



UREASE ACTIVITY AND KINETICS PARAMETERS IN SOIL WITH HEAVY METALS UNDER FIELD CAPACITY AND WATER-LOGGED REGIMES

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Abstract

An incubation experimental was conducted to reveal the effect of different heavy metals (Cr, Cd, Pb, Mn, Fe, Cu, Zn and Ni) added at critical concentrations to soils with different properties. On urease activity and parameters (V_{max} & K_m) treated and control soils were incubated at 30C for 14 days under field capacity and water-logged moisture conditions. Urease activity was assayed, then V_{max} and K_m values were calculated. Results showed that urease activity in soil treated with all heavy metals were significantly lower than control treatments. Data also revealed that V_{max} values of control soils were significantly higher (V_{max}) or lower (K_m) than these of soil treated with heavy metals in all soils under study and at both moistures regimes. Heavy metals showed most negative effect on studied parameters (activity, V_{max} and K_m) differs according to moistures regime and soil types.

Keywords: Urease, V_{max} , K_m , Heavy metals, Moistures level soil.

Introduction

Heavy metals pollution of the soil are considered one of the major sources of pollution of the soil Cu, Ni, Cd, Zn, Cr and Pb are major sources of soil pollutions (Effron *et al.*, 2004). Heavy metals can be hazardous to soil, plant and human health through the soil-crop-food chain (Shen *et al.*, 2017). Heavy metals cause hazardous effect on soil ecosystem and negatively effect soil biological processors (Kunito *et al.*, 2001; Lorenz *et al.*, 2006 ; Malley *et al.*, 2006). European community (CEC) has established permissible heavy metals limits in agricultural soil, for Hg 1-1.5, Pb 50-300 and Zn 150-300 mg Kg⁻¹ dry soil (CEC,1986). Khan *et al.* (2007) pointed that heavy metals have an inhibitory effect on soil enzymes activities. Effect of heavy metals on soil enzymes depend on soil properties such as clay contents silt, and organic matter contents and soil pH (Geiger *et al.*, 1998 ; Effron *et al.*, 2004). Most of the soil enzymes are microbial origin protein, and heavy metals concentration in soil reduce growth and reproduction of microorganism (Simon, 1999). Soil enzyme activities have been generally accepted as one of the diagnostics indices of soil fertility quality (Bandick *et al.*, 1999). Yang *et al.* (2007) reported that inhibition of soil enzymes and microbial activity in soil by heavy metals negatively affect soil fertility. Mohammad *et al.* (2018) reported that the response of different enzymes to the same pollutant may vary considerably, and the same enzyme may respond differently to different pollutions. Soil enzymes play major role in the biochemical functioning of soil such as organic matter formation and degradation, cycling of nutrients and energy transformation (Sahoo *et al.*, 2014). Variation in moisture content in soil change soil redox dynamic (Pal *et al.*, 2010) and conditions for microorganisms

living (Geisselr *et al.*, 2011). Shifting from aerobic to anaerobic conditions in soil change soil microbial community function, which resulted in reduced soil enzyme activity (Chambers *et al.*, 2016). Urease (Urea amidohydrolase, EC 3.5.1.5) is one of the hydrolases enzyme are involved in hydrolyses of amide applied as urea and animal waste to NH⁴⁺ (Fraser *et al.*, 2013). Most of above studies concerning effect of heavy metals on enzymes activity were carried under normal soil water content (field capacity), little informations are available about effect of heavy metals on urease activity in soil under water-logged conditions. Hence, this study was conducted. The purpose of this study is to present the effect of different heavy metals (Cr, Cd, Pb, Mn, Cu, Fe, Zn and Ni) on urease activity and its kinetics parameters (K_m and V_{max}) under normal water condition (F.C.) and water-logged conditions in soil with different properties.

Materials and Methods

Soil samples from three locations differ in their agricultural status located as 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 torrifuvent), silty clay loam classified (fine clayey, mixed, active, calcareous, hyperthermic, typic torrifuvent) and loamy sand classified (sandy, mixed, active, calcareous, hyperthermic, typic torripasmments). Collected samples were kept in a refrigerator (4C) for urease enzymes measure. Sub-samples were air dried, grounded and passed through 2mm mesh. Some physical and chemical properties of the soil were determined following standard procedures described by page *et al.* (1982) and presented in table1.

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

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

100 grams (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 and Pendias, 2001).

