cu, Fe, Zn and Ni (table 2) (Kabata-Pendias & Pendias, 2001).

Table (2): The critical concentrations of heavy metals in ppm added to the soil according to (Kabata-Pendias & Pendias, 2001).

Element	Critical concentration
Cd	3
Cr	100
Cu	100
Fe	200
Mn	100
Ni	50
Pb	100
Zn	300

Untreated soils were used as control, the soil moisture of all treatments were adjusted to either field capacity (F.C.) or water-logged using distilled water. Samples were incubated at 30°C for 14 days, then urease activity was determined. Desired moisture levels of incubated samples were maintained by periodic weighting of the containers. Urease activity of samples was determined following procedure of Tabatabai & Bremner (1972). Five grams of amended and control soils were incubated with 9 ml of 0.05M pH 9 tris (THAM) buffer, 0.2 ml of toluene, and 1 ml of 0.2M substrate (urea) solution at 37°C for 2 hours. After incubation, urea was inhibited by addition of KCl-Ag₂SO₄ solution, then NH₄⁺-N released was determined by

distillation procedure. Thermodynamic parameters were calculated from the results obtained at 0.6M urea. The activation energy (Ea) of urease in soils was calculated from enzymes activity obtained at 0.6M urea but temperatures of incubation varied from 10°C to 60°C. Ea values were calculated using Arrhenius equation plot of log K against 1/T (Tabatabai, 1994).

$$K = A \cdot \exp(-Ea/RT) \dots\dots(1)$$

Where A: pre exponential factors, Ea: energy of activity, R: gas constant, T: temperature (kelvin degree).

$$\text{Log } K = (- Ea/2.303 RT) + \text{Log } A$$

The temperature coefficient (Q10) was calculated by the formula of Frankenberger & Tabatabai (1983).

$$Q_{10} = \exp \frac{10000 E_a}{8.314 T(T + 10)}$$

The study was carried as factorial experiments with three replicates in complete randomized design. Data were analyzed by two-way analysis of variance (ANOVA) using GenStat program. Least significance difference (LSD) calculated for treatments means at 5% probability.

Results

Temperature effects on urease enzyme activity

Fig. (1) demonstrated increase in urease enzyme activity by increasing the incubation temperature from 10 to 50°C, but increasing incubation temperature more than 50°C resulted in a significant decrease in the enzyme activity in all studied soils, at both moisture levels and all studied heavy metals.

Fig. (1) also

illustrated that increasing moisture levels from field capacity to waterlogging conditions decreased urease activity in soils treated with different elements and incubated at different temperatures. Urease activity in silty clay was higher than the silty clay loam soil and sandy soil which showed lower urease activity under all treatments. Urease activity in control soils were higher than the activity in soils treated with heavy metals under both moisture levels (fig1). The negative effect of heavy metals on urease activity was in order of Zinc > Cadmium > Iron > Lead > Chromium > Copper > Manganese > Nickel under field capacity conditions, however under waterlogged condition the effect follows the order Zinc > Iron > Cadmium > Manganese > Lead > Copper > Chromium > Nickel..

