

**BIOAVAILABILITY OF PHOSPHORUS AND ZINC IN
CALCAREOUS SOILS OF THE KURDISTAN REGION OF
IRAQ**

MUHAMMED S. RASHEED

(**BSc.** Soil Science 2005; **MSc.** Soil Physiochemistry 2010)

Thesis submitted to the University of Nottingham for the
degree of doctor of philosophy

2019

I dedicate this work to:

- My father who he passed away when I was three years old
- My mother, brothers and sisters
- My wife (Snur) and daughters (Nza and Nma)
- All those who helped me in this work

ACKNOWLEDGEMENT

I would like to thanks the Kurdistan regional government who financially supported me in this work.

I would also like to thank my respectful supervisors Dr Scott Young and Dr Liz Bailey for their guidance and encouragement throughout my PhD.

I would like to express my thanks to my mother, brothers, sisters and wife who supported me throughout my PhD.

Most thanks to administrator and technical staff of school of bioscience, a division of agriculture and environmental science.

Thanks also extended to:

- Halabja technical college of applied science staff especially.
- Halabja technical college of applied science field staff who helped me during my field trial conducting.
- All my friends in the division of agriculture and environmental science, school of bioscience at the University of Nottingham
- My friends at the University of Salahaddin-Erbil-Faculty of agriculture Dr Tariq Faruq and Mr Muslim Arab.

CONFERENCES AND SYMPOSIUMS

1. The Nottingham Environment Conference at the University of Nottingham on 25th and 26th Jun 2018 Nottingham – Guest
2. 5th Symposium on Zinc in Soils and Plants (from Molecular Scale to Ecosystem) on 5-7 September 2018 Leuven Belgium - Oral presentation
3. 6th Symposium on Phosphorus in Soils and Plants on 10-13 September 2018 Katholieke Universiteit Leuven (Kulkarni et al.), Belgium – Poster presentation

TABLE OF CONTENTS

ACKNOWLEDGEMENT	iii
CONFERENCES AND SYMPOSIUMS	iv
TABLE OF CONTENTS.....	v
TABLE OF FIGURES	xii
TABLE OF TABLES	xx
VOCABULARY.....	xxiii
ABSTRACT.....	xxiv
1 INTRODUCTION.....	1
1.1 Zinc in soil and its availability	1
1.2 Factors affecting Zn availability.....	2
1.2.1 Soil pH	2
1.2.2 Soil texture	3
1.2.3 Soil mineralogy and calcium carbonate content.....	3
1.2.4 Phosphorus (P) in the soil	4
1.2.5 Kinetics of Zn fertilizer reaction	4
1.3 Plant uptake of Zn.....	5
1.3.1 Soil factors affecting Zn uptake	6
1.3.2 Plant controls over Zn uptake	7
1.3.3 Plant Zn uptake and interaction with P.....	8
1.3.4 Use of Zn fertilizers to enhance uptake	9
1.4 Zinc in human and animal nutrition	10
1.4.1 The physiological function of Zn.....	12

1.4.2	Effect of Zn deficiency	12
1.4.3	Plant phytic acid (PA) content.....	13
1.4.4	Phytic acid to Zn molar ratio	15
1.5	Chemistry of phosphorus (P) in soil	15
1.5.1	Soil pH value.....	16
1.5.2	Soil organic matter and soil texture.....	17
1.5.3	Soil calcium carbonate content	18
1.5.4	Kinetics of P fertilizer reaction with soil	19
1.6	Plant uptake of P.....	20
1.6.1	Soil factors controlling P uptake.....	22
1.6.2	Plant controls over P uptake	23
1.6.3	Use of P fertilizer to enhance uptake	23
1.7	Phosphorus in humans and animals.....	24
1.7.1	The physiological function of P.....	26
1.7.2	Effect of P deficiency on humans.....	27
1.7.3	Plant phytic acid content.....	28
1.8	An overview of the Kurdistan region of Iraq.....	29
1.9	Objectives of the current project.....	30
1.10	Hypothesis	30
2	Methods and Materials.....	32
2.1	Soil sampling and preparation	32
2.2	Soil analysis	32
2.2.1	Soil pH	32

2.2.2	Loss on Ignition (LOI %)	32
2.2.3	Soil digestion	33
2.2.4	Determination of Olsen-P	33
2.2.5	Determination of DTPA-Zn	34
2.2.6	Determination of soluble P and Zn	34
2.2.7	Phosphate solubility	35
2.2.8	Zn solubility	36
2.2.9	Phosphate Fractionation	37
2.2.10	WHAM speciation	38
2.3	Plant sampling and preparation	38
2.4	Plant analysis	38
2.4.1	Plant digestion	38
2.4.2	Determination of phytic acid (PA)	39
2.4.3	Determination of Phytic acid to Zn molar ratio (PA/Zn molar ratio)	40
2.4.4	Determination of human Zn bioavailability	40
2.5	Elemental analysis using ICP-MS	41
2.6	Statistical analysis	42
3	THE SOLUBILITY OF PHOSPHORUS AND ZINC IN THE CALCAREOUS SOILS OF KURDISTAN	43
3.1	Introduction	43
3.2	Material and methods	45
3.2.1	Soil survey in Sulaimanyah Province	45
3.2.2	Soil analysis	54
3.2.3	Soil inorganic carbon (SIC %)	54

3.2.4	Phosphorus and zinc solubility experiment	54
3.2.5	Geochemical speciation using WHAM 7	55
3.2.6	Statistical analysis	55
3.3	Results and discussion	56
3.3.1	Soil chemical characteristics	56
3.3.2	Soils phosphate	60
3.3.3	Soils zinc	69
3.3.4	Multivariate statistical analysis	78
3.4	Conclusions	82
4	TIME-DEPENDENT SORPTION OF PHOSPHATE AND ZINC BY CALCAREOUS SOILS OF KURDISTAN	83
	Abstract	83
4.1	Introduction	85
4.2	Methods and Materials	88
4.2.1	Soil sampling	88
4.2.2	Soil analysis	88
4.2.3	Adsorption isotherms	89
4.2.4	Adsorption isotherm models	90
4.2.5	Calculation of E_{value}	92
4.2.6	Geochemical speciation using the WHAM-VII program	93
4.2.7	Statistical analysis	94
4.3	Results and discussion	95
4.3.1	General soil characteristics	95
4.3.2	Phosphorus behaviour in two calcareous soils from Kurdistan	96

4.3.3	Zinc behaviour in two calcareous soils from Kurdistan	104
4.4	Conclusion	115
5	PHOSPHORUS AND ZINC INTERACTION IN MAIZE, COWPEA AND TOMATO PLANTS GROWN IN THE CALCAREOUS SOIL OF KURDISTAN	116
5.1	Introduction	116
5.2	Materials and methods.....	119
5.2.1	Site description.....	119
5.2.2	Maize, tomato, and cowpea experiments.....	119
5.2.3	Managing the trials	121
5.2.4	Soil analysis	122
5.2.5	Plant analysis	123
5.2.6	Determination of Zn contamination	124
5.2.7	Statistical Analysis	125
5.3	Results and discussion	126
5.3.1	Meteorological data and soil properties of the site	126
5.3.2	Soil pH at harvest	127
5.3.3	Available P (P_{Olsen} and P_{sol}) and solid-liquid partition coefficient	128
5.3.4	Soil exchangeable Zn (Zn_{DTPA})	131
5.3.5	Determining controls over phosphate and zinc solubility in soils	132
5.3.6	Phosphorus fractionation in soil	137
5.3.7	The concentration of P and Zn in crops	140
5.3.8	The interaction between P and Zn in shoots, seeds, and fruit.....	145
5.3.9	Proportion (%) of plant element concentration arising from soil dust	148
5.3.10	Effect of applied P and Zn fertilizers on PA and PA/Zn molar ratio	150

5.3.11	Estimated Zn bioavailability to humans.....	153
5.3.12	Effect of PA on the proportion of bioavailable Zn	157
5.4	Conclusions.....	159
6	PHOSPHORUS AND ZINC BIOAVAILABILITY IN WHEAT GROWN IN THE CALCAREOUS SOILS OF THE KURDISTAN REGION	161
6.1	Introduction	161
6.2	Methods and materials.....	163
6.2.1	Soil and wheat survey in Sulaimanyah province.....	163
6.2.2	Soil analysis	166
6.2.3	Wheat grain, flour, and bread analysis.....	167
6.2.4	Statistical analysis	167
6.3	Results and discussion.....	168
6.3.1	Hierarchical clustering analysis for surveyed soils.....	168
6.3.2	Soil characteristics	169
6.3.3	Solid $\rightleftharpoons$ liquid partition coefficient (Log K_d)	174
6.3.4	Phosphate and zinc solubility in surveyed soils.....	176
6.3.5	Phosphorus and Zn contents in wheat grain, flour, and bread	181
6.3.6	Hierarchical clustering analysis in wheat grains.....	190
6.3.7	PA concentration and PA/Zn molar ratio.....	192
6.3.8	Estimated Zn bioavailability.....	199
6.4	Conclusions.....	207
7	SUMMARY, CONCLUSION AND FUTURE WORK.....	210
	SUMMARY	210
7.1	Phosphorus status in soils and crops	210

7.2	Zinc status in the soil and crop plants.....	212
7.3	Phosphorus interactions with Zn	214
7.4	Phytic acid content and PA to Zn molar ratio	215
7.5	Estimated human Zn bioavailability	217
7.6	Future work and wider considerations.....	218
REFERENCES		220

TABLE OF FIGURES

Fig. 3.1: Map of Sulaimanyah province showing soil collection sites. (A) Sulaimanyah province showing selected sites and (B) Iraqi map highlighting Sulaimanyah province.	46
Fig. 3.2: Soil sampling around Tanjaro site in Sulaimanyah.	47
Fig. 3.3: Soil sampling location around Halabja vegetable site in Halabja location.....	48
Fig.3.4: Soil sampling location around the waste site in Halabja.	49
Fig. 3.5: Soil sampling locations around the vegetable field site in the Sirwan location.	50
Fig.3.6: Soil sampling locations around the Khormal site.	51
Fig. 3.7: Soil sampling locations around the Grdagozina site in Kalar.	52
Fig. 3.8: Soil sampling locations around the Sirwan site in Kalar.	53
Fig. 3.9: The average values of total P concentration in studied soils collected at seven different locations in Sulaimanyah province. The source of irrigation water in STA, KHO and HAL was water waste from cities, in KAS and KAG was leake and in SVF and HWA was well water (clean water).	58
Fig. 3.10: The relationship between total P and soil pH in studied soils collected in seven different sites in the agricultural activity fields.	58
Fig. 3.11: The average total concentration of Zn_T in 21 soils from seven locations (Table 3.3). The source of irrigation water in STA, KHO and HAL was water waste from cities, in KAS and KAG was leake and in SVF and HWA was well water (clean water).	60
Fig. 3.12: Correlation between loss on ignition and Zn_T in 21 soils from seven locations (Table 3.3).	60
Fig. 3.13: Correlations between (A) P_T and P_{Olsen} and (B) P_{Olsen} and P_{Sol} in soils from seven study locations (Table 3.3).	62
Fig. 3.14: Mean change in P_{Sol} ($mg\ L^{-1}$) over time after incubation for 1, 7, 14, 28 and 56 days.	64
Fig. 3.15: Correlation between P_{Olsen} and P_{Sol} in studied soils before incubation.....	65
Fig. 3.16: The average of P Quantity-intensity relationship for studied soils, expressing P intensity concentration (X-axis) (A) $HPO_4^{=}$ and (B) $H_2PO_4^{-}$ as a function of P quantity	

