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Heavy Metals Effects on Agricultural Soil Enzyme Activities of Fez, Morocco

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ABSTRACT

Enzyme activities in soils are influenced by soil characteristics and pollutants and could be indicator of soil quality. This study was undertaken to determine the relationship between physicochemical characteristics, heavy metals contents and enzyme activities (EA) in agricultural soils in the Saiss plain (Morocco). The analysis of six agricultural soil samples (sites 1–6), collected from urban and periurban agricultural soils of Fez region, showed that soils are generally alkaline with high $CaCO_3$ that rich 46%, EC (525–703 µS/cm), rich in organic matter (3.14–5.80%). The Cr, Cu, Ni, Pb and Zn concentrations in the studied area are generally greater than the Upper Continental Crust, with a deceasing order: Zn> Cr> Pb> Cu> Ni. Geo-accumulation index showed that soils are unpolluted to moderately polluted except site 5 and site 6 that are moderately to strongly polluted by respectively Cu, Pb, Zn and Pb. Potential ecological risk factors were below 40, which means low potential ecological risk except site 5 that has moderate potential ecological risk by Cu and Pb. Soil potential ecological risk indices were found <150 indicating low ecological risk. According to this index, Cu caused more serious pollution than the other elements. The enzyme activities of the six soil samples showed almost the similar ranges of values. These EA showed the highest values in site 5.The sensitivity of soil enzyme to heavy metals were observed UREA>PHOS>GALA. Pearson's correlation showed significant positive correlation between studied soil EA and between these EA and heavy metals (Cu, Pb and Zn), and significant negative correlation between EA and heavy metals (Cr and Ni). The results of this study enrich and provide data base of the impact of heavy metals on soil enzyme activities in agricultural soils in the Saiss plain.

Keywords: agricultural soil, soil enzyme activity, heavy metals, ecological risk assessment, Saiss plain.

INTRODUCTION

Soil enzymes are enzymes that are commonly found in soil and play significant role in holding physicochemical properties, ecology and fertility (Zorzona et al., 2009). Enzyme activities (EA) are a good soil quality indicators, because of their sensitivity to heavy metals content and direct relation with soil cycles (Aponte et al, 2020). Enzymes activities have an important role in C, N, P and S cycles in soils (Gianfreda and Ruggiero, 2006). With increasing concentrations of heavy metals, soil enzymes activities are inhibited (Stankovic and Stankovic, 2003), sensitive to environmental stress. As known, soil properties affect the enzyme activities. Physicochemical soil properties like organic matter and soil pH may have significant impacts and can strongly change on heavy metals effects on the soil enzyme activities (Xian et al., 2015; Aponte et al, 2020).

EA such as urease, phosphatase, protease, cellulase, invertase, galactosidase and β-glucosidase are sensitive to heavy metals (Oliveira and Pampulha, 2006). They are the most studied enzymes in contaminated soils by heavy metals. Urease is responsible for the hydrolysis of urea to carbon dioxide and ammonia and acts on carbon nitrogen bonds other than the peptide linkage bond (Bremner and Mulvaney, 1978). Phosphatase plays an important role in the transformation of organic phosphorus into inorganic form, suitable for plant uptake. The phosphatase activity can accelerate the rate of dephosphorisation of organic phosphorus and improve the soil phosphorus efficiency (Cang et al., 2009). Galactosidase activity has been found to correlate with soil reaction (Marsina et al., 1997). Indeed, changes in soil enzyme activities give informations on biogeochemical reactions and the impact of anthropogenic and agricultural practices on soil composition and health (Mkhinini et al., 2020).

The main aims of the present study were to (1) determine heavy metals concentrations in agricultural soils; (2) study the potential ecological risk; (3) assess enzyme activities of three enzymes namely urease, phosphatase and galactosidase; (4) study the effect of irrigation type (well and river) on soil enzymes activities and (5) determine the relationships between the heavy metals, physicochemical properties and enzymatic activities of agricultural soils of Fez. The results of this study enrich and provide data base for agricultural soil enzyme activity based assessment of heavy metals.

MATERIALS AND METHODS

Study area description and sampling sites

The study area location was at Fez-Meknes region that is considered one of the most agricultural regions of Morocco. The climate area is continental semi-arid. =Agricultural soil samples were collected from six surface agricultural soil samples site (Figure 1) at a depth of (0–20 cm) with a composite of pooling 5 sub-samples and then mixed. Soil samples were irrigated by Fez and Sebou rivers and also by well-water. All obtained soil samples were stored in polyethylene plastic zip-loc bags then transferred to the laboratory for analyses.

- Site 1 – located at the Fez-Upstream, irrigated by well-water and essentially cultured with potatoes;
- Site 2 – located at the Fez-Upstream, irrigated by well-water and essentially cultured with Cardon;
- Site 3 – located at the Fez-Upstream, irrigated by Fez-river and essentially cultured with Cardon;
- Site 4 – located at the Fez-Upstream, irrigated by well-water and essentially cultured with Cardon and Bell Pepper;
- Site 5 – located at the Fez-Downstream, irrigated by Fez-river and essentially cultured with Cardon;
- Site 6 – located at the Fez-Downstream, irrigated by Sebou-river and essentially cultured with Zucchini.