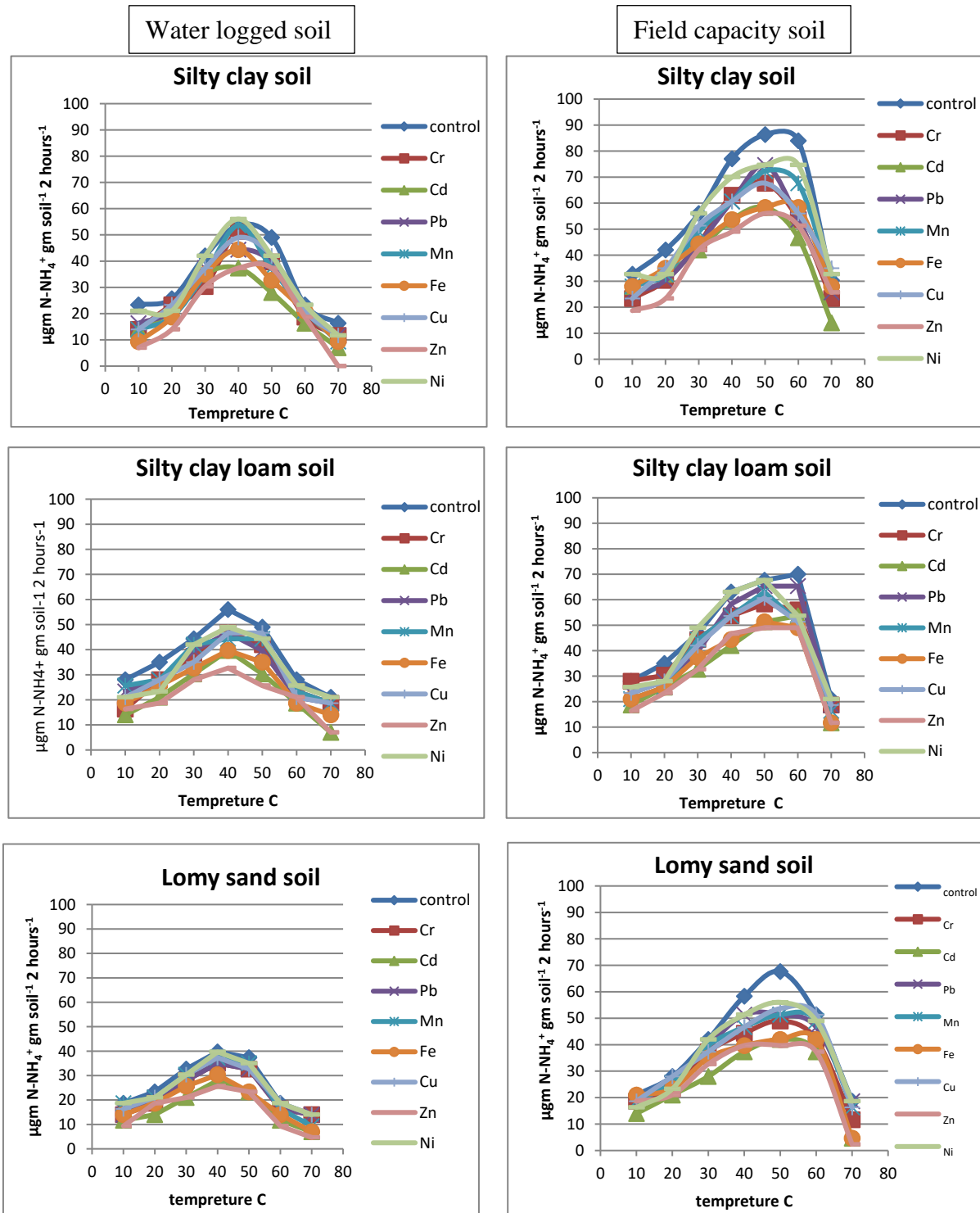


Fig. (1): Effect of temperature (°C) on urease activity of studied soils treated with heavy metals at two moisture levels.

Thermodynamic of urease enzymes: activation energy (Ea) and temperature coefficient (Q₁₀)

Fig. (2) showed a linear relationship between the logarithm of the ammonium amount resulting from the activity of urease enzyme and temperatures of 10-60 °C in all studied soils treated with different heavy metals and incubated at field capacity and water logged conditions and for all heavy metals. The Ea values of urease in the studied soils were calculated from the negative slope of the straight line (Tables 3 & 4).

Table (4) indicated that there is a significant difference in the values of Ea urease enzymes in all studied soils. Ea values ranged from 10.39 kJ mol⁻¹ in silty clay soil treated with Cd to 19.07 kJ mol⁻¹ in loamy sand soil treated with Ni under soil field capacity conditions and 11.36 kJ mol⁻¹ in silty clay loam soil treated with Zn to 33.36 kJ mol⁻¹ in silty clay soil treated with Zn under soil waterlogging conditions.

The data of table (4) revealed that Ea of urease enzymes (as average) in silty clay loam soil did not differ significantly from that of loamy sand soil, but both were higher than Ea value of silty clay soil when soils incubated under field capacity moisture level. However, when soil samples incubated under water logged conditions, Ea values were in order of silty clay > Loamy sand > silty clay loam.

Table (4) demonstrated that effect of heavy metals (as average) on Ea values was in order of: Nickel > Lead > Zinc > Manganese > Copper > Cadmium > Chromium > Iron. under field capacity conditions, but under waterlogged conditions the order was: Zinc > Chromium > Copper > Cadmium > Iron > Nickel > Manganese > Lead. The data of Table (5) displayed that the values of Q₁₀ of

urease enzymes ranged from 1.122 in silty clay loam treated with Zn at 20°C to 1.622 in silty clay soil treated with Zn at 10°C under field capacity conditions and from 1.111 in silty clay soil treated with Cr at 10°C to 1.319 in loamy sand soil treated with Ni under soil waterlogging conditions incubated at 10°C . Data of table5 indicated that Q₁₀ values (as average) were in order: silty clay soil > Loamy sand soil = silty clay loam soil. Under field capacity condition. However, no significant differences were recorded among Q₁₀ (as average) of soils under study.