'Olsen-P'. The studied soils from Kurdistan were incubated for (○) 1 day, (□) 7 days, (◇) 14 days, (△) 28 days and (x) 56 days.	65
Fig. 3.17: Correlations between solid-liquid partition coefficient in studied soil (n=21) with (A) soil pH, (B) SIC% content and (C) total Ca content.	67
Fig. 3.18: Phosphate solubility in the studied soils in relation to crystalline Ca-P minerals: monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; β-tricalcium phosphate, β-TCP; and hydroxyapatite, HAP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by Lindsay (1979). The points in the diagram separated with different color depending on its index (amount of available P (mg kg^{-1}) as P_{Olsen} in the soil); including index 2 (10-20), index3 (20-30), index4 (30-40), index5 (40-50), index6 (50-60) and index8 (>80) mg kg^{-1} P_{Olsen} in the soil.	69
Fig. 3.19: Correlation between (A) Zn_T and Zn_{DTPA} , (B) Zn_T and Zn_{Sol} , and (C) Zn_{DTPA} and Zn_{Sol} in the studied soils collected at seven deferent sites in the agricultural activity fields.	71
Fig. 3.20: Mean change in Zn_{Sol} ($\mu\text{g L}^{-1}$) over time following incubation for 1, 7, 14, 28 and 56 days.....	73
Fig. 3.21: Correlation between Zn_{DTPA} and Zn_{Sol} in studied soils.	73
Fig. 3.22: The average Zn Quantity-intensity relationship for studied soils, expressing Zn intensity concentration (Zn_{Sol} , X-axis) as a function of Zn quantity Zn_{DTPA} . The studied soils from Kurdistan were incubated for (○) 1 day, (□) 7 days, (△) 14 days, (◇) 28 days and (x) 56 days.	74
Fig. 3.23: Correlations between solid-liquid partition coefficient in studied soil (n=21) with (A) soil pH, (B) soil Ca content and (C) soil SIC% in studied soils (n=21).	76
Fig. 3.24: Stability or predominance diagram for soil data and the Zn minerals: amorphous Zn carbonate, hydrozincite, zincite, smithsonite and 'soil-Zn'. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Lindsay, 1979, Nazif et al., 2015, Urgan and Rimmer, 200). The points in the diagram separated with different color	

depending on its index (amount of available Zn (mg kg^{-1}) as Zn_{DTPA} in the soil); including index 1 (0-1), index2 (2-3), index3 (3-4), index4 (4-5), and index8 (>5) mg kg^{-1} Zn_{DTPA} in the soil. 78

Fig. 3.25: Hierarchical cluster analysis of elemental analysis data of soils ($n=21$) collected in Sulaimanyah province, Kurdistan at seven different sites. 81

Fig. 4.1: The percentage of adsorbed phosphorus in the two calcareous soils collected from Sulaimanyah province, Kurdistan region, Iraq: Soil 1 (filled symbols) and Soil 2 (open symbols). 97

Fig. 4.2: Effect of phosphate fertilizer application rates on adsorption of P in the two calcareous soils collected from Sulaimanyah province, Kurdistan region, Iraq: Soil 1 (filled symbols) and Soil 2 (open symbols). 98

Fig. 4.3 Phosphate adsorption isotherms for Soils 1 and 2, expressing solution P concentration (X-axis) as a function of 'adsorbed Olsen-P' (A and C) and 'total adsorbed P' (B and D). The two calcareous soils from Kurdistan were incubated with phosphate for (●) 1 day, (▲) 7 days, (■) 14 days, (○) 28 days, (△) 56 days and (□) 84 days respectively. In graphs A and C three models are fitted to the entire 'reactive phosphate' dataset across all incubation times. In graphs B and D the best-fit model line achieved for the Olsen P data is shown to illustrate the progressive adsorption (fixation) reaction against a consistent relationship between solution P and reactive adsorbed P. 102

Fig. 4.4: Phosphate solubility in the studied soils in relation to crystalline Ca-P minerals; monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; β -tricalcium phosphate, β -TCP; and hydroxyapatite; HAP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by Lindsay (1979). The two calcareous soils from Kurdistan were incubated with phosphate for (black) 1 day, (blue) 7 days, (red) 14 days, (orange) 28 days, (yellow) 56 days and (green) 84 days respectively. 103

Fig. 4.5: The percentage of ^{70}Zn adsorbed by two calcareous soils, (●) Soil 1 and (○) Soil 2, from Sulaimanyah province, Kurdistan region, Iraq. Adsorption was determined from

the difference between ^{70}Zn added and the solubility measured in 0.01 M $\text{Ca}(\text{NO}_3)_2$ suspension.	105
Fig. 4.6: Effect of ^{70}Zn isotope application rates on the amount of adsorbed Zn in Soil 1 (●) and Soil 2 (o).	105
Fig. 4.7: Zinc adsorption isotherms for Soils 1 and 2, expressing solution ^{70}Zn concentration (X-axis) as a function of 'adsorbed DTPA- ^{70}Zn ' (A and C) and 'total adsorbed ^{70}Zn ' (B and D). The two calcareous soils from Kurdistan were incubated with ^{70}Zn for (●) 1 day, (▲) 7 days, (■) 14 days, (○) 28 days, (△) 56 days and (□) 84 days respectively. In graphs A and C three models are fitted to the entire 'reactive ^{70}Zn ' dataset across all incubation times. In graphs B and D the best-fit model line achieved for the DTPA- ^{70}Zn data is shown to illustrate the progressive adsorption (fixation) reaction against a consistent relationship between solution ^{70}Zn and reactive adsorbed ^{70}Zn	112
Fig. 4.8: Zinc solubility in studied soils in relation to crystalline Zn minerals; amorphous (ZnCO_3), hydrozincite, zincite, soil- zinc and smithsonite. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Nazif et al., 2015, Lindsay, 1979, Urgan and Rimmer, 2000). The two calcareous soils from Kurdistan were incubated with phosphate for (grey) 1 day, (blue) 7 days, (orange) 14 days, (yellow) 28 days, (red) 56 days and (green) 84 days respectively.	114
Fig. 5.1: The average values of P_{Olsen} for maize, cowpea and tomato plots at harvest as a function of fertilizer P application. For direct comparison, applied fertilizer values (kg ha^{-1}) were converted to units of mg kg^{-1} assuming 2500 t ha^{-1} of topsoil. (□) maize plots, (Δ) cowpea plots and (O) tomato plots.	130
Fig. 5.2: Correlations between P_{Sol} and P_{Olsen} in (□) maize, (o) cowpea and (Δ) tomato plots.	130
Fig. 5.3: Solubility diagram comparing soil phosphate with a range of crystalline Ca-P minerals: monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; and β -tricalcium phosphate, β -TCP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by	

Lindsay (1979). The soil was from plots used to grow (A) maize, (B) cowpea and (C) tomato; soil sampling was at harvest.....	134
Fig. 5.4: Solubility diagram comparing test soils with a range of Zn minerals: amorphous, hydrozincite ($\text{Zn}_5(\text{CO}_3)_2(\text{OH})_5$), zincite (ZnO), and smithsonite (ZnCO_3). Equilibrium lines for mineral phases were calculated based on K_{sp} values reported by Nazif et al., (2015), Lindsay, (1979) and Urgun and Rimmer, (2000). For (A) maize, (B) cowpea and (C) tomato plots at harvesting.	137
Fig. 5.5: Fractionation (%) of P in soils: $\text{Ca}_2\text{-P}$ = Dicalcium phosphate; $\text{Ca}_8\text{-P}$ = P associated with OCP; Re-adsorbed-P; Al-P = associated Al oxides; Occluded P; $\text{Ca}_{10}\text{-P}$ = HP; Organic-P.....	139
Fig. 5.6: The concentration of phosphorus (mg kg^{-1} dry matter) in ($\square$), root, (o) shoot and (Δ) grain, seed or fruit of (A) maize, (B) cowpea and (C) tomato plants at harvest....	142
Fig. 5.7: Concentration of Zn (mg kg^{-1} dry matter) in the shoot, grain, seed and fruit of maize, cowpea and tomato plants at harvest and at different P and Zn application rates; (0, 50, 100 and 150) kg P ha^{-1} and (($\square$) 0, (Δ) 0.8 and (O) 1.2) kg Zn ha^{-1} for maize and (0, 40, 80 and 120) kg P ha^{-1} and (($\square$) 0, (Δ) 0.4 and (O) 0.8) kg Zn ha^{-1} for cowpea and tomato.....	145
Fig. 5.8: Correlations between V and Fe elements in maize, cowpea and tomato (A) shoots and (B) roots.....	149
Fig. 5.9: Effect of P and Zn fertilizer application on (A) PA and (B) PA to Zn molar ratio in maize grain, cowpea seed, and tomato fruit; (0, 50, 100 and 150) kg P ha^{-1} and (($\square$) 0, (O) 0.8, and (Δ) 1.2) kg Zn ha^{-1} for maize grain and (0, 40, 80 and 120) kg P ha^{-1} ($\square$) control, (O) 0.4, and (Δ) 0.8) Zn ha^{-1} for cowpea grain and tomato fruit.	153
Fig. 5.10: Effect of P and Zn fertilizers application rates on estimated bioavailable Zn in maize grain, cowpea seed, and tomato fruit; (0, 50, 100 and 150) kg P ha^{-1} ; (($\square$) 0, (O) 0.8, and (Δ) 1.2) kg Zn ha^{-1} for maize grain and (0, 40, 80 and 120) kg P ha^{-1} and (($\square$) control, (O) 0.4, and (Δ) 0.8) Zn ha^{-1} for cowpea grain and tomato fruit. Using three	

different sets of parameter values for K_R and K_P from (A) Imran et al. (2016), (B) Brown et al. (2010) and (C) Miller et al. (2007).	156
Fig. 5.11: Effect of the concentration of PA intake (mg d^{-1}) on the percentage of estimated Zn bioavailability in 300 g of (O) maize grain, ($\square$) cowpea seed and (Δ) tomato fruit; using three different parameters of K_R and K_P from (A) Imran et al. (2016), (B) Brown et al. (2010) and (C) Miller et al. (2007).	158
Fig. 6.1: Surveyed wheat and corresponding soil samples locations in Sulaimanyah province, Kurdistan region, Iraq.	164
Fig. 6.2: Wheat and corresponding soils sampling design.	165
Fig. 6.3: Dendrogram for hierarchical cluster analysis of soil characteristics, macro and microelement contents in surveyed soils ($n=120$), collected at ten locations in Sulaimanyah province, Kurdistan region, Iraq.	169
Fig. 6.4: The distribution of P_T in the studied soils ($n=120$) collected from wheat farms at ten locations in Sulaimanyah province, Kurdistan region, Iraq.	172
Fig. 6.5: The distribution of Zn_T in the studied soils ($n=120$) collected from wheat farms at ten locations in Sulaimanyah province, Kurdistan region, Iraq.	173
Fig. 6.6: Correlations between (A) P_{Olsen} and pH, (B) P_T and pH, (C) P_{Olsen} and P_T , (D) Zn_T and pH, and (E) Zn_T and LOI in soils ($n = 120$), collected in wheat fields at ten locations in Sulaimanyah province, Kurdistan region, Iraq.	174
Fig. 6.7: Correlation between solid-liquid partition coefficient (Zn_T/Zn_{Sol}) and loss on ignition (LOI) in surveyed soils.	176
Fig. 6.8: Solubility diagram for phosphate minerals in the surveyed soils in relation to crystalline Ca-P minerals: dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; β -tricalcium phosphate, β -TCP; and hydroxyapatite; HA. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Lindsay, 1979).	178
Fig. 6.9: The solubility of Zn in the studied soils in relation to possible Zn minerals: amorphous (amorphous, $ZnCO_3$), hydrozincite, zincite, soil-Zn, and Smithsonite.	