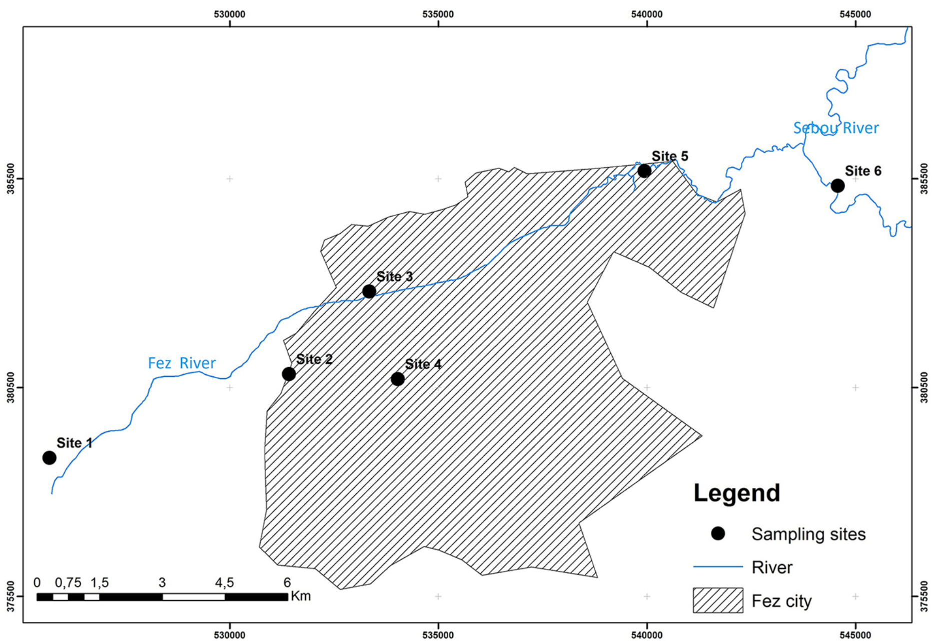

Figure 1. Sampling sites location

After homogenisation, soil samples were manually crumbled, and then sieved with 2 mm sieve and stored at 4 °C for microbiological analysis. A part of soil samples were air-dried then sieved with 2 mm sieve for chemical analysis.

Soil physicochemical properties and heavy metals contents

Physicochemical properties of the six agricultural soils were evaluated by determining soil pH with soil-water ratio according to NF X 31-103 standard, electrical conductivity (EC) by NF ISO 11 265, carbonate content ($CaCO_3$) determined using NF ISO 10693 and organic matter (OM) matter contents determined by combustion (Nelson and Sommers, 1996). The total concentration of heavy metals Cr, Cu, Ni, Pb and Zn in agricultural soil samples were extracted by tri-acid attack combination (HNO_3:$HClO_3$:HF) in open system according to NF-ISO 14869-1 standard, heavy metals concentrations were measured by ICP-AES. All analyses were performed in triplicates.

Soil enzyme activities measurement

Three enzyme activities were analyzed in this study: urease (UREA) used for N-cycling enzyme, β-D-galactosidase (GALA) used for C-cycling enzyme and phosphatase (PHOS) used for P-cycling. UREA, GALA and PHOS were assayed based on principal of incubating an aliquot of soil with a substrate. Then, the amount of metabolite was measured. Enzyme activities were expressed in mUg^{-1}dry soil. The enzymes, substrate, incubation time, metabolite and references are in Table 1. One U of enzyme activity was determined as the quantity of enzyme that catalysed 1 μmol substrate in 1 min. All analyses were carried out in triplicates.

Soil contamination assessment

Soil contamination index

Geo-accumulation index (Igeo) was adopted to evaluate soil contamination of heavy metals in topsoils. The Igeo were measured by the following equation defined by Muller (1969).

$$Igeo_x = \log_2(C_x/1.5bg_x) \quad (1)$$

where: the background (*bg*) values used are the Upper Continental Crust UCC (Wedepohl, 1995). *C* concentrations are measured for an element *x* in soil samples; 1.5 is the correction factor for the background due to lithospheric effects.

Assessment of the degree of heavy metal contamination

The contamination factor (CF), the degree of contamination (DC) and the pollution load index (PLI) were calculated to assess the degree of heavy metal contamination according to Hakanson (1980) and Tomlinson et al. (1980) the calculations of these indices were briefly shown as followings:

$$CF_x = (C_x/bg_x) \quad (2)$$

$$DC_x = \sum CF \quad (3)$$

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{1/n} \quad (4)$$

where: *n* – equals the number of contamination factors.

Soil potential ecological risk (PER)

To assess potential ecological risk of heavy metals, PER factor (*Er*) and PER index (*RI*) were adopted. They serve as a direct indicator of soil toxic level. RI is the sum of *Er*. *Er* and *RI* were calculated using following equations according to Hakanson (1980):

$$Er = T \times CF \quad (5)$$

$$RI = \sum Er \quad (6)$$

where: *CF* – the contamination factor, *T* – the toxic response factor for each element. The standardized values was $T_{Cu}=T_{Pb}=T_{Ni}=5$, $T_{Cr}= 2$ and $T_{Zn}= 1$.