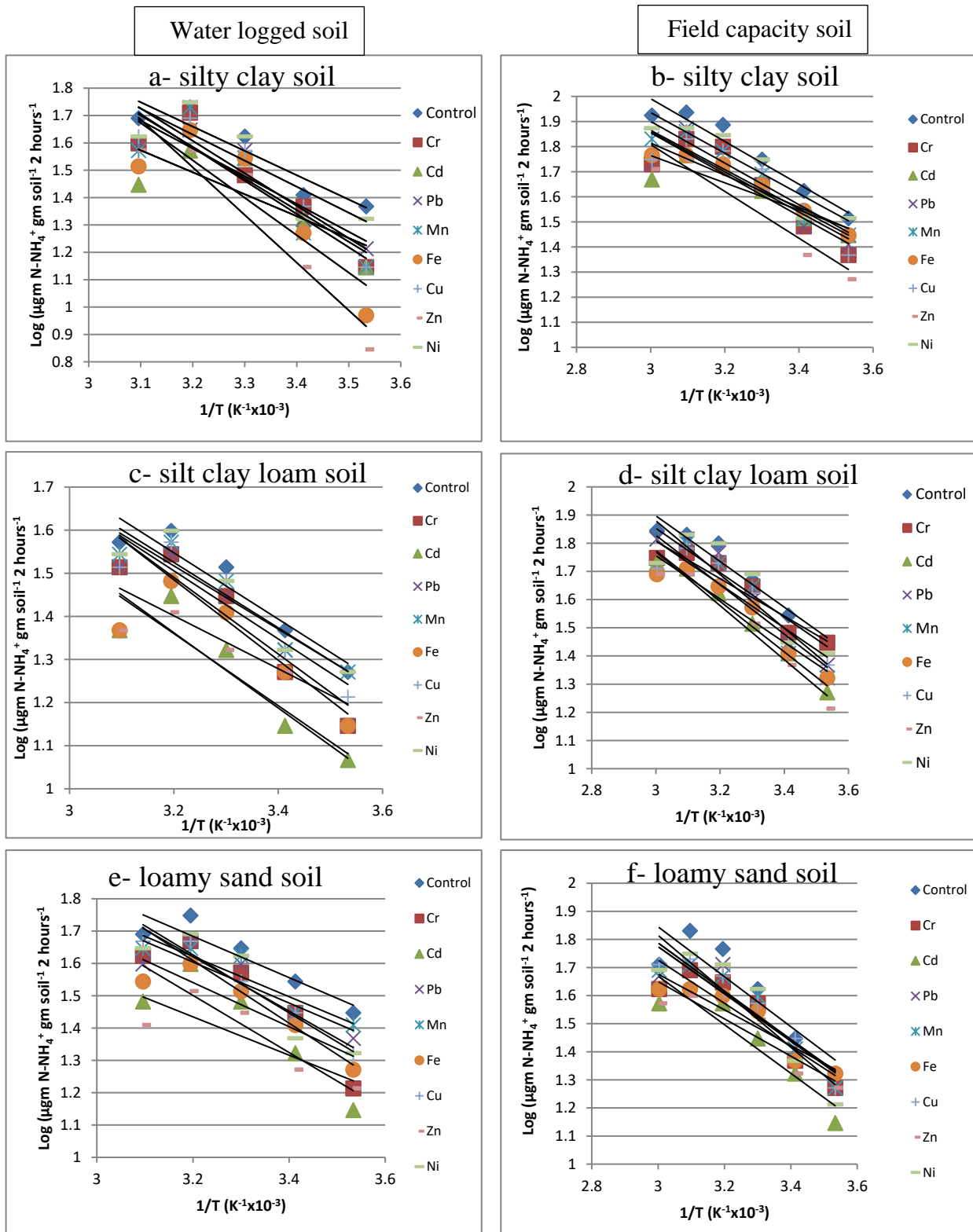


Fig. (2): The linear relationship between 1/T and log NH₄⁺ released of urease activity of studied soils treated with heavy metals on two moisture levels.