Equilibrium lines for mineral phases were calculated based on the Ksp values reported by (Nazif et al. 2015).....	180
Fig. 6.10: The concentraion of P_T and Zn_T in ($mg\ 300g^{-1}$) in wheat, flour and bread in the studied samples (120 wheat, 50 flour and bread samples).....	181
Fig. 6.11: The concentrations of phosphorus in wheat grain samples ($n=120$) collected at ten different locations in Sulaimanyah province, Iraq.	182
Fig. 6.12: Correlations between P concentration in wheat grain samples with (A) soil pH and (B) available P in soils across all the surveyed wheat grain samples ($n=120$) collected at different locations in Sulaimanyah province, Iraq.....	183
Fig. 6.13: The concentrations of zinc in wheat grain samples ($n=120$) collected at ten different locations in Sulaimanyah province, Iraq.	184
Fig. 6.14: The correlation between (A) P_{Olsen} and (B) P in wheat grain with Zn concentrations in the wheat grains and ($n=120$).	186
Fig. 6.15: Correlations between P (A) and Zn (B) concentration in flour and corresponding bread samples collected at locations in Sulaimanyah province.....	190
Fig. 6.16: Dendrogram analysis of the hierarchical cluster analysis of soil characteristics with the elemental composition of wheat grain samples ($n=120$).	191
Fig. 6.17: Correlation between Fe and Al, and Fe and V, across all studied wheat grain samples.	192
Fig. 6.18: Distribution of phytic acid (PA) in wheat grain samples ($n=120$), collected at ten different locations in Sulaimanyah province, Kurdistan region, Iraq.	193
Fig. 6.19: Correlation between PA content in wheat grains and available P in corresponded soil ($n=120$).	194
Fig. 6.20: Distribution of phytic acid to zinc molar ratio (PA/Zn) in wheat grain samples ($n=120$), collected at ten different locations in Sulaimanyah province, Kurdistan region, Iraq.....	195
Fig.6.21: Estimated Zn bioavailability in $mg\ d^{-1}$ for consumption of 300 g wheat grain. Samples were collected from ten different locations in Sulaimanyah province; Zn	

bioavailability was estimated using trivariate models with three different values for K_{MAX} , K_P and K_R reported by (Δ) Imran et al., (2016), (o) Brown et al., (2010) and ($\diamond$) Miller et al., (2007). 201

Fig. 6.22: Correlations between estimated Zn bioavailability (%) and PA intake from consumption of 300 g day⁻¹ of wheat grain. Samples were collected from ten different locations in Sulaimanyah province; human Zn bioavailability was calculated using trivariate models with different A_{MAX} , K_R and K_P values reported by (Δ ; $R^2= 0.6647$) Imran et al. (2016), (O; $R^2=0.8288$) Brown et al. (2010) and ($\diamond$; $R^2=0.823$) Miller et al. (2007).. 201

Fig. 6.23: Estimated Zn bioavailability and measured PA intakes from 300g day⁻¹ of flour or bread. Samples were collected from seven different locations in Sulaimanyah province; estimated human Zn bioavailability was calculated using trivariate models with different A_{MAX} , K_R and K_P values reported by (O) Imran et al. (2016), (Δ) Brown et al. (2010) and ($\diamond$) Miller et al. (2007)..... 205

Fig. 6.24: Correlation between estimated Zn bioavailability from consumption of 300g day⁻¹ of bread or corresponding flours. Values were estimated from trivariate models with different A_{MAX} , K_R and K_P values reported by (O) Imran et al. (2016), (Δ) Brown et al. (2010) and ($\diamond$) Miller et al. (2007). 206

Fig. 6.25: Correlation between phytic acid and the concentration of P in four (O) and bread (Δ) samples collected from seven different locations in Sulaimanyah province. 206

TABLE OF TABLES

Table 1.1: Daily phosphorus intake depending on age during 1999-2000 in the USA. ..	25
Table 1.2: Dietary intake reference and tolerable upper levels for P according to (Gal and Dahl, 2013; Kiela et al., 2017).....	26
Table 2.1: Calcium phosphate phases, reactions and Ksp used in the construction of a phosphate solubility diagram.	36
Table 2.2: Zinc minerals, reactions and Ksp used to draw a Zn solubility diagram.	37
Table 2.3: Modified sequential fractionation approach for phosphate fractionation.	37
Table 2.4: Reference values (A_{MAX} , K_R , and K_P) used for the calculation of human Zn bioavailability.	41
Table 3.1: Soil sample locations and sites details.	45
Table 3.2: Sections and chapter used for soil analysis properties.....	54
Table 3.3: Values of soil pH, %LOI and %SIC; (n=21). Data are organized by the field code.	56
Table 3.4: The average values of P_T , P_{Olsen} in ($mg\ kg^{-1}$) and P_{Sol} ($mg\ L^{-1}$) in studied sites.	62
Table 3.5: Values of P_{Olsen} and P_{Sol} in studied soils; mean values with standard deviations.	63
Table 3.6: The average values of $\log K_d$ for $H_2PO_4^-$ in studied sites.	66
Table 3.7: Average values of Zn_T , Zn_{DTPA} and Zn_{Sol} in 21 soils collected from seven different locations.	70
Table 3.8: The values of Zn_{DTPA} and Zn_{Sol} and standard deviation in 21 soils sampled...	72
Table 3.9: The average and standard deviation of $\log K_d$ Zn values for studies sites at Sulaimanyah province.....	75
Table 3.10: Correlation matrix presenting significant positive and negative relationships (r values) among soil pH, %LOI, %SIC, macro and micro elements in the studied soils (n=21).	80

Table 4.1: Soil characteristics of the two selected calcareous soils used in the experiment.	95
Table 4.2: The proportion (%) of phosphorus recovery ($\pm$ SEM) as NaHCO_3 -extractable P (P_{Olsen}) in two soils used in the study.	99
Table 4.3: Correlations between P_{Olsen} and P_{Sol} in the two studied soils incubated up to 84 days.	100
Table 4.4: The recovery of $^{70}\text{Zn}_{\text{DTPA}}$ in (mg kg^{-1}) in the two soils used in the study, which were incubated for days and at laboratory condition with different applied amount of ^{70}Zn	107
Table 4.5: Effect of time of incubation and Zn application rates on Zn E-values ($\pm$ standard deviation) for the two soils.	108
Table 4.6: The proportion of Zn_E of total added ^{70}Zn extracted by DTPA in the two studied soils which were incubated for 84 days with different level of applied ^{70}Zn isotope.....	109
Table 5.1: Meteorological data for growing season (March-August, 2016)*.....	126
Table 5.2: Average soil chemical properties of the experimental plots.	127
Table 5.3: The average pH values for maize, cowpea and tomato plots at harvest.	128
Table 5.4: Effect of applied phosphate fertilizer on the solid-liquid partition coefficient of applied P ($\log K_d P$) for maize, cowpea and tomato plots. Values of $\log K_d P$ were calculated as $\log_{10}(P_{\text{Olsen}}/P_{\text{Sol}})$	131
Table 5.5: The average concentrations of Zn_{DTPA} in maize, cowpea and tomato plots.	132
Table 5.6: Phosphorus fractions in maize, cowpea and tomato plots.	139
Table 5.7: The concentration of P and Zn in (A) maize, (B) cowpea and (C) tomato roots, shoots, seeds and fruit.....	146
Table 5.8: The estimated proportion (%) of macro and micro-element concentration in maize, cowpea and tomato roots, shoots, grain, seed and fruit arising from soil dust.	150
Table 6.1: Wheat varieties collected at ten different locations in Sulaimanyah province.	163

Table 6.2: Bread type with corresponded flours collected at seven different locations in Sulaimanyah province.....	166
Table 6.3: Maximum, median and minimum values of P_T , Zn_T and Ca , P_{Olsen} , Zn_{DTPA} , P_{Sol} , Zn_{Sol} , soil pH and LOI for all the studied soils (n=120): Data are arranged in order of decreasing average Ca concentration for each location.....	170
Table 6.4: The logarithm of the solid-liquid partition coefficient ($\log K_d$) for P and Zn adsorption in surveyed soils (n=120) cultivated with wheat grain at ten locations in Sulaimanyah province, Kurdistan region, Iraq.	176
Table 6.5: The concentration of P and Zn in flour and corresponding bread samples collected at seven different locations in Sulaimanyah province. Data are arranged in descending order of the average P concentration in flours.	188
Table 6.6. Comparison of Phytic acid content in wheat grain from several countries...	193
Table 6.7: Phytic acid and phytic acid to Zn molar ratio in 50 flour and corresponding bread samples collected in Sulaimanyah province, Kurdistan region, Iraq.....	198
Table 6.8: Estimated Zn bioavailability contents for flour and corresponding bread samples collected from seven different locations in Sulaimanyah province. The estimated values of Zn bioavailability were calculated using trivariate models with different A_{MAX} , K_R and K_P	203

VOCABULARY

Zn: Zinc

Zn_T: total zinc in soils or in plants

Zn_{DTPA}: available zinc extracted by diethylene triamine penta acetic acid

Zn_{sol}: soluble zinc in soils

%Zn: proportion of zinc

Zn_E: zinc E-value

⁷⁰Zn: zinc 70 isotope

P: Phosphorus

P_T: total phosphorus in soils or in plants

P_{Olsen}: available phosphorus extracted by Olsen method

P_i: inorganic phosphorus

%P: proportion of phosphorus

WHO: Water holding capacity

LOS: Loss on ignition

ICP-MS: Inductively coupled plasma mass spectrometry

DIC: dissolved inorganic carbon

DOC: dissolved organic carbon

SIC: soil inorganic carbon

SOC: soil organic carbon

Ca: calcium

CaCO₃: calcium carbonate

Fe: Iron

Al: aluminium

DCP: dicalcium phosphate

DCPD: dicalcium phosphate dihydrate

OCP: octacalcium phosphate

β-TCP: beta tricalcium phosphate

HA: hydroxyapatite

PA: phytic acid (phytate)

PA/Zn molar ratio: phytic acid to zinc molar ratio

Log K_d: logarithm of solid⇌liquid partition coefficient

CRBD: completely randomized block design

ABSTRACT

The key objectives of the present project were to determine (i) the chemical form, solubility and bioavailability of zinc (Zn) and phosphorus (P) in the calcareous soils of Iraqi Kurdistan and their possible interactions, (ii) the uptake of Zn and P by crop plants and the viability of biofortification and (iii) the transfer of Zn and P into the human diet and their possible interactions through Zn binding to phytate.