Data analysis

Data were presented by the means obtained from three replicates and was processed with Microsoft excel and XLSTAT software 2016 was used for all data analyses. Pearson correlation was used to define the level of correlation between soil properties, heavy metals and enzyme activities. Box-plots were used to indicate the interquartile and to represent mean value. Principal component analysis (PCA), cluster analysis (CA) and hierarchical cluster were performed for physicochemical analyses, heavy metals content and enzyme activities to facilitate the interpretations of the results by minimizing the number of variables and to identify their sources and origins.

Table 1. Soil enzyme activities method

Enzyme	Substrate	Incubation(h)	Metabolite	References
Urease	Urea	3	NH_4-N	Sinsabaugh et al., 2000
Phosphatase	4-Nitrophenyl phosphate	0.5	p-Nitrophenol	Tabatabai and Bremner, 1969
Galactosidase	4-Nitrophenyl β-D-galactopyranoside	3	p-Nitrophenol	Eivazi and Tabatabai, 1988

RESULTS AND DISCUSSION

Soil physicochemical properties and heavy metals contents

Physicochemical properties, heavy metals concentrations and enzyme activities values are represented in Table 2 and Table 3. Overall, the soil pH of agricultural soils did not vary across various sites; the grade was slightly to moderately alkaline in this study (USDA, 1999). The average soil pH value was, ranging from 7.70 to 8.07. Conductivity ranged from 525 µS·cm^{-1} to 702.67 µS·cm^{-1}. All studied area was rich in $CaCO_3$ ranging from 28.4% to 46.23% except site 4 was the most impoverished soil with 12.31%, which can be explained by the soil type that is an isohumic soil. The sample soils were rich in OM that reach (5.8%) with the lowest OM contents (3.14%). Concerning pH soil and EC, the most acidic pH (7.7) and the highest EC measured (702.67 µS·cm^{-1}) was found in site 5 urban site in downstream irrigated by Fez River. Meanwhile, for the other sites no distinction due to their geographical location neither to their irrigation mode. Site 4 is the most depleted soil by $CaCO_3$, which can be explained by the fact that it is an isohumic soil, which in Morocco is known to be a decalcified soil at the surface (Billaux and Bryssine, 1967).

The Cr content ranged from 49.16 µg·g^{-1} to 120.18 µg·g^{-1}. Ranges of concentration were from 18.99 µg·g^{-1} to 180.13 µg·g^{-1} for Cu. Ni concentrations ranged from 15.37 µg·g^{-1} to 44.69 µg·g^{-1}. Pb concentrations ranged from 10.12 µg·g^{-1} to 140.71 µg·g^{-1}. Zn concentrations ranged from 58.18 µg·g^{-1} to 332.46 µg·g^{-1}. Overall, with the exception of Ni, the highest values of Cu (180.13µg·g^{-1}), Pb

Table 2. Heavy metals content (mean value± standard error) of soil samples in (µg.g^{-1})

Sites	Cr	Cu	Ni	Pb	Zn
Site 1	66.5±3.4	19.74±1.3	26.4±1.5	19.5±1.7	63.8±2.6
Site 2	57.4±0.9	22.5±1.4	23.9±1.3	21.5±0.4	79.8±5.1
Site 3	78.9±2.1	19.0±0.6	29.8±1.8	10.1±1.2	86.2±15.2
Site 4	120.2±2.7	47.6±5.0	44.7±3.6	31.6±4.2	58.2±4.5
Site 5	99.2±2.2	180.1±0.9	23.1±0.6	140.7±52.8	332.5±14.1
Site 6	49.2±1.2	32.8±1.0	15.4±1.0	113.5±0	64.3±2.2
Study area	78.56	53.63	27.21	56.16	114.12
UCC (Wedepohl, 1995)	35	14.3	18.6	17	52
World soil (Kabata-Pendias, 2011)	59.5	38.9	29	27	70

Table 3. Soil properties and enzyme activities

Properties	Unit	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
pH	-	7.83±0.1	8.07±0.1	8.07±0.1	7.83±0.1	7.70±0.1	8.03±0.1
EC	µS·cm^{-1}	630±162.8	681±81.3	534.67±115.9	525±16.4	702.67±111.3	603.67±87.8
$CaCO_3$	%	28.40±1.4	46.04±0.5	34.67±0.6	17.05±0.4	38.48±0.6	38.00±1
OM	%	5.64±0.1	5.76±0.5	3.35±0	5.81±0.1	5.51±0	3.14±0.1
PHOS	mU·g^{-1}	3.98±0.5	15.81±2.2	0.00	0.00	20.71±0.4	14.64±1.1
GALA	mU·g^{-1}	0.77±0.3	1.76±0.4	0.70±0.1	1.14±0	3.10±0.1	1.53±0.1
UREA	mU·g^{-1}	15.17±2.41	43.14±1	14.31±0.4	19.88±0.9	46.13±3.3	36.79±4.0

(140.71 μg·g^{-1}), Zn (332.46 μg·g^{-1}) and to a lesser extent Cr (99.22 μg·g^{-1}) are to be noted for site 5 (urban and downstream) site where the effects of urban pollution of Fez are concentrated. For the other sites, two exceptions should be noted: a very high content for Cr (120.18 μg·g^{-1}) from site 4 and Pb (113.47 μg·g^{-1}) from site 6. This argues for a probably anthropogenic origin for Cu, Zn and Pb and to a lesser extent Cr.