Table (3): Straight-line equation and coefficient of determination (r) for relationship between temperature and urease activity by ($\mu\text{g N-NH}_4^+$ gm soil⁻¹ 2 hor⁻¹).

Field capacity soil						
Treatments	silty clay soil		silty clay loam soil		loamy sand soil	
	Equation	r	Equation	r	Equation	r
Control	$y = -0.8517x + 4.5459$	0.968	$y = -0.8103x + 4.3281$	0.973	$y = -0.8887x + 4.511$	0.899
Cr	$y = -0.8271x + 4.3363$	0.894	$y = -0.6651x + 3.8028$	0.944	$y = -0.7705x + 4.038$	0.904
Cd	$y = -0.5428x + 3.3927$	0.870	$y = -0.8968x + 4.4637$	0.989	$y = -0.866x + 4.2674$	0.936
Pb	$y = -0.7987x + 4.2551$	0.887	$y = -0.9524x + 4.7368$	0.969	$y = -0.8273x + 4.2559$	0.904
Mn	$y = -0.8289x + 4.3869$	0.960	$y = -0.8768x + 4.4585$	0.906	$y = -0.8416x + 4.2991$	0.936
Fe	$y = -0.6387x + 3.73$	0.969	$y = -0.7686x + 4.0616$	0.959	$y = -0.635x + 3.5814$	0.939
Cu	$y = -0.7824x + 4.2121$	0.886	$y = -0.7744x + 4.1322$	0.919	$y = -0.8593x + 4.3651$	0.955
Zn	$y = -0.9325x + 4.6055$	0.928	$y = -0.9581x + 4.6444$	0.957	$y = -0.6636x + 3.6407$	0.903
Ni	$y = -0.8079x + 4.359$	0.933	$y = -0.7869x + 4.2137$	0.868	$y = -0.996x + 4.8025$	0.895
Water logged soil						
Treatments	silty clay soil		silty clay loam soil		loamy sand soil	
	Equation	r	Equation	r	Equation	r
Control	$y = -0.8889x + 4.504$	0.930	$y = -0.6395x + 3.7306$	0.921	$y = -0.7694x + 4.0097$	0.948
Cr	$y = -1.1553x + 5.2822$	0.918	$y = -0.9695x + 4.7117$	0.916	$y = -0.933x + 4.4704$	0.948
Cd	$y = -0.8018x + 4.0585$	0.791	$y = -0.8843x + 4.331$	0.872	$y = -0.8355x + 4.0336$	0.912
Pb	$y = -1.0147x + 4.8267$	0.918	$y = -0.6348x + 3.6357$	0.884	$y = -0.8879x + 4.3317$	0.942
Mn	$y = -1.216x + 5.4747$	0.884	$y = -0.6225x + 3.6136$	0.924	$y = -0.7335x + 3.8643$	0.942
Fe	$y = -1.3679x + 5.9137$	0.870	$y = -0.6834x + 3.7279$	0.912	$y = -0.614x + 3.366$	0.814
Cu	$y = -1.1856x + 5.4014$	0.923	$y = -0.8426x + 4.3173$	0.977	$y = -0.79x + 4.0337$	0.920
Zn	$y = -1.7424x + 7.0865$	0.942	$y = -0.5933x + 3.3333$	0.819	$y = -0.8729x + 4.1553$	0.865
Ni	$y = -0.9511x + 4.6736$	0.845	$y = -0.8977x + 4.4993$	0.906	$y = -0.7583x + 3.9515$	0.927

Table (4): Ea values (KJ mal⁻¹) of urease in studied soils treated with heavy metals on two moisture levels.

Treatmen t.	Ea values (KJ mal ⁻¹)							
	Field capacity				Water-logged			
	Silty clay soil	Loamy clay soil	Loamy sand soil	Average	Silty clay soil	Loamy clay soil	Loamy sand soil	Average
Control	16.31	15.51	17.02	16.28	17.02	12.24	14.73	14.66
Cr	15.84	12.73	14.75	14.44	22.12	18.56	17.86	19.51
Cd	10.39	17.17	16.58	14.71	15.35	19.93	16.00	17.09
Pb	15.29	18.24	15.84	16.45	19.43	12.15	17.00	16.19
Mn	15.87	16.79	16.11	16.26	23.28	11.92	14.04	16.41
Fe	12.23	14.72	12.16	13.04	26.19	13.09	11.76	17.01
Cu	14.98	14.83	16.45	15.42	22.70	16.13	15.13	17.99
Zn	17.85	18.34	12.71	16.30	33.36	11.36	16.71	20.47
Ni	15.47	15.07	19.07	16.54	18.21	17.19	14.52	16.64
Average	14.91	15.93	15.63		21.96	14.73	15.31	

LSD heavy metals = 0.162, soil = 0.408 , moisture level = n.s , heavy metals* soil = 0.429 , heavy metals* moisture level = 2.156 , moisture level * soil = 2.642 , heavy metals* soil* moisture level = 2.646.