A survey was conducted including a collection of 21 soil samples at seven different agricultural activity sites in Sulaimanyah province, Iraqi Kurdistan. The availability of both P and Zn nutrients for plant uptake was determined through their exchangeable and soluble amounts in the studied soils. Time-dependent changes in solubility were determined by incubation of selected soils up to 56 days at $20 \pm 2^\circ\text{C}$ and at half of their water holding capacity (WHC). The impacts of soil factors, including soil pH, inorganic carbon content (SIC) and soil organic carbon content (SOC), on the plant-available and soluble fractions of P and Zn and the solid $\rightleftharpoons$ liquid partition coefficient ($\log K_d$) of both elements were determined. Total P (P_T) and total zinc (Zn_T) concentrations in all the studied soils were within the range of 622-1210 and 43-187 mg kg^{-1} , respectively, which were in the range of P and Zn concentrations reported globally. The pH values were 7.33-8.27, soil inorganic carbon was in the range of 0.67-4.92% indicating the calcareous nature of the studied soils, and SOC was in the range 5-12%. Approximately 8% of P_T was in the form of available P (sodium bicarbonate extractable P; P_{Olsen}) and 3% of Zn_T was in the form of available Zn (extractable with 0.005 M DTPA; Zn_{DTPA}). Furthermore, only about 2% of P_{Olsen} was in the form of soluble P (soluble in 0.01 M $\text{Ca}(\text{NO}_3)_2$; P_{sol}) and 0.3% of Zn_{DTPA} was in the form of soluble Zn (Zn_{sol}). Average values of $\log k_d$ when available divided by soluble were 2.30 for P and 3.25 for Zn, indicating stronger adsorption of Zn than P. This is indicative of the lack of Zn fertilizer application to soils in Kurdistan because farmers only apply NPK fertilizers. A negative correlation was determined between P_T and soil pH value whereas Zn_T was positively correlated

with loss on ignition. Values of P_{Olsen} , P_{Sol} , Zn_{DTPA} and Zn_{Sol} were positively correlated with their total soil concentrations, P_T and Zn_T . Similarly, positive correlations were also observed between P_{Sol} and P_{Olsen} as well as between Zn_{Sol} and Zn_{DTPA} , while all four variables were negatively correlated with soil pH value. Log k_d for Zn was negatively correlated with LOI but there was no correlation between soil pH value and Log K_d values possibly due to a narrow range of pH values.

To further examine P and Zn solubility, soils were incubated at half their water holding capacity (50% WHC) for 1, 7, 14, 28, and 56 days, without P and Zn application. P and Zn solubilities in soil suspended in 0.034 M $\text{Ca}(\text{NO}_3)_2$ were measured; the Ca concentration was determined from a Q/I relation as the natural equilibrium Ca concentration. Following solution speciation, using the geochemical speciation model WHAM-7, P and Zn solubility data were presented on stability diagrams comprising (i) phosphate potential ($\log \text{H}_2\text{PO}_4^- - \text{pH}$) against lime potential ($\log \text{Ca}^{2+} + 2\text{pH}$) and (ii) the $\log_{10}$ activity of Zn ($\log \text{Zn}^{2+}$) against pH. Results indicated that P and Zn solubility decreased with increasing incubation time and increasing soil pH, and increased with increasing 'available' P and Zn (P_{Olsen} and Zn_{DTPA}). Construction of solubility diagrams and determination of P_{Sol} and Zn_{Sol} fractions suggested that P release was controlled by a series of potential minerals, including dicalcium phosphate (DCP), dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), β -tricalcium phosphate (β -TCP) and hydroxyapatite (HP), depending on the phosphate status of the soil. By contrast, Zn was undersaturated with respect to Smithsonite.

A study was conducted to evaluate the effect of time of incubation on P and Zn adsorption and solubility in the calcareous soils. Two calcareous soils (pH 8.10 & 8.24, CaCO_3 content 6.13 & 41.0%, and clay content 43.0 & 31.4%) from Sulaimanyah province, Iraqi Kurdistan, were treated with several levels of P (0, 50, 100, 200, and 300 mg kg^{-1}) as KH_2PO_4 and the enriched stable isotope ^{70}Zn (0, 2, 5, 10, and 20 mg

kg⁻¹) and incubated for 1, 7, 14, 28, 56, and 84 days at 50% of their water holding capacity under laboratory conditions with three replicates. The results indicated that adsorbed P and Zn, P_{Olsen}, Zn_{DTPA}, P_{sol} and Zn_{sol}, significantly increased with increase in the application rates but decreased with time of incubation. The 'apparent' E-value of ⁷⁰Zn (Zn_E) increased with increasing Zn application and incubation time in both soils as the added isotope gradually mixed with increasingly non-labile soil Zn pools.

At the end of the experiment (84 days), about 80 and 72% of applied P and 97.7 and 99.5% of applied ⁷⁰Zn were adsorbed by soil components at 300 mg P kg⁻¹ and 20 mg ⁷⁰Zn kg⁻¹ applied levels in Soils 1 and 2 respectively. The Langmuir-Freundlich model described the 'available' P and Zn solubility data (P_{Olsen} vs P_{sol} and Zn_{DTPA} vs Zn_{sol}) well for both studied soils – regardless of incubation time. By contrast, adsorption isotherms constructed using total adsorbed P and Zn showed a systematic change with incubation time, indicating prolonged time-dependent P and Zn fixation in the calcareous soils. The lability (%Zn_E) in the two selected soils was 70.4 and 90.5% of extracted ⁷⁰Zn and 13.8 and 8.86% of total applied Zn recovered when the all the applied Zn levels were considered in Soils 1 and 2 respectively. At the end of the experiment, the solubility of P appeared to be controlled by OCP and β-TCP while the solubility of Zn was controlled by Smithsonite or was undersaturated. Application of Zn fertilizer directly to the calcareous soils of Kurdistan would appear to be non-viable due to their high capacity for continued time-dependent Zn adsorption and fixation.

To investigate the interaction between P and Zn and viability of Zn biofortification in a range of crops grown in the calcareous soil of Kurdistan region, a field experiment was carried out during the early season (March-August 2016) at the technical college of Halabja, Sulaimanyah, Iraqi Kurdistan. The trial was a completely randomized block design (CRBD), with twelve treatments and five replicates. The treatments included (i) four levels of P (0, 50, 100 and 150 kg ha⁻¹) as triple super phosphate (TSP 20% P) and three levels of Zn (0, 0.8 and 1.2 kg ha⁻¹) applied as foliar Zn-EDTA (5% Zn) to

maize and (ii) four levels of P (0, 40, 80 and 120 kg ha⁻¹) and three levels of Zn (0, 0.4 and 0.8 kg ha⁻¹) applied to maize, cowpea and tomatoes. At harvest, both P_{Olsen} and P_{sol} significantly increased with increasing rates of applied P, but the availability and solubility of P and Zn also decreased as time passed. Phosphorus solubility was undersaturated with respect to OCP and Zn solubility was undersaturated with respect to Smithsonite. The result of P fractionation showed that most of the applied phosphate had been re-distributed between P fractions in the same proportion as the native soil P.

The nutrient concentrations in shoots, grain, seed and fruits were determined, along with the phytic acid (PA) content of maize grain, cowpea seed and tomato fruit. The P and Zn concentrations in shoots, grains and fruits were increased by the application of P and Zn fertilizers compared with control, but the applications were more effective in shoots than in either grain, seed or fruit when compared with controls. The shoot P and Zn application were more effective in single P and Zn application than in a combined P+Zn treatment. The PA and PA/Zn molar ratio in the edible part of the crops increased with the increased rate of P applied and decreased with increasing Zn rates. Furthermore, the estimated human Zn bioavailability in grain, seed and fruit also increased with increasing Zn rates and decreased with P rates, indicating that foliar application of crops with Zn fertilizer may be a viable strategy for increasing bioavailable Zn in the crops grown in the calcareous soils of Kurdistan.

To evaluate the bioavailability of P and Zn in the soil, wheat, flour and bread and PA, PA to Zn molar ratio and estimated Zn bioavailability contents of wheat grain grown in the calcareous soils, a survey of flour and bread samples was undertaken in Sulaimanyah province, Kurdistan region, Iraq. Since wheat is the staple carbohydrate source in the region, a total of 120 farmers' cereal fields were surveyed for soil and

wheat grain samples along with 50 flour samples and corresponding bread samples which were collected in bakery markets.

At harvest, the soil samples were analysed for P_T , Zn_T , P_{Olsen} , Zn_{DTPA} , P_{Sol} , and Zn_{Sol} . The values ($mg\ kg^{-1}$) were within the range of 403 -3740 (P_T), 42.0-203 (Zn_T), 2.13-28.1 (P_{Olsen}), 0.14-5.23 (Zn_{DTPA}), 0.07-9.91 $mg\ P\ L^{-1}$ (P_{Sol}) and 0.21-9.46 $\mu g\ Zn\ L^{-1}$ (Zn_{Sol}); pH was in the range 7.46-8.67, indicating that all the soils were calcareous. The values of Log K_d were in the range 1.65 ± 0.47 - 0.40 ± 0.28 for P and 3.00 ± 0.30 - 2.32 ± 0.29 for Zn. The results of soils analysis showed that P and Zn concentrations were in the range reported globally. However, about 83% and 53% of soil samples contained low concentrations of P_{Olsen} and P_{Sol} , below-recommended values of 10 $mg\ kg^{-1}$ P_{Olsen} and 0.5 $mg\ L^{-1}$ P_{Sol} . Furthermore, about 78% and 98% of soil samples contained a Zn_{DTPA} and Zn_{Sol} below-recommended values of 0.5 $mg\ kg^{-1}$ Zn_{DTPA} and 4-270 $\mu g\ L^{-1}$ Zn_{Sol} . Again these figures reflect the high $CaCO_3$ content and high pH values in the surveyed soils and the absence of Zn fertilization by farmers. Analysis of solubility indicated that TCP and OCP are probably the main calcium phosphate minerals controlling the solubility of P and Smithsonite is the main Zn mineral which controls Zn solubility in the studied soils or probably indicates adsorption of Zn on the surface of $CaCO_3$, Fe oxides and on humus when undersaturated with respect to Smithsonite.