For the case of site 4 which shows abnormally high contents of Cr and Ni compared to the other sites could be partly explained by the isohumic nature weakly carbonated and more enriched in clays probably more enriched by natural Cr and Ni origins. It should also be noted that for all the sites, with the exception of site 5, the other sites, whether irrigated by the well (1, 2 and 4) or surface water (3 and 6) show in all the comparable values. The heavy metals concentrations were higher than the limit values of Upper Continental Crust (UCC) (Wedepohl, 1995) except Pb in site 3 and Ni in site 6. In general compared to world soil (Kabata-Pendias, 2011), the heavy metals were above world soil except Ni. The highest Cu, Pb and Zn concentrations were found at site 5 and the highest Cr and Ni concentrations were determined on site 4.

Soil contamination assessment

The soil contamination index results are listed in Table 4 and soil contamination assessment of the study area is given in Figure 2. The Igeo values of the studied heavy metals varied related to the metal and to the studied site; Ni (-0.27 to 0.82), Cr (-0.10 to 1.19), Zn (-0.43 to 2.09), Pb (-1.34 to 2.39) and Cu (-0.12 to 3.07). Site 1 (-0.39 to 0.34), site 2 (-0.25 to 0.13), site 3 (-1.3 to 0.59), site 4 (-0.43 to 1.19), site 5 (-0.27 to 3.07) and site 6 (-0.28 to 2.15). Based on Igeo ranking (Muller, 1986), Ni was the lowest Igeo values for all sites, it is more natural origin than anthropogenic. For the other elements the values vary strongly from one site to another, the Igeo values are higher for Cu and Cr for sites 4 and 5, Pb for sites 5 and 6 and Zn for site 5.

Concerning the sites, site 1 shows the lowest Igeo values, except for Cr, this site is unpolluted for all other metals (Cu, Ni, Pb and Zn). In contrast, site 5 shows the highest Igeo values, except for Ni, this site is moderately polluted by Cu, Pb, Zn and to a lesser extent Cr. The other sites show intermediate Igeo values: sites 2 and 3 are unpolluted to slightly polluted for all elements, site 4 is moderately polluted by Cr and Cu and site 6 is moderately to heavily polluted by Pb. The Igeo mean values of Cr, Cu, Ni, Pb and Zn of the study

Table 4. Soil pollution assessment of heavy metals across different locations of study area

Index	Heavy metals	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Igeo	Cr	0.34	0.13	0.59	1.19	0.92	-0.10
	Cu	-0.12	0.07	-0.18	1.14	3.07	0.61
	Ni	-0.08	-0.23	0.09	0.68	-0.27	0.82
	Pb	-0.39	-0.25	-1.34	0.30	2.39	2.15
	Zn	-0.29	0.03	0.13	-0.43	2.09	-0.28
CF	Cr	1.90	1.64	2.26	3.43	2.83	1.40
	Cu	1.38	1.58	1.33	3.33	12.60	2.30
	Ni	1.42	1.28	1.60	2.40	1.24	2.64
	Pb	1.15	1.26	0.60	1.86	8.28	6.67
	Zn	1.23	1.53	1.66	1.12	6.39	1.24
DC		7.08	7.30	7.44	12.14	31.34	14.25
PLI		1.39	1.45	1.36	2.24	4.68	2.34
Er	Cr	3.80	3.28	4.51	6.87	5.67	2.81
	Cu	6.90	7.88	6.64	16.63	62.98	11.48
	Ni	7.11	6.42	8.02	12.01	6.21	13.21
	Pb	5.75	6.31	2.98	9.30	41.39	33.37
	Zn	1.23	1.53	1.66	1.12	6.39	1.24
RI		24.78	25.42	23.80	45.93	122.64	62.11

area were 0.51, 0.77, 0.17, 0.18 and 0.21, respectively. However, the study area is unpolluted to moderately polluted.

The minimal and maximal CF of heavy metals in the sampling sites were Cr (1.4–3.43), Cu (1.33–12.6), Ni (1.24–2.64), Pb (0.6–8.28) and Zn (1.12–6.39). Based on the classification defined by Hakanson (1980): sites 1, 2, 3 and 4 showed moderate contamination factor except Pb(0.6) in site 3 that has the lowest contamination factor and site 4 that has considerable contamination by Cr and Cu. In contrast, site 5 showed very high contamination factor by Cu, Pb and Zn and site 6 showed a very high contamination by Pb. Ni was an exception, because all the sites have CF between 1 and 3 therefore moderate contaminations. For the other heavy metals it varies according to the site. CF data results confirm Igeo results. The CF mean value of Cr, Ni and Zn are 2.24, 1.77 and 2.2, respectively witch mean the study area has moderate contamination. Meanwhile the study area has considerable contamination factor by Cu and Pb. Sites 1, 2 and 3 had low degree of contamination. Site 4 and site 6 showed moderate degree of contamination. However, site 5 showed the highest value DC = 31.34 indicating a considerable degree of contamination. This value could probably be due to the concentration of Cu, Pb and Zn. The DC shows increasing values from site 1 to site 5, the degree of contamination increases from Fez-upstream (low urban pollution) to Fez-downstream (high because it is the outlet of the urban pollution of Fez), this DC is not correlated with the nature of the irrigation water (rivers or well).