Table 2 the concentrations of heavy metals in ppm added to the soil according to (Kabata – Pendias and Pendias, 2001).

element	Zn	Cu	Ni	Fe	Mn	Pb	Cd	Cr
critical concentration	300	100	50	200	100	100	3	100

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 30C 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 (Tabatabai and 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 37C for 2 hours. After incubation, urea was inhibited by addition of KCl-Ag₂SO₄ solution, then NH⁴⁺-N released was determined distillation procedure. Kinetic parameters (Vmax and Km) were calculated from the results obtained in studies of the effect of varying substrate concentrations in assay of urease activity. The parameters were obtained by Line weaver-Burk transformation of Michaelis-Menten equation:

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]} \quad (1)$$

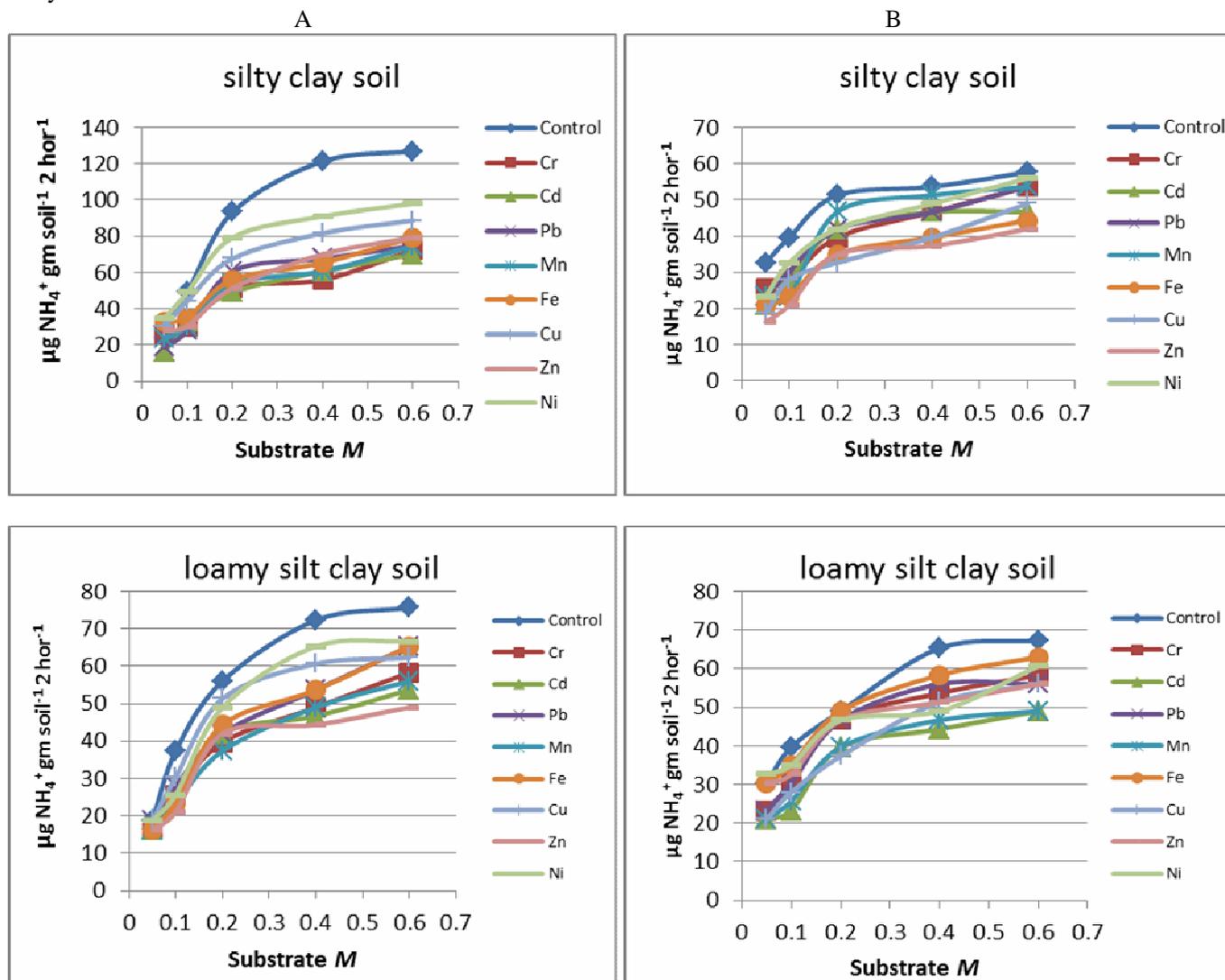
Where: V is initial velocity, (S) is substrate concentration, Km is the Michaelis constant, and Vmax is the maximum velocity.