The concentrations of P, Zn, PA, PA/Zn molar ratio and estimated Zn bioavailability was also determined for wheat grain, flour and bread samples in various bread types including Hawrami, Tiry, Lawasha, Mashini and Samoon which are consumed in the Sulaimanyah community. The values of P, Zn and PA concentrations ($mg\ kg^{-1}$) were 3131-3625, 1051-1872 and 1195-1631 (P), 26.3-35.0, 7.04-17.2 and 8.90-14.9 (Zn) and 3000-11000, 1600-3100 and 800-1800 (PA), giving a PA/Zn molar ratio of 15.7-30.6, 17.8-22.9 and 8.96-14.6 for whole wheat grain flours, flours and breads respectively. The results suggest that the concentrations of P and PA are high, about

40% of wheat grains contain higher amounts than the recommended value of 3570 mg kg⁻¹ of P. This is probably due to extensive applications of phosphate fertilizers by farmers, typically 100-200 kg P₂O₅ ha⁻¹ in every growing season. However, Zn concentration in 88% of wheat grain samples was lower than the recommended concentration of 41.6 mg kg⁻¹ in whole wheat grains due to low available Zn in the surveyed soils.

A trivariate model was used to estimate intake of available Zn. Estimated bioavailable Zn (mg d⁻¹) was estimated assuming an average wheat, flour and bread consumption of 300 g day⁻¹ in the region. Consideration of the PA and Zn intake suggest only 21.5±2.9% of grain Zn, 43.6±6.52% of flour Zn, and 52.1±6.56% of bread Zn were bioavailable so that the effective Zn intake from wheat grain, flour and bread are only 1.84-2.63, 0.78-2.26 and 1.25-2.05 mg d⁻¹ for the local population respectively. The results indicated low Zn absorption from wheat grain, flour and bread. However, the Zn absorption from a common flatbread, 'Tiry', was higher than Mashini. The bread making processing had a major influence on estimated bioavailable Zn in the final product, producing reductions of 15-47% in PA and 17.0-61.0% in the PA/Zn molar ratio. The estimated bioavailable Zn increased between 2-41% in white flours and bread depending on the amount of daily PA intake concentration. Overall results suggest that available dietary Zn is below recommended levels (11 mg d⁻¹) which is partly due to low uptake by wheat but also due to the presence of large concentrations of PA in wheat grains and bread.

In general, the total concentration of P and Zn were found to be adequate in the Kurdistan soils. However, the bioavailability of both nutrients is low due to the calcareous nature of soils because of high calcium carbonate (CaCO₃) content, high pH value, and high adsorption and 'fixation' capacities. The concentrations of P and PA in the crop plants and wheat grain grown in the soils of Kurdistan were high due to the

application of phosphate fertilizers, while the Zn content was typically low, and under the recommended value in the case of wheat grain which is the staple daily food for the population of the Kurdistan region. The low concentration of Zn is due to some factors such as low soil Zn bioavailability, high application of phosphate fertilizers, P interaction with Zn in soils and plants, and absence of Zn biofortification as an agronomic strategy in the region. The daily Zn intake from crop plants is not adequate to meet the recommended Zn level in human populations due to a high level of PA and PA to Zn molar ratio. Biofortification of Zn is viable, but a key recommendation may be to reduce the application of phosphate fertilizer. The low intake of bioavailable Zn could cause health problems in the region. Given the current status of Zn in crop plants grown in Kurdistan, biofortification of Zn to increase Zn in staple crops is recommended. A crop breeding program combined with targeted agronomic management methods is also required to enhance both Zn uptake and its bioavailability in grains of cultivated wheat and crops.

1 INTRODUCTION

1.1 Zinc in soil and its availability

Total Zn concentration in uncontaminated soil varies depending on the mineralogy, the parent material, and particularly the concentration of quartz, which tends to dilute most elements (Kabata-Pendias and Mukherjee, 2008). The source of Zn in the soils is release from magmatic and sedimentary rocks to the soil during the weathering processes. The average amount of Zn in the Earth's crust is less than 1000 mg kg⁻¹ (Siddiqui, 2010). However, Zn concentration in a range of soils varied from 10 to 300 mg kg⁻¹ with the highest concentrations in calcareous and organic soils (Kabata-Pendias and Mukherjee, 2008). The lowest Zn concentration reported was 4 mg kg⁻¹ found in an Australian non-calcareous soil (<2% CaCO₃) and the highest concentration recorded in England and Wales across various soil types was 3550 mg kg⁻¹ (Alloway, 2009).

The surface soil Zn content increases with agricultural practice or application of organic and inorganic Zn fertilizers (Xiang et al., 1995). For example, agricultural soils in Japan had Zn concentrations between 59 and 99 mg kg⁻¹ caused by differences in agriculture practice (Kabata-Pendias and Mukherjee, 2007). The total quantity of Zn in soils is mainly distributed between five operational fractions: (i) water-soluble present in soil solution, (ii) exchangeable bound to soil particle surfaces, (iii) organically bound by humus, (iv) adsorbed into metallic oxides and clay mineral edges, and (v) in primary minerals (Alloway, 2008a). Zinc in the soil is present in all possible fractions but is often mainly in the residual fraction (60-99%) (Xiang et al., 1995).

There are several soil parameters which control Zn availability and behaviour. Kabata-Pendias and Mukherjee (2007) reported that in the soil solution Zn occurs as free and complex forms, including both anions (ZnCl₃⁻, Zn(OH)₃⁻, and ZnO₂²⁻) and cations (Zn²⁺,

ZnHCO_3^+ , ZnCl^+ , and ZnOH^+). Ahmed et al. (2012) reported that less than 1 mg Zn kg⁻¹ was dissolved in the soil solution in most soil types.

1.2 Factors affecting Zn availability

1.2.1 Soil pH

It is recognised that Zn availability to plants is affected by a number of soil factors, but the most significant factor is probably soil pH (Watts-Williams et al., 2013). At alkaline pH, Zn is most strongly adsorbed on humus and soil oxides (Lindsay, 1979) because of increased negatively surface charge (Nystrom, 2012) which then causes a decrease in Zn availability to plants. Sadeghzadeh (2013) showed that Zn concentration in soil solution varies systematically with pH; for example, at pH 8.0 the soil solution Zn concentration was around 10^{-10} M, whereas, at pH 5.0 it was around 10^{-4} M. Similarly, Hashemi and Baghernejad (2009) suggested that the solubility of Zn decreases about 100-fold for every unit increase in soil pH. Milivojevic et al. (2011) evaluated the impact of soil physical and chemical properties on Zn availability and distribution in Vertisols in Serbia. They found that exchangeable Zn (extracted with 0.1 M CaCl_2 at pH 7) in Vertisol increased with a decrease in the soil pH. However, the effect of soil pH is not entirely straightforward. In acid soils Zn^{2+} may precipitate as franklinite ($\text{ZnFe}^{\text{III}}_2\text{O}_4$) or, more likely, be adsorbed on organic matter that then controls Zn availability (Catlett et al., 2002, Duffner et al., 2012). On the other hand, Obreza et al. (2015) considered that alkaline pH values above 7 did not necessarily cause a decline in Zn availability. This was thought to be due to a lower pH in the rhizosphere ($\text{pH} < 7$), compared to the bulk soil, because of H^+ ions released from the plant roots. Furthermore, at alkaline pH values, complex forms of Zn occur in solution involving Zn^{2+} combinations with OH^- and HCO_3^- ions and reversible Zn adsorption on CaCO_3 .

1.2.2 Soil texture

Large clay contents in soils cause an increase in Zn sorption and decrease in Zn bioavailability due to increased soil cation exchange capacity (CEC) (Nystrom, 2012). Several reports have shown a significant correlation between soil clay content and Zn adsorption capacity or strength (de Matos et al., 2001). However, the proportion of exchangeable Zn has also been shown to increase with increasing sand and silt content (Milivojevic et al., 2011).

1.2.3 Soil mineralogy and calcium carbonate content

Despite substantial understanding of Zn chemistry in the soil, Zn deficiency still remains common in calcareous soils (Wenming et al., 2001, Cakmak, 2009, Kumar and Qureshi, 2012). In this type of soil, the availability of Zn is low due to both precipitation and adsorption processes (Jalali and Zinli, 2012) which can render about 90% of applied Zn fertilizer unavailable (Abid et al., 2013). Zinc is thought to precipitate as ZnCO_3 (Dahiya et al., 2005), Zn_2SiO_4 and $\text{Zn}(\text{OH})_2$ (Sadeghzadeh, 2013) and is adsorbed on soil colloids (Abid et al., 2013) and the surface of carbonate minerals (Jalali and Zinli, 2012). These processes reduce the concentration of soluble Zn available for plant uptake (Nystrom, 2012). It is generally recognised that adsorption is more important at low Zn concentrations and precipitation occurs as Zn concentration rises (Jalali and Zinli, 2012).

In calcareous soils, several factors other than CaCO_3 content may control the availability of Zn, such as the soil geocolloidal minerals present, soil organic matter content and soil solution anion concentration (Obrador et al., 2003, Jalali and Zinli, 2012). The results of Siddiqui and Khattak (2010) suggested that, in calcareous soils, Zn was mainly associated with oxides of Fe, Mn and Al. However, they derived this result from a sequential extraction procedure which are notoriously operational in nature. Sharma

et al. (2014) found that soil organic matter tended to increase available Zn when applied to incubated samples in an Aridisol.

1.2.4 Phosphorus (P) in the soil

Zinc availability in the soil is reduced due to high levels of P (Mousavi and Galavi, 2012, Drissi et al., 2015a, Zhu et al., 2001a) and Zn deficiency indicators in plants are often noted in the presence of large amounts of soil P (Soltangheisi et al., 2013). Phosphate-induced Zn deficiency can occur in various crops (Gianquinto et al., 2000) due to restriction of Zn uptake by P (Mousavi and Galavi, 2012).

Interactions between Zn and P in soils have been studied by many researchers but the relative importance of different mechanisms is still not absolutely understood (Drissi et al., 2015b, Zhu et al., 2001a, Watts-Williams et al., 2013). It has been shown that interaction occurs between P and Zn in the soil solution. But, P application also reduces Zn availability due to the precipitation of mixed Zn-phosphate minerals (Lambert et al., 2007).

The effect of P on Zn availability also depends on the type of P fertilizer. The application of rock phosphate and triple superphosphate both cause reduced Zn uptake into plant shoots (Wang et al., 2008b). However, Gupta et al. (2014) reported that application of di-hydrogen phosphate salts, such as KH_2PO_4 , NaH_2PO_4 , $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Ca}(\text{H}_2\text{PO}_4)_2$, significantly *increased* Zn uptake by *S. alfredii* in paddy soils. This seemed to be caused by an increase in Zn availability following a decrease in the soil pH value after application of the phosphate.