PLI provide comparative information in order to assess heavy metals pollution. The mean values of PLI in all sampling sites ranged from site 3 with PLI = 1.36 in Fez-upstream to site 5 with PLI = 4.68 in Fez-downstream. All sampling sites were above 1 which indicates pollution. PLI showed the same results as DC, the results were not correlated with irrigation methods but it could probably depend to industry. Site 5 showed the highest pollution in the study area. The Er values of studied heavy metals ranged from 1.12 to 62.98. Based on Er classification (Hakanson, 1980), the study area were under low potential ecological risk (Er<40) except site 5 where Pb with Er_{Pb}= 41.39 and Cu with Er_{Cu}= 62.98 indicating moderate potential ecological risk. The mean value ecological risk factor by heavy metals of the study area is under 40 low potential ecological risks. The potential ecological risk index RI of heavy metals ranged from 23.80 to 122.64.The results indicated that the agricultural soils in the study area were characterized by low ecological risk index (RI<150). RI results shows increasing values from site 1 to site 5, in agreement with DC and PLI results.

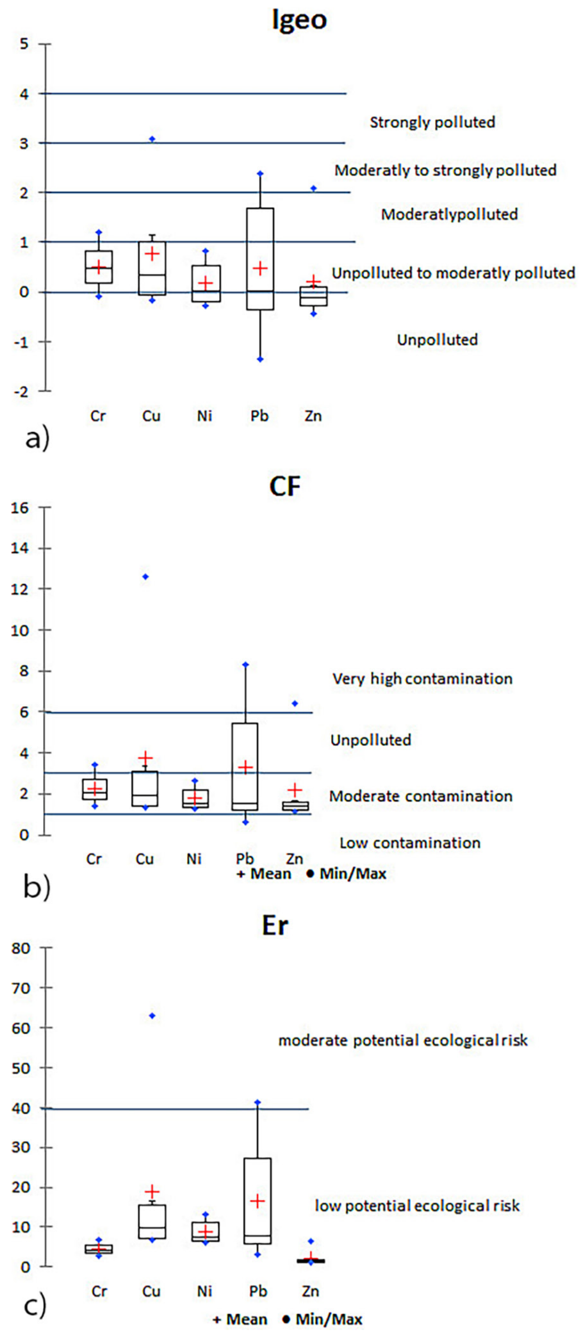

Figure 2. Box and whisker plots of: (a) geoaccumulation index, (b) contamination factor and (c) ecological risk index. (+) mean values and (.) min-max

Enzyme activities

The studied agricultural soils enzyme activities, UREA, GALA and PHOS related to N, C and P cycling, respectively of the six samples are given in Table 3 and Figure 3. Overall, PHOS, GALA and UREA activities showed the same trends from site to another. They show similar behavior Figure 3. The sites (1, 3 and 4) in the upstream zone of Fez showed lower EA values than the site 2 in the upstream and the sites 5 and 6 in the downstream zone. Urease activity ranked highest, followed by phosphatase activity then galactosidase. The values of urease, phosphatase and galactosidase ranged from 14.31 mU·g^{-1} to 46.13 mU·g^{-1}, 0 mU·g^{-1} to 20.71 mU·g^{-1} and 0.7 mU·g^{-1} to 3.1 mU·g^{-1}, respectively.