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

Urease activity

Figure1 shows that at all treatments the relationship between substrate concentrations (S) and velocity of urease enzymes activity (V) exhibited typical Michaelis-Menten kinetics behaviors. The velocity of enzymes activity increased as substrate concentrations increased til it reached its maximum. Concentration at which maximum activity was reached varied between 0.4 and 0.6 M. Increasing substrate concentration beyond these values did not significantly affected enzymes activity. Figure1 also indicates that urease reaction velocity was lower in presences of different heavy metals when compared to control treatment at all treatments. At all treatments urease activity in soil under field capacity were higher than under water-logged condition.



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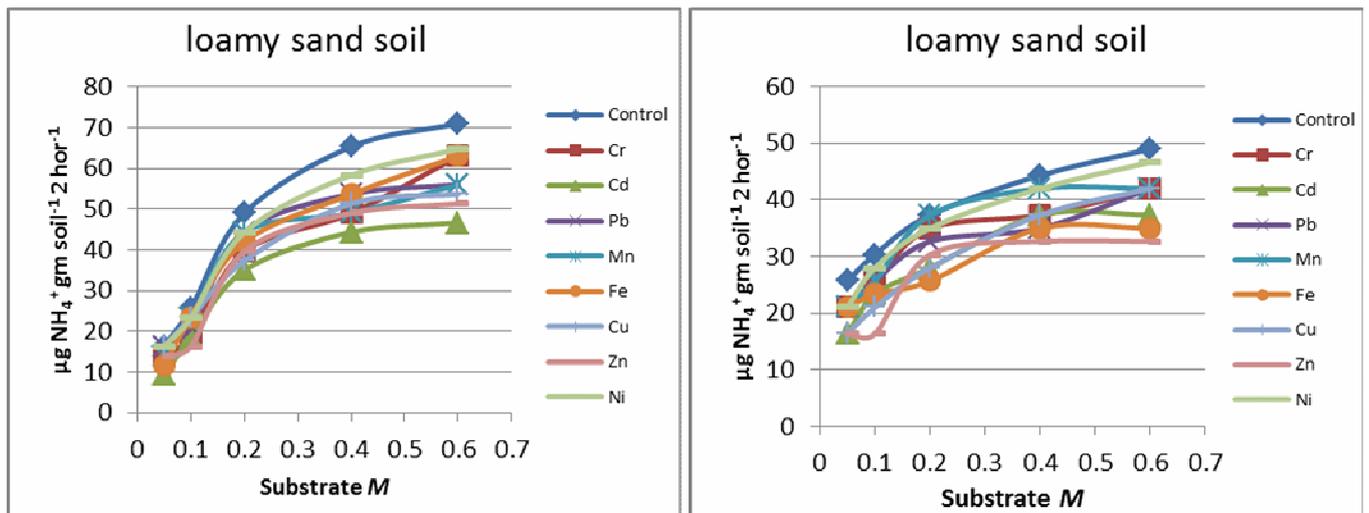


Fig. 1 : Effect of substrate concentrations on urease activity in soils treated with different heavy metals at field capacity (A) and water logged (B) soil moisture levels.

Kinetics parameters (V_{max} & K_m)

The ANOVA results of kinetics parameters showed that moisture levels, soil types, and heavy metals and their interaction had significant effect on V_{max} and K_m values (table 3). Michaelis-Menten equation was linearized according to line-weaver transformation and illustrated in Fig. 2 and Fig. 3 for field capacity and water-logged conditions, respectively. Correlation coefficient (r) values of transformation range between 0.920 and 0.998. V_{max} and K_m values of urease were calculated from data presented in Fig. 2 and Fig. 3 and presented in Fig. 4 and Fig. 5 respectively. V_{max} values of urease in all soils in presence of heavy metals were significantly lower than control and were higher at field capacity moisture level than water-logged condition

under field capacity condition lowest V_{max} values in silty clay soil were recorded at soil treated with Cr^{+2} but at silty clay loam soil and loamy sand soil lowest values were recorded at soils treated with Zn^{+2} . However, at water-logged soil conditions lowest V_{max} values were obtained at soil treated with Fe at silty clay and loamy sand soils and Cd in silty clay soil Fig. 4. Data of Fig. 4 also show that, at most but not all treatments, under field capacity condition. V_{max} values were in the order: Silty clay soil > loamy sand soil > silty clay loam, while under water-logged condition the order was silty clay loam soil > Silty clay soil > loamy sand V_{max} values of all treatments at field capacity moisture level were higher their corresponding values under water-logged condition.