Table (5): Q₁₀ values of urease in heavy metals contaminated studied soil.

Q ₁₀ values - Field capacity soil																		
Treat.	Silty clay soil						Silty clay Loam soil						Loamy sand soil					
	10	20	30	40	50	Aver.	10	20	30	40	50	Aver.	10	20	30	40	50	Aver.
Contr.	1.280	1.188	1.241	1.224	1.210	1.229	1.194	1.132	1.168	1.157	1.147	1.160	1.238	1.160	1.205	1.192	1.179	1.195
Cr	1.378	1.250	1.324	1.301	1.281	1.307	1.309	1.206	1.265	1.247	1.231	1.252	1.296	1.198	1.254	1.237	1.221	1.241
Cd	1.249	1.168	1.215	1.200	1.187	1.204	1.278	1.187	1.240	1.223	1.209	1.227	1.261	1.175	1.225	1.210	1.196	1.213
Pb	1.326	1.217	1.279	1.260	1.243	1.265	1.193	1.131	1.167	1.156	1.146	1.158	1.280	1.187	1.241	1.224	1.209	1.228
Mn	1.402	1.265	1.344	1.319	1.297	1.325	1.189	1.128	1.163	1.152	1.143	1.155	1.226	1.152	1.195	1.182	1.170	1.185
Fe	1.462	1.303	1.394	1.366	1.340	1.373	1.209	1.141	1.181	1.168	1.158	1.171	1.186	1.126	1.161	1.150	1.141	1.153
Cu	1.390	1.258	1.334	1.310	1.289	1.316	1.264	1.177	1.227	1.212	1.198	1.215	1.245	1.165	1.212	1.197	1.184	1.201
Zn	1.622	1.401	1.527	1.487	1.452	1.498	1.179	1.122	1.155	1.145	1.136	1.147	1.274	1.184	1.236	1.220	1.206	1.224
Ni	1.302	1.202	1.260	1.242	1.226	1.246	1.283	1.190	1.244	1.227	1.212	1.231	1.234	1.158	1.202	1.189	1.176	1.192
Aver.	1.379	1.305	1.324	1.301	1.281		1.092	1.233	1.201	1.177	1.175		1.249	1.167	1.215	1.200	1.187	
Q ₁₀ values – water-logged soil																		
Treat.	Silty clay soil						Silty clay Loam soil						Loamy sand soil					
	10	20	30	40	50	Aver.	10	20	30	40	50	Aver.	10	20	30	40	50	Aver.
Contr.	1.267	1.174	1.222	1.214	1.200	1.215	1.252	1.170	1.218	1.203	1.190	1.206	1.280	1.188	1.241	1.274	1.210	1.238
Cr	1.258	1.111	1.141	1.207	1.194	1.182	1.203	1.137	1.175	1.164	1.153	1.166	1.239	1.161	1.206	1.224	1.179	1.202
Cd	1.163	1.167	1.214	1.132	1.123	1.160	1.283	1.189	1.243	1.227	1.212	1.231	1.272	1.182	1.234	1.192	1.204	1.217
Pb	1.248	1.174	1.223	1.200	1.187	1.206	1.303	1.202	1.260	1.242	1.226	1.247	1.258	1.174	1.223	1.218	1.194	1.213
Mn	1.259	1.132	1.168	1.208	1.194	1.192	1.276	1.185	1.237	1.221	1.207	1.225	1.263	1.177	1.227	1.207	1.198	1.214
Fe	1.194	1.163	1.209	1.157	1.147	1.174	1.238	1.160	1.205	1.191	1.179	1.195	1.193	1.131	1.167	1.211	1.146	1.169
Cu	1.243	1.198	1.254	1.195	1.182	1.214	1.240	1.162	1.207	1.193	1.180	1.196	1.270	1.181	1.232	1.156	1.202	1.208
Zn	1.296	1.169	1.217	1.237	1.221	1.228	1.305	1.204	1.262	1.244	1.228	1.248	1.202	1.137	1.175	1.216	1.153	1.177
Ni	1.252	1.179	1.230	1.202	1.189	1.210	1.244	1.164	1.211	1.196	1.184	1.200	1.319	1.212	1.241	1.163	1.238	1.235
Aver.	1.242	1.163	1.209	1.195	1.182		1.260	1.175	1.224	1.209	1.195		1.255	1.171	1.216	1.207	1.191	