Site 1 the least contaminated by Cu, Pb and Zn showed low EA values and site 5 the most contaminated by Cu, Pb and Zn showed the highest values. Furthermore, sites 3 and 4 showed a great similarity of their EA, especially for PHOS, however, they did not show any similarities concerning physicochemical properties including soil pH, $CaCO_3$ and OM, neither soil type: vertisol for site 3 and isohumic for site 4 nor irrigation water: river for site 3 and well for site 4. The results showed that there was no correlation established between the nature of water irrigation and EA.

For the EA, site 5 is the exception with the highest values of enzymes activities. Sites 2 (Fez-upstream, well-water irrigation) and site 6 (Fez-downstream, Sebou river irrigation) show close EA values. Sites 1 (Fez-upstream, well-water irrigation), site 3 (Fez-upstream, Fez-river irrigation) and site 4 (Fez-upstream, well-water irrigation) show comparable and lowest EA values. EA does not depend on the nature of the irrigation water (rivers or wells).

Soil enzyme activities are an important index to evaluate soil pollution (Ciarkowska, 2018; Aponte et al., 2020). Furthermore, EA was used for predicting heavy metals pollution (Alkorta et al, 2003; Kouchou et al., 2017). The soil enzyme activities could be controlled by OM content, soil pH and microbial biomass. It could be probably due to moisture (Jin et al., 2016). EA in soils are important, they are associated with physical, chemical and biological soil properties (Shukla and Varma, 2011), particularly heavy metals that effects their presence and activities in soils (Xian et al., 2015; Singh et al., 2020). Table 5 summarizes the correlation between soil

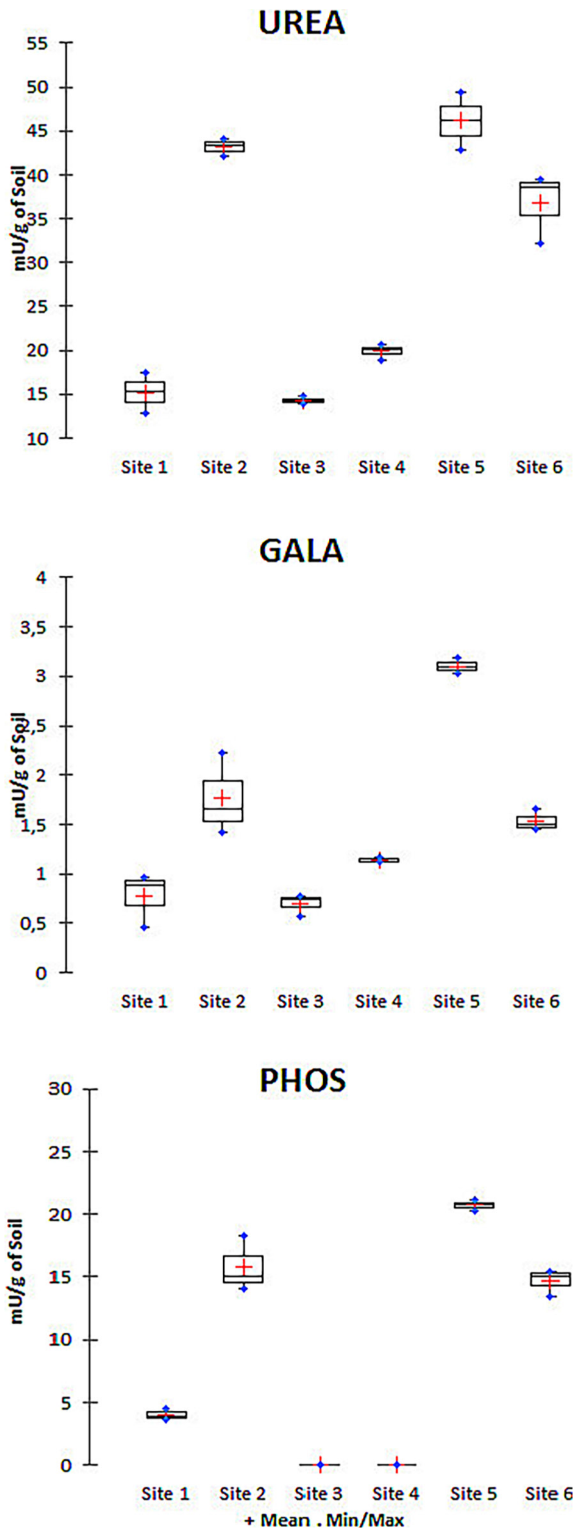

Figure 3. Box and whisker plots of the soil enzyme activities

enzyme activities, heavy metals and soil properties. Pearson's correlation between enzyme activities showed positive correlation between GALA-UREA-PHOS activities, more noticeable there is a strong positive correlation between PHOS-UREA of P-N cycling. The positive correlation

Table 5. Correlation between soil characteristics, enzyme activities and heavy metals