Table 3 : Analysis of variance kinetics parameters of soil urease.

Source of variation	d.f.	V_{max}		K_m	
		ms	RLSD	ms	RLSD
moisture	1	76206.66	1.55	.923839090	0.0020
soil	2	2465.31	1.90	.046861420	0.0024
element	8	4771.37	3.29	.019862090	0.0042
moisture.soil	2	3434.82	2.69	.073239810	0.0035
moisture.element	8	2902.46	4.65	.024569220	0.0060
soil.element	16	850.51	5.70	.012029660	0.0073
moisture.soil.element	16	734.53	8.06	.013151840	0.0104
Error	108	24.79		.000041140	
Total	161				

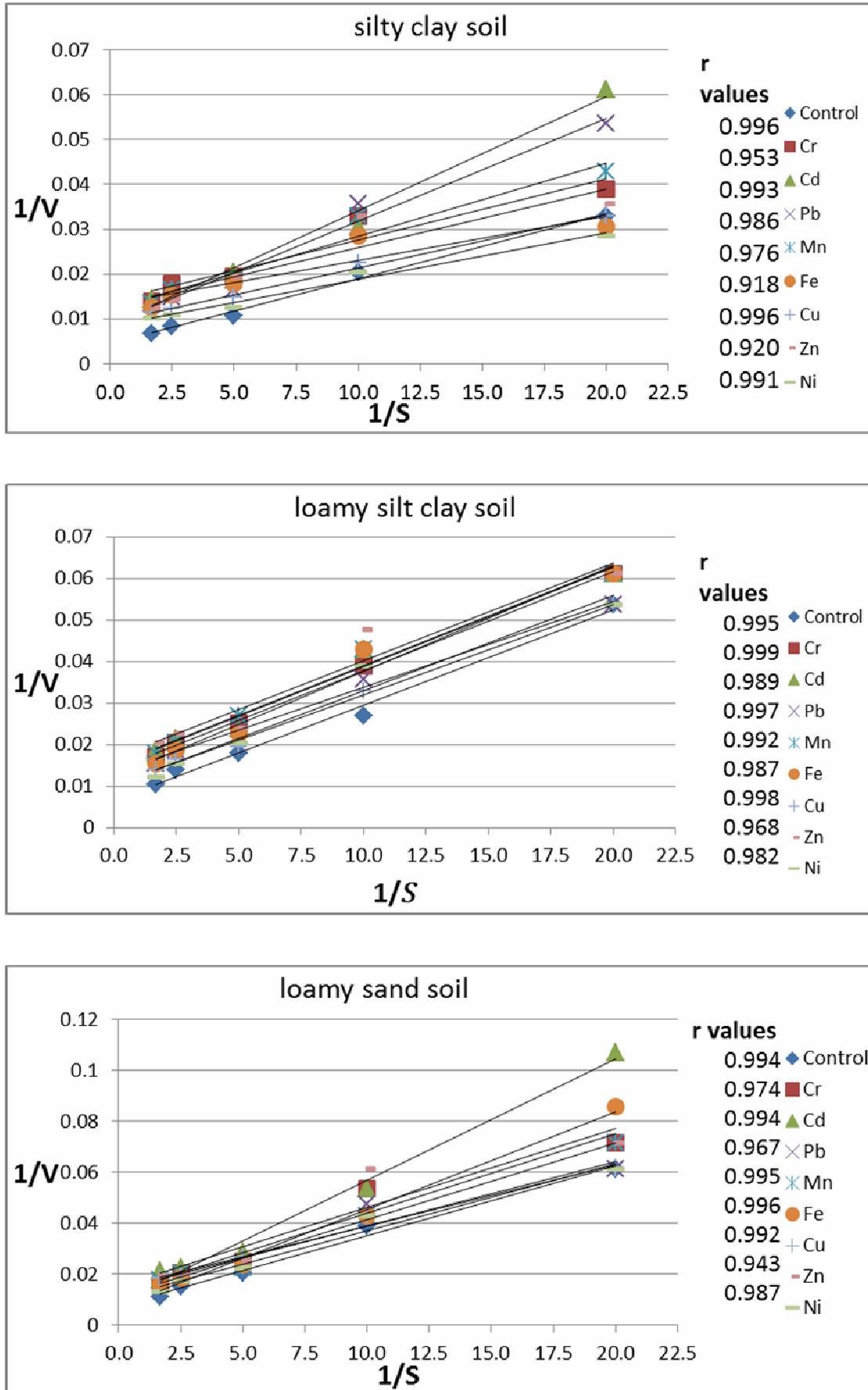


Fig. 2 : Linear plots Michaelis-Menten equation for urease in soils treated with different heavy metals at field capacity soil moisture level.