1.2.5 Kinetics of Zn fertilizer reaction

To provide available Zn (soluble and exchangeable) for plant uptake, ideally, Zn fertilizer should remain available for several years after application. However, a high

proportion of applied Zn is rendered unavailable for plant uptake because of reactions between the Zn applied and soil constituents such as strong adsorption on the surface of soil particles and precipitation (Alloway, 2009). Information about such reactions is required to understand the performance of different Zn fertilizers and the kinetics of their reactions with soil.

Zinc fertilizers applied to soil may undergo a range of time-dependent chemical, physical and biological reactions. Alvarez et al. (2008) suggested that the reaction between soil components and applied Zn may continue for more than several weeks but mechanistic investigations of Zn kinetics remain inconclusive (Reyhanitabar and Gilkes, 2010). In calcareous soils, the time-dependent precipitation of Zn hydroxide or carbonate and surface-adsorption by CaCO_3 are two major processes that regulate the availability of Zn (Hashemi and Baghernejad, 2009). This is well known that the efficiency of applied Zn fertilizers is gradually reduced with increasing soil CaCO_3 content because of increase in Zn adsorption (Reyhanitabar and Gilkes, 2010).

1.3 Plant uptake of Zn

It is estimated that almost 50% of the World's vital cereal-growing soils have sub-optimal amounts of plant available Zn (Watts-Williams et al., 2013) and in calcareous soils about 60- 85% of applied Zn may be rapidly converted to a fixed state (Xue and Huang, 1994). Zinc is one of several essential micronutrients needed by plants for development (Mousavi et al., 2013), growth (Wang et al., 2017, Alloway, 2009) and reproduction (Ahmed, 2013). Zinc is involved in many physiological functions of plants (Hafeez et al., 2013) and is an important element as a regulatory co-factor of a large number of enzymes (Ahmed, 2013). These enzymes are essential in biological processes involved in photosynthesis, conversion of sugars to starch and carbohydrate metabolism (Ahmad et al., 2012). In the soil, plants absorb Zn ions through their root system; Zn then moves through the xylem system to be stored in leaves and may then

be transported to grain through the phloem (Saha et al., 2017). Plant phenological stage, plant Zn and nitrogen status and variation between cultivars are important influences on translocation. Developed leaves are capable of nutrient export via the phloem to developing grains and other organs (Shahid et al., 2017). However, there are impairment factors, such as chelation processes and the high pH of phloem so that Zn movement from leaves to the edible parts of the plant is more efficient if foliar Zn application is used as a fertilizer source (Fernandez and Brown, 2013). Furthermore, the efficiency of Zn uptake differs between plants. Hart et al. (1998) reported that durum wheat is less 'Zn efficient' than bread wheat.

From soils, plants can only take up Zn in the form of Zn^{2+} ions (Mousavi, 2011), simple inorganic complexes such as $ZnOH^+$ and some low molecular weight organic complexes (Alloway, 2009) which are present in the exchangeable and water-soluble fractions (Dvorak et al., 2003, Reyhanitabar et al., 2011). Thus, in semi-arid and calcareous regions, Zn is a common nutritional problem for cereals and grains (Erenoglu et al., 2002) because only a small proportion of soil Zn is available to plants (Ahmad et al., 2012). Characterization of Zn 'availability' by various soil test methods represent attempts to measure potential soil Zn supply to plants (Cakmak and Marschner, 1993). A glasshouse trial conducted by Obrador et al. (2003) showed that plant Zn uptake from different Zn sources was associated with soluble and extractable Zn as determined by extraction with DTPA.

1.3.1 Soil factors affecting Zn uptake

Soil constituents and properties control Zn availability (Wang et al., 2017) through control over Zn concentration in the soil solution (Bouain et al., 2014). Generally, the soil factors identified as controlling availability of Zn to plants, include content of organic matter, sesquioxides and clay particles and soil pH (Dvorak et al., 2003, Ramesh et al., 2004, Obaid and Al-Hadethi, 2013). The quantity and type of anions in the soil solution

(Obrador et al., 2003), available P, soil moisture conditions (Karami et al., 2009, Ma et al., 2016) and the speciation of Zn dissolved in the soil solution (Tani and Barrington, 2005) have also been identified as factors. In the case of high pH soils, Zn is absorbed as Zn^{2+} or ZnOH^+ , but uptake is metabolically regulated, especially in the case of low Zn concentrations (Ahmed et al., 2012). In addition to the influence of soil characteristics, Mousavi et al. (2013) reported an antagonistic effect on Zn uptake by Fe and P. By contrast, Tani and Barrington (2005) reported that Cu^{2+} has a synergistic effect on Zn uptake by plants.

1.3.2 Plant controls over Zn uptake

The uptake of Zn by plants is increased due to some factors such as Zn fertilizer application and release of organic acids (e.g. malic and citric acids) to dissolve soil Zn (Sadeghzadeh, 2013). To characterise uptake of Zn by plants, Mousavi (2011) divided plants into three categories depending on their sensitivity to Zn deficiency: 'resistant' includes plants such as alfalfa, carrots, forage plant and radish; 'moderately sensitive' applies to sugar beet, tomato, barley, lettuce and soybean and 'most sensitive' includes rice, beans, onion, sorghum and corn.

Accumulation of Zn beyond the optimal range causes damage of various kinds. Accumulation of more than 300 mg kg^{-1} is toxic, causing growth inhibition and physiological alteration (Barrameda-Medina et al., 2017). Zinc accumulation in maize roots and shoots significantly increased when Zn concentration in the soil solution exceeded 3.25 mg L^{-1} (Islam et al., 2014). They also found that total soluble protein and chlorophyll content decreased with increasing Zn concentration. Cherif et al. (2011) divided the damage caused by excessive Zn in plants into three categories (i) leaf chlorosis, (ii) diminished root development, and (iii) reduced growth.

Plants can protect themselves to a degree against the negative impacts of excess Zn by limiting Zn uptake by roots (Stoyanova and Doncheva, 2002). Pre-soaking with a Zn-tolerant bacterial strain decreased the negative effect of Zn on plant growth (Islam et al., 2014). Also, plants can resist or control heavy metal toxicity by physiological mechanisms and an inter-related network of molecular strategies differ between plant species (Shahid et al., 2017). Using these strategies plants can decrease metal absorption and in-vivo transportation.

1.3.3 Plant Zn uptake and interaction with P

Phosphate has an antagonistic influence on Zn uptake by plant roots (Mousavi et al., 2013, Watts-Williams et al., 2013), although there is little information about specific mechanisms. Zhu et al. (2001a) showed that increasing P applications to soil decreased the uptake of Zn by plants. Zinc movement from plants roots to shoots also decreases with increasing application rate of P fertilizer (Zhang et al., 2016). Bogdanovic et al. (1999) suggested several mechanisms for the effect of P on Zn uptake: (i) simple dilution of the concentration of Zn in the upper parts of the shoots in response to the growth response of P (ii) P–Zn interactions in the soil, (iii) slower Zn translocation from the root to shoot of plants, (iv) metabolic changes inside plant cells correlated to a difference in the ratio Zn:P and (v) a large P concentration interfering with the metabolic function of Zn at certain sites in the cell. Furthermore, root morphology could be altered by P fertilization which would have an effect on plant uptake of Zn (Zhang et al., 2016). It has been suggested that the main interactions between P and Zn appear as change in plant metabolism (Bogdanovic et al., 1999) rather than a reduction in Zn solubility in the soil. Mousavi et al. (2011) reported three major explanations (i) within the plant, Zn ion valence is reduced due to increasing P concentration in the plants, (ii) a high concentration of P reduces Zn transmission from roots to shoots of plants and (iii) Zn will dilute in plant tissues due to an induced P growth response.

Ryan et al. (2008) showed that Zn concentration in wheat grain decreased by 36% when P concentration increased by 17%. However, the source of P fertilizer is another factor which has a role in this interaction. Zwonitzer et al. (2003) found that Zn concentration in Sorghum-Sudangrass *decreased* with increasing amounts of P from (solid) rock phosphate, possibly due to greater Zn adsorption in the soil, but *increased* with increasing amounts of P from (soluble) KH_2PO_4 , possibly due to increased root extension caused by improved P status.

1.3.4 Use of Zn fertilizers to enhance uptake

Zinc fertilizers can play a vital role in improving plant Zn content (Saha et al., 2017). Ahmad et al. (2012) reported that concentrations of Zn in roots, leaves, and stems increased with application of Zn fertilizers. Du et al. (2015) demonstrated a positive response of Zn concentration in tomato plants to foliar application of Zn. Differences have also been shown between genotypes with different Zn remobilization capacities (Mabesa et al., 2013).

Plant response to Zn fertilization supply differs depending on the Zn fertilizer source (Obrador et al., 2003). Various Zn compounds are used as Zn fertilizers and their effect depends on the fertilizer composition and soil type (Mousavi et al., 2013). Typically, however, the plant use efficiency of Zn applied does not exceed 2% of the total amount of applied Zn (Saha et al., 2017).

Generally, micronutrients are more available to plants when foliar applications are used because they are not fixed by soil components (Fahad et al., 2014). Zhao et al. (2016) showed that Zn fertilizer and foliar applications significantly increased the concentration of Zn in cereal grain. Weisany et al. (2014) showed that Zn treatment caused increase root, shoot, and seed Zn concentrations in soybean plants under saline conditions.

However, although foliar application of Zn fertilizers to plants can ameliorate shoot deficiency, it does not improve Zn deficiency in roots (Swietlik, 2002).

Zhao et al. (2016) studied the effect of different Zn fertilization methods on translocation and absorption in wheat. The result of their field experiment showed that application of DTPA-Zn did not increase Zn concentration in grain. By contrast, in their hydroponic experiment Zn foliar spray, and application to roots, increased the grain Zn concentration. Additionally, Cakmak (2009) found that application of Zn fertilizers such as Zn-enriched urea to rice and wheat improved both grain Zn concentration and grain yield. Similarly, Wang et al. (2015) found that the Zn concentration in different parts of winter wheat significantly improved with Zn fertilizer rate.

Biofortification through foliar Zn use has also been proposed for raising grain Zn concentration in crop plants grown in both Zn-deficient and Zn-sufficient soils (Poblaciones and Rengel, 2016). Zhang et al. (2012) studied the influence of Zn biofortification on concentration of Zn in crops and found that foliar application is more effective than soil application in the enhancement of wheat grain Zn. Similarly, Manzeke et al. (2014) found that application of Zn increased yield and micronutrient quality of maize.

1.4 Zinc in human and animal nutrition

Zinc is one of several micronutrient elements required by humans and animals for their physical development (Maret and Sandstead, 2006), growth (Alloway, 2009, Po et al., 2016), brain development (Hemalatha et al., 2005), health (Chen et al., 2017b) and resistance to illness (Po et al., 2016). Zinc is the second most prevalent transition element in organisms after iron (Broadley et al., 2007), the fourth most vital trace element after vitamin A, iron and iodine (Hemalatha et al., 2007) and it plays a major role in the immune system (Salgueiro et al., 2000). Zinc deficiency is a worldwide

challenge for human populations (Wang et al., 2015a). About 30% of the World population suffers from insufficient consumption of Zn (Alloway, 2009).