	PHOS	GALA	UREA	Cr	Cu	Ni	Pb	Zn	pH	EC	OM	CaCO3
PHOS	**1**											
GALA	**0.854**	**1**										
UREA	**0.946**	**0.865**	**1**									
Cr	-0.320	0.157	-0.202	**1**								
Cu	**0.595**	**0.868**	**0.564**	**0.475**	**1**							
Ni	**-0.704**	-0.359	**-0.555**	**0.804**	-0.106	**1**						
Pb	**0.713**	**0.741**	**0.665**	0.016	**0.729**	**-0.490**	**1**					
Zn	**0.627**	**0.857**	**0.571**	0.338	**0.965**	-0.225	**0.682**	**1**				
pH	-0.147	-0.434	-0.083	**-0.607**	**-0.694**	-0.248	-0.348	**-0.597**	**1**			
EC	**0.530**	0.378	0.446	-0.185	0.327	-0.363	0.231	0.355	-0.194	**1**		
OM	0.090	0.268	0.142	0.436	0.277	0.424	-0.154	0.214	**-0.541**	0.245	**1**	
CaCO3	**0.730**	0.438	**0.686**	**-0.676**	0.120	**-0.794**	0.263	0.291	0.394	0.401	-0.229	**1**

between enzyme activities is in line with the study carried out by Kouchou et al. (2017) on Fez-downstream. In general, the studied enzyme activities galactosidase, urease and phosphatase, were positively correlated with heavy metals, Cu, Pb and Zn, these heavy metals are anthropogenic therefore more bioavailable which are beneficial for the growth of EA, especially Cu and Zn. Heavy metals such as Cu and Zn were reported to have nutritional value and do not inhibit enzyme activities (Singh et al., 2020). However, there was significant negative correlation between PHOS-Ni, UREA-Ni and negative but not significant between GALA-Ni. This result agreed with Tejada et al., (2008) that reported that soil enzyme activities decreased with increasing Ni concentration. There is no significant correlation between Cr and EA. This could be explained by the fact that Cr and Ni have mostly natural origin, Therefore they are not very bioavailable, so they have no effect on EA growth. There was high positive correlation between Ni-Cr, Cu-Pb, Cu-Zn and significant positive correlation between Cu-Cr, Zn-Pb and negative correlation between Pb-Ni. There was significant negative correlation between pH-Cr, pH-Cu and pH-Zn.

The soil pH gave negative but not significant correlation with studied enzymes activities, Ni and Pb. Negative significant correlation between soil pH, Cr, Cu and Zn. In accordance with our findings, it has been reported that soil pH gave negative correlation with soil enzymes activities (Angelovicova et al., 2014; Liu et al., 2020). Soil pH is another factor that could have significant impacts on soil heavy metal bioavailability thus the soil enzyme activities (Xian Y. et al, 2015). Besides this, soil pH and soil OM modify the impact of heavy metals on enzyme activities (Karaca et al, 2010). The results indicate that there was no correlation between OM and EA. It was reported by (Kouchou et al., 2017) that dissolved organic carbon (DOC) has a positive correlation with EA. However, DOC is an important bioavailable fraction of the OM and provide source of energy for microorganism. The effect of heavy metals content on enzyme activities can be mediated by soil pH (Dick, 2011) and organic matter (Tang et al., 2020). The increasing pH leads to the decreasing bioavailability of Cu, Cd, Pb and Zn in soil, which results in lower heavy metals toxicity for microorganisms and reduced inhibition of enzyme activities (Aponte et al., 2020).

The $CaCO_3$ was positively correlated with PHOS and UREA. It could be explained by the fact that in carbonate nature of soil, the fixation of cations is favored in alkaline environment. Moreover, such conditions allow to small percentages of heavy metals to became easily soluble and provide nutrients for microorganisms (Kouchou et al., 2017).The $CaCO_3$ was also found to be negatively correlated with Cr and Ni; this may be explained by the fact that carbonated soil is poor in clays and poor in geogenic Cr and Ni. Besides, less carbonated soil is richer in clays, richer in geogenic Cr and Ni (isohumic soil).

Aponte et al., (2020) reported that all studied enzymes activities strongly decreased under heavy metals contamination. Among the sampling sites of the study area, the site 5 was most contaminated and the enzyme activities were not the lowest in