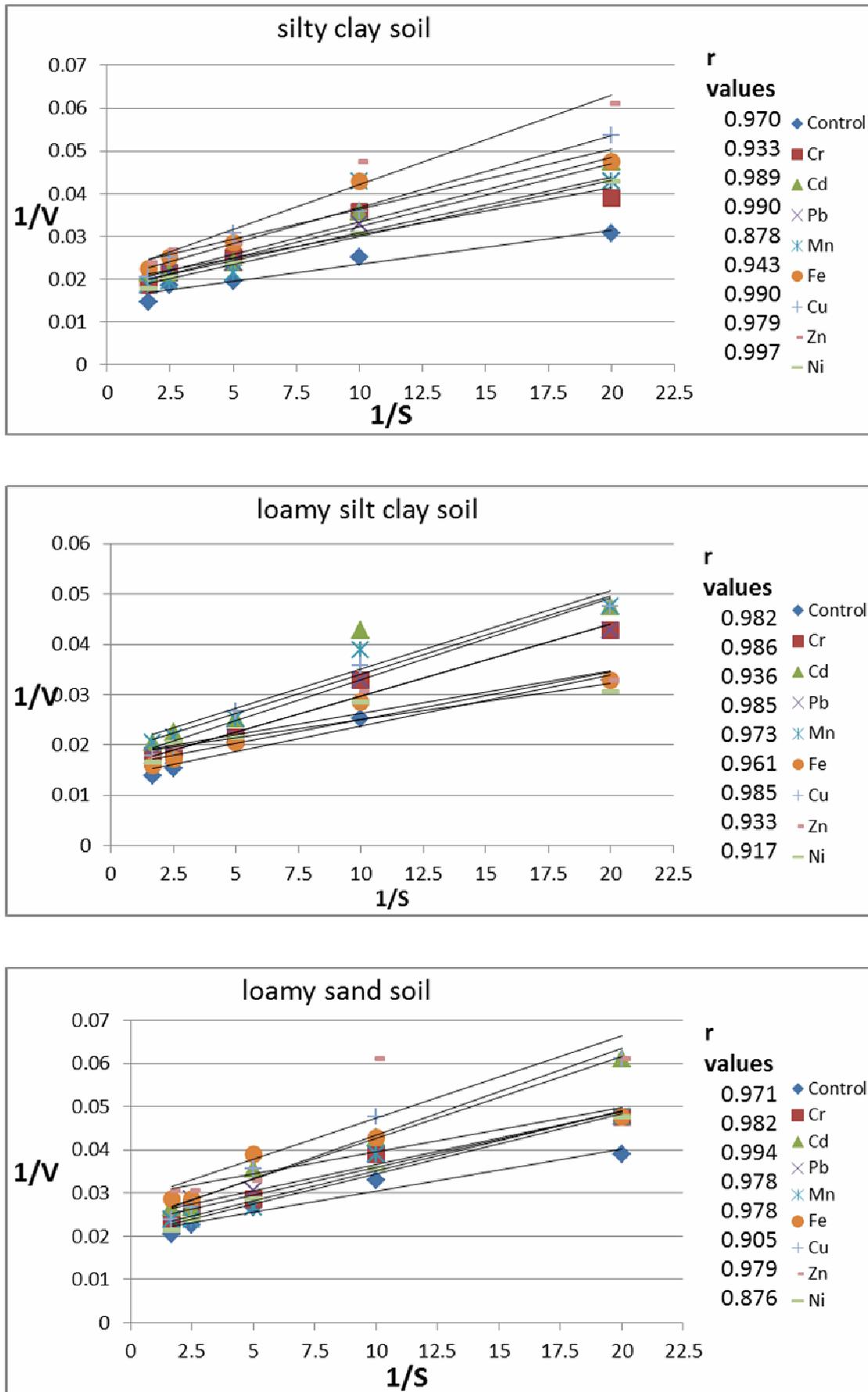


Fig. 3 : Linear plots Michaelis-Menten equation for urease in soils treated with different heavy metals at water-logged conditions.