Discussion

The results in fig (1) demonstrated that increasing the incubation temperature from 10 to 50°C increased the urease enzyme activity, however further increasing of temperature reduced the urease activity at all treatments. These results are in accordance with the results of Kizilkaya & Ekberli (2008) which showed that the highest activity of urease enzyme obtained at a temperature between 40 and 50 °C. Meng *et al.* (2006) has attributed the reduced activity of enzymes in the soil at high temperatures to high energy reactive molecules absorption leading to a change in the enzyme triple structure, its nature and losing part of its effectiveness. Data of figure 1 also indicated that urease activity in soil treated with different heavy metals incubated under field capacity level were higher than those incubated under water logged moisture levels. This result is similar to that reported by Pulford & Tabatabai (1988); Al-Jabri (2010) and Ou *et al.* (2019) who reported that urease activity decreased with increasing soil moisture content from field capacity to soil waterlogging limits. Burke *et al.* (2011) stated that soil water saturation reduces gas exchange between soil and the periphery that reduces soil aeration, and induced anaerobic conditions which negatively affect biological activity and consequently a significant decrease in enzymes activity. The organic substances, as ethanol, ethylene short-chain fatty acid ...etc accumulated under anaerobic condition may have adverse effect on the activity of soil enzyme (Setter *et al.*, 2009).

The results of fig. (1) showed difference in urease activity in different soils being highest for silty clay soil and lowest for loamy sand soil. These findings are consistent with the findings of Busto & Mateos (2000), Tawil

(2016) and Al-Ansari *et al.* (2019) which showed higher urease activity in silty clay loam soil than in loamy sand soils. The decrease in the activity of enzyme urease in loamy sand soil may be due to the low proportion of organic matter and clay content, which exposes the enzyme to the direct impact of thermal changes which will change the nature of the enzymatic protein and causes loss of activity, and the lack of organic matter leads to a lack of energy sources for the organisms and consequently a decrease in the number and activity of the organisms producing the enzyme (Al-Ansari, 2000; Al-Jabri, 2010).

The results in figure 1 showed negative effect of heavy metals on urease activity at all temperatures at both moisture levels as compared to control treatment. These results are consistent with Zaher *et al.* (2010) and Al Harkani (2018) who showed that decrease in urease activity of the soil treated with some heavy metals due to the effect of these elements on the activity of microorganism. Ofoegbu *et al.* (2013) reported that high concentration of heavy metals in soil reduce the number of soil microorganisms which is reflected on enzyme activity in soil.

Wyszkowska *et al.* (2006) showed that soil contamination with heavy metals plays a negative role in reducing the activity of microorganisms enzymes produced outside the cell. Dick (1997) indicated that heavy metals inhibit urease enzyme by binding it to complexes with the controlled substance, active groups in the enzyme or its effect on the enzyme complex- the controlled substance.

The results of the study in table (4) indicated significant differences in Ea values among different soils at both moisture levels.

Low values of E_a are an indicator of the catalytic efficiency of the enzyme in converting the substrate into a product. The reaction is more rapid because of the role of the enzyme which reduces the activation energy needed by the reaction and the rise in E_a values needs more energy to activate the enzymes bound within the enzyme- controlled substance and enzyme release.

The results of table (5) presented that the Q_{10} values of urease enzymes differed according to heavy metals and soil used at both moisture levels. Significant difference in Q_{10} values were also noticed between soils incubated under field capacity level or waterlogged condition. Zaffren & Hall (1973) reported that enzymatic reactions are sensitive to temperature change compared to chemical reactions, thus the values of Q_{10} for the enzymatic reaction are less than 2 while their value is greater than 2 in chemical reactions. Zhang *et al.* (2010) showed that values of activation energy (E_a) and temperature coefficient (Q_{10}) did not differ significantly when used four soils of different characteristics.

Conclusions:

The highest activity of urease enzyme was at a temperature 40-50°C and that its activity has varied with different heavy metals and different moisture levels. Effect of heavy metals on urease activity and thermodynamic parameter differ according to the temperature of incubation and the moisture level.

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