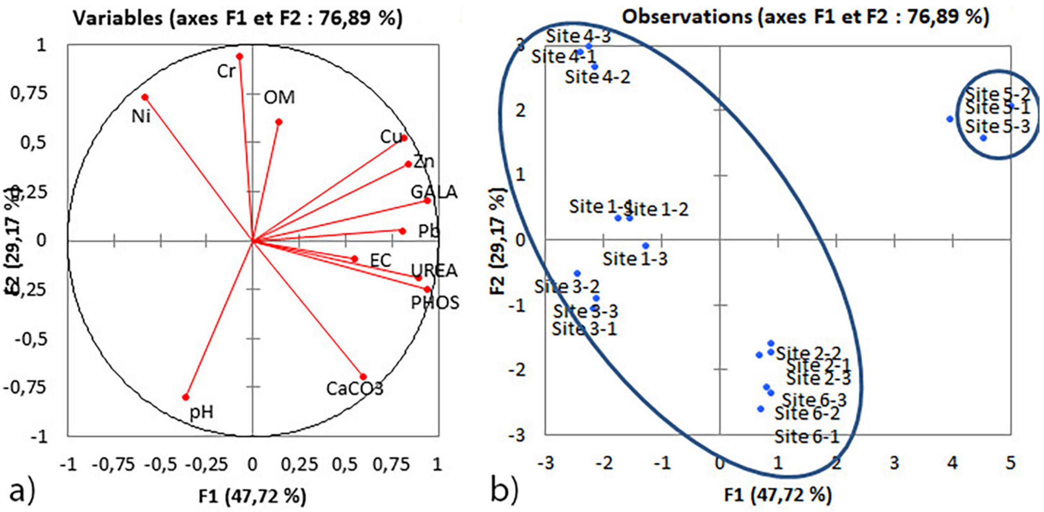

Figure 4. Principal component analysis of enzymatic activities, heavy metals and soil physicochemical properties (a) and sampling sites map (b)

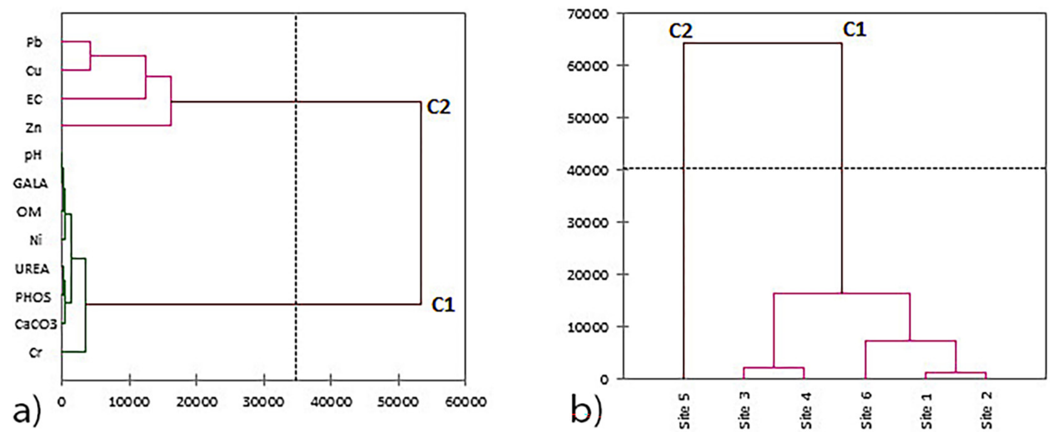

Figure 5. The dendrogram of cluster analysis of soil characteristics, heavy metals and enzyme activities (a) and sampling sites (b)

this study area. Contrarily to research, the EA in present study increased in responses with increasing heavy metals contents. Some authors have also shown that treated wastewater used for long-term irrigation can significantly improve soil enzyme activities by providing extra nutrients (Mkhinini et al, 2020). The study done in Fez-downstream by Kouchou et al. (2017) showed that the highest enzyme activities were recorded at the site with the highest levels of heavy metals, which confirms the results recorded in the present study. Furthermore, other studies has been reported the opposite trend in soil EA, Heavy metals presented positive correlation with EA (Hagmann et al., 2015; Yang et al., 2016). The principal component analysis PCA of all sites was represented in Figure 4. Two components model PC1 and PC2 using PCA that accounted 76.89% of the data variation. The first component with a variance of 47.72% was mainly associated with PHOS, GALA, UREA, Cu, Pb and Zn, while the second component with a variance of 29.71% was associated to Cr, Ni, pH and $CaCO_3$. The cluster analysis calculated using Euclidian distance and ward's grouping method in order to determine similarity between 6 studied sites based on soil characteristics, heavy metals (Cr, Cu, Ni, Pb and Zn) and soil enzyme activity (UREA, PHOS and GALA) Figure 5. The dendrogram identify 2 classes. Class 1 groups enzyme activities, Cr, Ni, pH, OM and $CaCO_3$ indicating natural source. Meanwhile, Class 2 includes CE, Zn, Cu and Pb indicating anthropogenic origin. Sampling sites dendrogram, identify similar groups. The sampling sites were grouped in two clusters. C1 represent 83% of the sampling sites and C2 represent site 5.

CONCLUSION

Heavy metals concentrations in this study area were above upper continental crust and world soil. The elements Cr and Ni were highest in site 4 thus elements Cu, Pb and Zn were highest in site 5. The soil pollution assessment for the studied agricultural soils showed a moderate potential ecological risk by Cu and Pb in site 5 and low ecological risk grade. In general, there was reported high positive correlation between heavy metals Cu, Pb and Zn and soil enzyme activities that indicates the same origin source. Among the enzymes, phosphatase activity was the most sensitive indicator however urea activity were insensitive to heavy metals. Soil enzyme activity increased in downstream area. The relationship between soil properties, heavy metals and enzymatic activities do not only depend on heavy metals concentration but on speciation of the metal (chemical form). On the basis of this study, the results support the findings of (Kouchou et al, 2017) focused on semiarid calcareous soils.

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