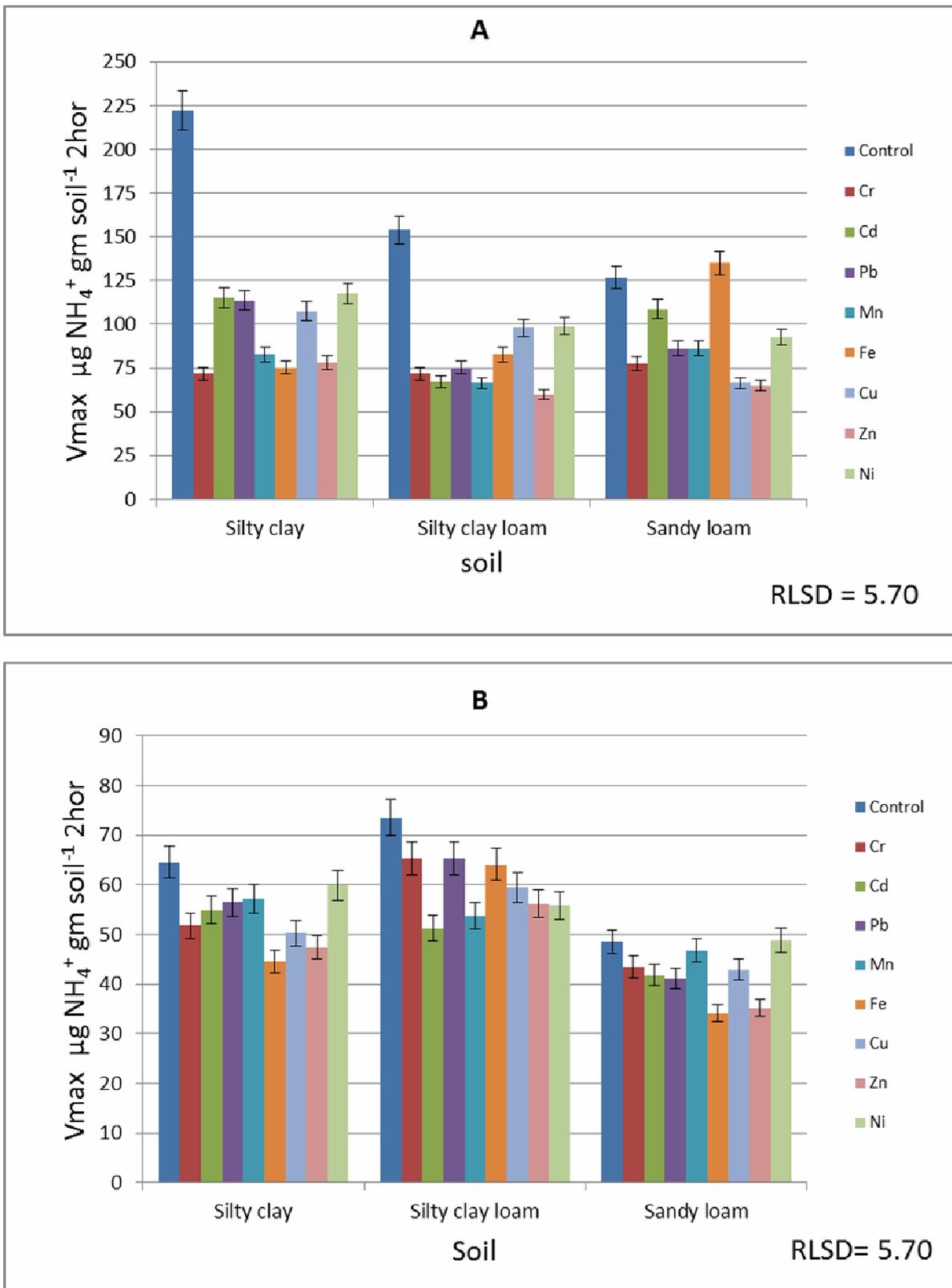


Fig. 4 : Vmax values of urease in soils treated with different heavy metals under field capacity (A) and water-logged condition (B).

Figure 5 shows that Km values of soil urease in presence of heavy metal were significantly higher than control at all soil and at both moisture levels. The effect of test heavy metals on Km values differ with moisture levels and soil types. Under field capacity condition highest Km values were recorded at soil treated with Cd⁺ in silty clay soil, Ni²⁺ at silty clay loam and Fe in loamy sand. However, under water logged condition highest Km values were obtained in soil treated with Zn²⁺ in silty clay and Cu at both

loamy silt clay and sandy loam soils. Km values of all treatment under field capacity condition (range 0.1 - 0.42M) were higher than under water-logged condition (range 0.03 - 0.10M). Figure5 also shows that Km values, in most but not all treatments, were in the order of: loamy sand soil > loamy Silt clay soil > Silty clay soil. However, under water-logged condition the order was Silty clay > Silty clay loamy > Sandy loam soil.