Zinc deficiency is the 11th most important factor causing death and disease (Poblaciones and Rengel, 2016). However, in developing countries, Zn is fifth in relative importance among the 10 leading risk issues related to disease and illness (Stacey et al., 2008). In some developing countries, Zn deficiency is even considered to be the main health problem (Luo and Xie, 2012). Mortality and sub-optimal growth are several of the severe indicators related to Zn deficiency in children globally (Manzeke et al., 2014). Also, some authors reported that high rates of virulent sicknesses and other difficulties are related to Zn deficiency in pregnant women during pregnancy and at birth. And yet, the essentiality of Zn was first established only in 1963 (Prasad, 2012).

In the human body, unlike Fe, there is no reservoir of Zn that can be easily mobilized when consumption is insufficient; this highlights the need for a diet which is consistently adequately supplied with Zn (Lowe et al., 2009). The total amount of Zn in an adult body is between 2-3 g (Das and Green, 2013) mostly dispersed in the musculoskeletal system (Trame et al., 2018). The concentration of Zn in humans differs between organs (Kabata-Pendias and Mukherjee, 2007a). They reported that Zn concentration in tissues varies between 13 and 310 mg kg⁻¹, being lowest in the skin and highest in the liver and kidneys. Saunders et al. (2012) reported that about 60% of total body Zn is in the skeleton and 30% in bone mass.

A daily intake of around 1.1-11.2 mg day⁻¹ in children, 3-19 mg day⁻¹ in adults or 11 and 8 mg d⁻¹ for man and women respectively is recommended (Manzeke et al., 2014). Also, Alloway (2009) recommended 3-16 mg Zn daily intake depending on gender, age,

and type of diet. While, Trame et al. (2018) reported recommended daily intakes (mg kg^{-1}), of 0.14 for male and 0.12 for females.

1.4.1 The physiological function of Zn

The importance of Zn as a vital nutrient has been established for several years but only recently have academics understood the full importance of this nutrient on human and animal health (Ahmad et al., 2012). Researchers have classified more than 200 to 300 Zn-dependent enzymes. All of these are key to biochemical pathways in the body (Ahmed et al., 2012, Prasad et al., 2016, Manzeke et al., 2014). Furthermore, Zn prosthetic groups are involved in about 3000 proteins in the higher human and animals (Alloway, 2009).

Zinc is essential for the largest number of biological processes in human (Cakmak, 2009). Consequently, sufficient Zn consumption is important for normal reproduction and healthy growth (Po et al., 2016, Chen et al., 2017b). In cellular processes, the main role of Zn includes cell proliferation, cognition/behaviour, vision, intermediary metabolism, DNA and RNA metabolism, repair, reproduction, taste, immune function and defence against free radicals, has been extremely recognized (Ho, 2004, Cakmak, 2009, Maret and Sandstead, 2006, Lowe et al., 2009). Hence, in the human body, decreased Zn status causes impairment and cellular disturbance such as: (i) inhibited growth of children, (ii) high susceptibility to disease and immune dysfunctions, and (iii) delayed mental growth (Black, 2003). Women of childbearing age and young children are the most vulnerable to Zn malnutrition (Dickinson et al., 2009).

1.4.2 Effect of Zn deficiency

Zinc deficiency is the most important nutritional problem globally which influences around two million people, mainly children below 5 years old and pregnant women (Gargari et al., 2007, Chen et al., 2017b). At the worldwide level, Zn is one of several

micronutrient elements with the lowest level of acceptable intake (Beal et al., 2017). In developing countries, Zn deficiency is greater than developed countries because diets are low in vegetables and fruits (Saunders et al., 2012).

The most recognised physiological indicators involve dry and rough skin, brittle fingernails, white spots on nails, dull hair, reduced smell and taste, loss of hunger, reduced adaptation to darkness, mood swings, normal diseases, deferred injury recovery and acne dermatitis (Das and Green, 2013). Additionally, in a study by Sandstead and Freeland-Graves (2014) memory, eye-hand coordination and reasoning ability in women, and immunity and lean-mass in children, were significantly affected by Zn deficiency.

1.4.3 Plant phytic acid (PA) content

In plants, PA is a recognised anti-nutrient factor and an effective chelator of nutrient metal ions which are fixed to the inositol ring (Okazaki and Katayama, 2014, Brankovic et al., 2015). Phytate can bind di- and tri-valent metals very strongly (Sandstead and Freeland-Graves, 2014) because it contains six negatively charged phosphate groups (Lopez et al., 2002b). The binding of Zn causes the formation of insoluble Zn-phytate which then prevents systemic assimilation of Zn (Gupta et al., 2015, Gabaza et al., 2018). The daily intake of PA is about 2 g for developing countries and 0.2 to 0.8 g for industrialized countries (Wu et al., 2010a). The highest concentration of PA is found in cereal grains, nuts, legumes (Gargari et al., 2007), pulses and oleaginous crops (Febles et al., 2002).

The phytase enzyme hydrolyzes PA into inositol tetrakisphosphate, pentakisphosphate, tetrakisphosphate, triphosphate, diphosphate, and monophosphate in germination, fermentation, food processing, storage and digestion in the human digestive system (Garcia-Esteva et al., 1999). They reported that only inositol tetrakisphosphate (InP4) and

inositol pentaphosphate (InP5) have a negative effect on mineral bioavailability. The absence of intestinal phytase enzymes in the human gastrointestinal system causes decreased absorption of Zn (Abebe et al., 2007). In standard physiological conditions, the amount of absorbed Zn is affected by the total PA content (Gupta et al., 2015), the amount and type of protein, and the amount of Zn in foods (Lopez et al., 2002b). The latter authors reported that PA is the main factor affecting Zn absorption especially in foods with low animal protein content. Several strategies can be used to address the problem of PA in cereals, such as biofortification with Zn. Wang et al. (2015b) also found that application of Zn fertilizer actually caused a decrease in PA content of winter wheat.

There are several methods to reduce the negative effects of PA in food. It is possible to hydrolyse PA by thermal processing, using a phytase enzyme, soaking seeds and grains, and fermentation during bread making process. Angela et al. (2012) reported that during the baking process, heat destroys more than 50% of PA; about 60% of PA is hydrolyzed by phytase during the bread making processing (Tavajjoh et al., 2011). They reported that by these methods the bioavailability and the bio-accessibility of Zn could be increased. Bio-accessibility can be defined as the proportion of element converted to soluble forms in the gastrointestinal system (Khouzam et al., 2011). Moreover, PA content is influenced by the rate of P application (Tavajjoh et al., 2011). Application of P fertilizer could be decreased to lower the PA concentration in the edible parts of plants. Approximately, 30% of human beings are at risk of Zn deficiency due specifically to large PA contents in grains and processed cereals (Sandstead and Freeland-Graves, 2014) - most of this is thought to be due to the high rate of P application to crops.

1.4.4 Phytic acid to Zn molar ratio

To predict Zn absorption from edible foods (crops and cereals) containing PA, it has been usual to use their molar ratio in the food as an indication of the likely effect on Zn bioaccessibility (Roohani et al., 2012, Wang et al., 2015b). Karunaratne et al. (2008) divided diets into three groups based on PA: Zn molar ratio; < 5 high, 5-15 moderate, and >15 low bioavailability. In food, a PA/Zn ratio of 25-30 is considered a critical value for (restricted) Zn adsorption (Tavajjoh et al., 2011). To increase Zn bioavailability in foods by lowering the PA/Zn molar ratio Zn fertilization is one of several methods which may be used. Wang et al. (2015b) showed that application Zn fertilizer causes a decrease in the PA/Zn molar ratio in crops down to under 15.

Calcium and iron content of foods and pharmacological intake of folic acid are other factors which they have an effect on Zn absorption (Nriagu, 2007). Luo and Xie (2012) reported that Zn absorption from grains increases with daily vegetable intake. Some vegetables such as spinach and carrot promote Zn absorption because these contain β -carotene.

1.5 Chemistry of phosphorus (P) in soil

Approximately, 42% of agricultural soils in the world are influenced by P deficiency. In developing countries, P deficiency is even more; commonly 70% of cultivated soils in China contain a low level of P (Zhang et al., 2014). The availability of P is an important determinant of crop growth.

The total P concentrations (mg kg^{-1}) in surface soils (0-30 cm) have been reported as 200-5000 with an average of 600 (Lindsay, 1979) and 50-3000 in 0-15 cm for calcareous soil (Zhu et al., 2018). Inorganic P (P_i) accounts for about 60-80% of P in agricultural soils, while organic (P_o) constitutes 30-50% in most soils (Zhu et al., 2018). The P_i is distributed among several fractions including various forms of the mineral

apatite ($\text{Ca}_5(\text{PO}_4)_3[\text{F}, \text{OH} \text{ or } \text{Cl}]$) and other Ca phosphates, secondary precipitates of Fe and Al, a surface-adsorbed exchangeable pool and free and complexed ions in the soil solution phase (Jalali and Ranjbar, 2010). Organic P in most soils exists as orthophosphate monoesters, phosphonate, organic polyphosphates and DNA nucleotides (Zhu et al., 2018). The physio-chemical properties of soil types are the main factors determining the forms of P present and their retention in soil (Jalali and Ranjbar, 2010).

Only a very small proportion of soluble P is dissolved in soil water (Tunesi et al., 1999, Soinne, 2009, Hanrahan et al., 2005). The orthophosphate forms of HPO_4^{2-} and H_2PO_4^- ions are plant-available P forms in the soil solution. The pKa value for these protonated anions is 7.2 and so in slightly acidic soils (pH 5-7) the H_2PO_4^- ion is dominant, while at $\text{pH} > 7.2$ the HPO_4^{2-} ion is the main form of soluble P (Zhu et al., 2018, Jalali and Tabar, 2011). Tunesi et al. (1999) showed that it is important to differentiate between different forms of soluble P in soils, because P speciation influences P availability to plants.

1.5.1 Soil pH value

pH is one of the main factors affecting the availability and forms of P in the soil (Holliday and Gartner, 2007). Devau et al. (2011) studied the effect of pH on P availability in alkaline soils. They found that increasing soil pH resulted in the formation of insoluble Ca-phosphate mineral types which then caused a decrease in P availability (McDowell et al., 2003, Gustafsson et al., 2012). At high pH, several calcium phosphate minerals may form. Superphosphate fertilizer contains monocalcium phosphate (MCP) but this is rapidly transformed to dicalcium phosphate (DCP) and then to octacalcium phosphate (OCP) and hydroxyapatite (HA) as the soluble P gradually mixes with the Ca-rich soil matrix. The minerals with a greater Ca:P ratio (OCP and HA) are less soluble and their formation therefore decreases P availability (Shen et al., 2011, Wadu, 2015b).