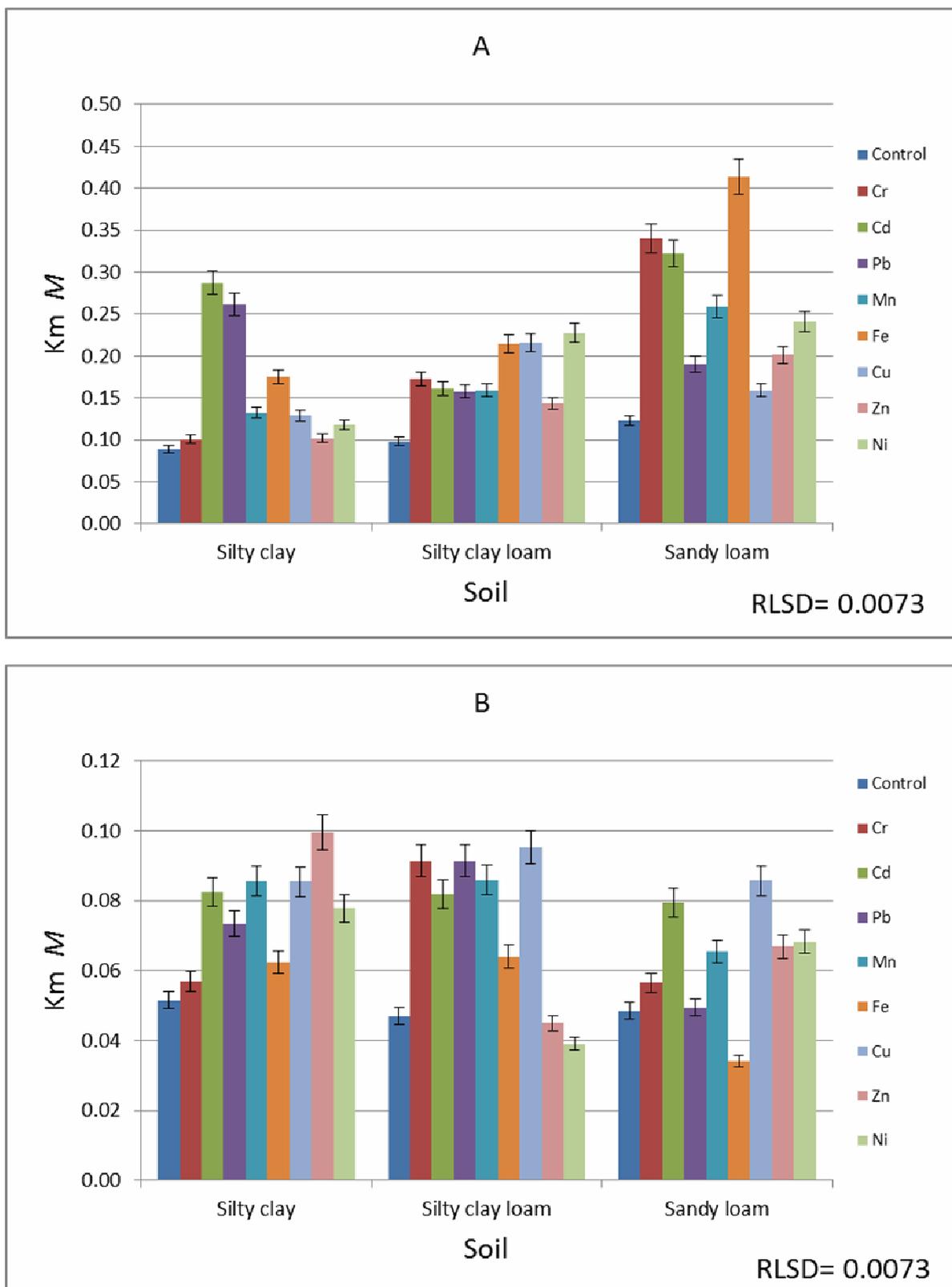


Fig. 5 : Km values of urease in soils treated with different heavy metals under field capacity (A) and water-logged condition (B).

Discussion

Results of the study showed negative relationship between activity and Vmax values of urease enzyme and heavy metals applied to soils under both moisture levels (field capacity and water-logged) at all soils under study (Fig. 1-2-3-4). Earlier studies showed negative effect of high concentration of heavy metals on urease activity in soils (Tabatabai, 1977; Baath, 1989; Antiel *et al.*, 2001; Sahoo *et al.*, 2014). Heavy metals contamination reduced microbial urease activity by altering microbial community which synthesis enzymes (Kandeler *et al.*, 2000), reaction of metal

ion with sulfhydryl groups in the catalytic site of urease (Deng and Tabatabai, 1993; Nies, 1999). Free ions can directly affect microbial cells and multiplicity of interactions can occur between microbial cell and heavy metals ion (Visvalavicius *et al.*, 2006). Ofoegbu *et al.* (2013) attributed the reduction of soil enzyme activity by heavy metals to their negative effected on the number of soil microorganisms and direct effect on soil enzymes. Gadd (1993) reported that organo metals are generally more toxic to microorganisms than corresponding free metal ions and their toxicity varies with number and identify of organic groups. However, Stuczynski *et al.* (2003) indicated that heavy metals input to

soils does not always inhibit soil enzymes activities. More ever, result of Sandrin and Maier (2003) and Moreno *et al.* (2001) showed that enzyme activity in soils could be stimulated when the soil heavy metals only slightly exceed natural values but excessive heavy metal showed negative effect on enzyme activity. Showing same trend, Yan *et al.* (2013) reported that low concentrations of Pb^{+2} and Cd^{+2} ($0.5 \text{ mg kg}^{-1} Pb^{+2}$ and $0.5 \text{ mg kg}^{-1} Cd^{+2}$) showed positive effected on urease activity, however, increase concentrations beyond these values decreased urease activity. Results of the this study indicated that urease activity in soils under field capacity where higher than those under water-logged condition (Fig. 4). This results are in accord with that of Antile *et al.* (1993), Antile *et al.* (2006) and Ou *et al.* (2019) who reported that urease activity decreased as soil moisture content increased from field capacity to flooding in polluted and unpolluted soils. When soil water content is relatively high, soil permeability is poor, urease is strongly inhibited, and its activity level in soil is low (Singh, 1985). On other hand, contrary results were reported by Tao *et al.* (2018) which showed increase moisture content from 60% 100 field capacity decreased urease activity. Urease activity in different soils were significantly differ being higher in fine texture soils than coarse texture soils (Fig. 1). Results of Doelman and Haanstra (1984) and Hattori (1992) showed larger effect of heavy metals on urease activity in coarse-textured soils than in soil of high clay or organic matter content. Other study gave positive correlation between urease activity and clay content, but was not significantly corrected with organic C or sand content (Kızılkaya *et al.*, 2004). The significant correlation of urease activity with clay content of soils may be results of adsorption capacity of enzyme by clay fraction which retain and protect urease either active extracellular form or through the protection of the ureolytic microbial biomass (Burns, 1982). Disagree with other, Haanstra and Doelman (1991) indicated that, in most studies, it was not possible to established relationships between the magnitude of the adverse effect of heavy metals and soil properties as pH, clay or organic matter. Results of this study showed that heavy metals applied to all soils under field capacity and water logged conditions increased Km values of urease enzymes compared to control treatment (Fig. 5). Increase Km values noticed could be attributed to the formation of heavy metals-urease complex decreasing the affinity of urease substrate. This finding aggress with results of Zhao and Zhao (1991), Watsan *et al.* (1994), Varel (1997) and Juan *et al.* (2009), which indicated that applying urease inhibitors with urea increased Km values and retired urea hydrolysis. Lai and Tabatabai (1992) stated that formation of inhibitor-enzymes complex decreasing the formation dissociation of enzyme-substrate complex. Juan *et al.* (2010) attributed differences in Km values of soil urease in different soils to the differences in soils physic chemical properties. Km and Vmax are constant for the enzyme, but they may vary independently of each other under different condition (Frankenberger and Tabatabai, 1980). Km value of the reactive catalyzed by enzymes in soil is desirable because it could be used to predict the reaction rate and substrate concentration needed for certain condition. The reaction velocity (V) can be calculated for any substrate concentrations by using linear transformation of Michaelis-Menten equation. Michaelis-Menten equation is such that approximately 10 and 90% of Vmax is obtained at substrate

concentration corresponding to $Km^* 10^{-1}$ and Km^*10 respectively (Tabatabai, 1994).

Conclusions

It could be concluded from this study that heavy metals in all soils under study and at both moistures regimes. Heavy metals showed most negative effect on studied parameters (activity, Vmax and Km) differs according to moistures regime and soil types.

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