Maximum P solubility is found at pH 6.5 (Naeem et al., 2013). The solubility of P in acid soils (pH <6) is low due to P fixation by Fe and Al (Zhang et al., 2014) and adsorption on Fe/Al oxide surfaces (Onwonga et al., 2008). Gustafsson et al. (2012) reported that at low pH, mineral phases such as strengite ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$) and variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$) may form due to the reaction between P ions with Fe^{2+} and Al^{3+} ions and adsorption to hydroxyl- Al polymers in the interlayer space of clay minerals or the surface of Fe^{III} and Al^{III} oxides. At pH values below 5.5, deficiency of P is mainly due to solubilisation of Al^{3+} ions (Desalegn et al., 2016) from gibbsite. Yang et al. (2013) showed that fulvic acid (FA) increased available P through complexation of solubilised Al ions in acid soils. In acid soil, fixed P can be released for plant uptake after liming to raise the soil pH (Desalegn et al., 2016) and precipitate exchangeable Al^{3+} (Haynes and Mokolobate, 2001)

1.5.2 Soil organic matter and soil texture

A significant amount of potentially available P is returned in organic wastes and crop residues added to soil. For example, in western European half of all available P in agricultural soils is from the return of animal manure and a quarter in the United States (Smil, 2000). The amount of available P in the soil, which is derived from organic matter depends on the chemical composition of applied organic matter and the P:C ratio (Ziadi et al., 2013). The rate of decomposition of organic matter residues in soil is the major influence on the availability of P; the decomposition rate is dependent on microbial activity and the quality of organic residues (Salas et al., 2003).

To reduce the fixation of P in soil, organic materials can be used (Mkhabela and Warman, 2005). They assumed that the addition of urban-waste leads to a reduction in immobilization of phosphate by coating sesquioxides with humus acids, which causes an increase in P solubility. The presence of organic molecules in the soil solution has also been shown to decrease P fixation by interfering with fixation reaction processes

(Leytem and Mikkelsen, 2005). Organic matter provides oxy-acid functional groups and microbial products such as citrate that can efficiently chelate Ca^{2+} ions and thus prevent Ca-phosphate precipitation (AL-OD, 2011). Finally, the application of organic matter to agriculture soil increases the soil organic P fraction. Annaheim et al. (2015) report that organic matter input to agricultural soil increased sequential extractable organic P in cropping systems in the north of America. Delgado et al. (2002) found that application of organic matter to calcareous soils increased the amount of inorganic P in the soil solution due to inhibition of the precipitation of hydroxyapatite by organic acids and favoured the formation of dicalcium phosphate dihydrate (DCPD).

Soil physical properties, principally texture, have an important role in determining P availability and solubility. Thus, Ziadi et al. (2013) and Schimel et al. (1985) reported greater reservoirs of available P in the finer-textured soils. However, Shariatmadari et al. (2006) found that greater amounts of clay in soil causes a decrease in P release to the soil solution. Gustafsson et al. (2012) showed an inverse relationship between P solubility and soil clay content and Smil (2000) showed that clayey soils have a high P fixation capacity compared to sandy soils. All these findings can be simply reconciled in terms of a Q/I (quantity/intensity) relationship. Clay soils will yield more extractable P but also adsorb more added P. Thus, fine textured soils have a higher P buffer capacity than sandy soils and will sustain a more conservative level of P solubility in the face of depletion by plant uptake or leaching.

1.5.3 Soil calcium carbonate content

Many researchers have found a significant correlation between P fixation, available P, and the soil CaCO_3 content (Rawajfih et al., 2010). In calcareous soils, calcium carbonate transforms P into insoluble calcium phosphate complexes or surface adsorbed forms on calcite (Shariatmadari et al., 2006, Carey et al., 2011, Pizzeghello et al., 2011, Braschi et al., 2003, Zhang et al., 2014). With time, adsorbed P is probably transformed

to crystalline calcium phosphate minerals with very low solubility. About 80% of P in calcareous soils may be rendered unavailable for plant uptake because of these processes (Neset et al., 2008). As noted in section 1.5.2, (Delgado et al., 2002) the presence of organic acids, such as fulvic and humic acids, in soil could raise the efficiency of applied P fertilizer by increasing the portion of applied P that remains as more soluble Ca-phosphates.

1.5.4 Kinetics of P fertilizer reaction with soil

The kinetics of P fertilizers applied to soils have been widely studied. Given the time-dependent fixation of P in soils it is important to understand the likely changes in P solubility during the course of a growing season, following fertilizer application.

Samadi (2006) found that the rate constant (k) of P precipitation increased with increasing clay content and this was the main influence on P retention. Tunesi et al. (1999) found that the time of reaction was the major influence in Ca-P formation along with pH and Ca-ions concentration. Over time, the reaction of applied P with soil decreases and become slower but without necessarily reaching an equilibrium state (Sinegani and Sedri, 2011).

Less soluble forms of P fertilizer can become more available with time. Zhang et al. (2014) reported that plant available P is slowly released from rock phosphate which gradually increases the amount of available P. Rock phosphate (apatite) needs time for decomposition and then released P to soil as available P for plant uptake – but this is more likely to apply to acidic, rather than calcareous, soils.

Various models have been used to describe the kinetics of P dynamics in soils (Sinegani and Sedri, 2011, McDowella and Sharpley, 2003). These studies have provided some

understanding of mechanisms controlling P sorption but also have practical application (Samadi, 2006).

1.6 Plant uptake of P

Phosphorus is one of several macronutrients required by plants (Nadeem et al., 2014) for their metabolic processes (Smith et al., 2003) and optimal growth (Ziadi et al., 2013). Phosphorus in plants is important for numerous functions. Mullins (2009) separated P functions in the plant into (i) key processes of energy storage and transfer, cell division, respiration, photosynthesis, cell enlargement, (ii) a component of many cell constituents, and (iii) a physiological function of energy transfer. Also, P has a primary role in energy transfer for metabolic processes through cellular products such as ATP and ADP from the first stages to the end of a plant's life (Ali et al., 2014).

Phosphorus is involved in photosynthetic constituents, including proteins, sugars, lipids, and nucleic acids. The major components of cell membranes are phospholipids, and the building blocks of DNA and RNA are phosphate-containing nucleotides (Shih et al., 2015). Plants accumulate P typically as the salt of PA (myoinositol-1, 2, 3, 4, 5, 6-hexaphosphoric acid), mainly in seed (Ziadi et al., 2013). This accounts for around 50-80% of total P in crops (Steiner et al., 2007), which is the major anti-nutrient factor (Liu et al., 2006). Moreover, given that photosynthesis is the main indicator of plant productivity, Danyagri and Dang (2014) reported that P has an impact on plant productivity indirectly through the total size of leaf and directly by a decrease in Ribulose 1,5-bisphosphate regeneration and Rubisco activity.

In plants, the P concentration is typically in the range 0.05-0.5% (Vance et al., 2003). Its availability is considered one of the key growth-limiting factors for plants in several natural ecosystems (Liu et al., 1998), and it is one of the two critical growth-limiting nutrients after nitrogen (Ma et al., 2016b). In natural soils, P uptake efficiency by plants is related to P availability (Bhattacharyya et al., 2015a). The inorganic P fraction (P_i) is

the major source of P for plants (Smith et al., 2003). Plants absorb P_i in the form of orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) which exists in the soil solution in extremely low concentrations $0.03\text{--}0.31\text{ mg L}^{-1}$ (Vance et al., 2003).

Phosphate ions in the soil solution transfer to roots by the comparatively slow process of diffusion (Smith et al., 2003). And then, plants absorb P via their root hairs, root tips and outer layers of root cells (name, 1999). In the root, P is acquired via high-affinity inorganic P transporters (Liu et al., 1998). Thus, plants must have specific transporters at the root/soil interface to absorb P_i from the soil solution (Schachtman et al., 1998). The plant's ability to absorb soluble P from the soil solution varies depending on plant genotype (Yang et al., 2011). At the first stage of plant growth, root length is a major factor governing P uptake (Schroder et al., 2011), through their morphological and physiological characteristics. Fernandes et al. (2014) showed that P uptake by plants is influenced by root branch distribution within the soil. They studied the effect of potato root morphology on P uptake. Results showed that the factors determining P uptake included a balance between root morphological parameters (length and surface area) and physico-chemical parameters (P 'intensity'). However, P ion concentration in the soil solution and the root surface absorbing area in contact with soil solution are probably the main factors controlling the ability of plants to take up P (Grant et al., 2005).

Other plant factors that influence P uptake when foliar application of P fertilizers are used include (i) leaf age and wetting (ii) the nature of cuticle layer on leaf surfaces and (iii) stomatal opening (Ali et al., 2014b). Furthermore, (Maruyama and Wasaki, 2017, Neumann and Martinoia, 2002, Cordell et al., 2011) reported five 'strategies' which can be used to enhance P uptake by plants, including (i) infection with mycorrhizal fungi (ii) increase in the root surface by change of root architecture, (iii) solubilisation of P_i by decreasing rhizosphere pH and exudation of organic acid, (iv) up-regulation of high-affinity P_i transports, and (v) mineralization of organic P. Strategies ii – iv would be

achievable through plant breeding. The amount of available P to plants is increased by solubilization of soil P by citrate excreted from plant roots in some plants (Richardson et al., 2011).

1.6.1 Soil factors controlling P uptake

Soil characteristics which have an effect on P uptake by plants can be divided into chemical, physical and biological factors (Armstrong, 1999). The source of P, soil available P (Mohasses, 2015) and interaction with other nutrients are also important factors affecting P uptake by plants (Armstrong, 1999). Soil P is largely unavailable to plants (Vance et al., 2003), especially when P is applied to the soil surface where the highest amount of P fixation takes place (Akhtar et al., 2016). Plant P uptake is limited by the fixation mechanisms already discussed - formation of poorly soluble Ca-P minerals in calcareous soils (Hopkins and Ellsworth, 2005) and fixation with Fe and Al oxides in acidic soils (Tran et al., 2010). Thus P uptake by plants is usually low due to inadequate mobility and solubility of P_i sources (Lambers et al., 2008).

Soil pH is the most important soil property affecting P uptake by plants. The forms in which P_i exists in the soil solution change according to pH value given that the pKa values for H_3PO_4 dissociation into $H_2PO_4^-$ and then into HPO_4^{2-} are 2.1 and 7.2, respectively. Numerous studies on the pH dependency of P_i uptake by plants have found that the highest uptake occurs between pH 5.0 and 6.0 when $H_2PO_4^-$ is dominant (Liu et al., 1998, Ziadi et al., 2013); this may suggest that P_i is taken up as the monovalent form. However, P uptake by plants from soil greatly depends on the soil buffering capacity, ease of P_i desorption from the solid phase, and subsequent diffusion to the root surface (Holford, 1997).

1.6.2 Plant controls over P uptake

Phosphate toxicity symptoms occur in leaves when P uptake exceeds the tissue storing capacity because the capacity of plant stem tissues is limited (Shane et al., 2004). Phosphate toxicity causes enhanced leaf necrosis and senescence, induced micronutrient deficiency and reduced growth (Hawkins et al., 2008). The plant growth rate increases to resist excess uptake of P (Abdolzadeh et al., 2010). However, when plants absorb more P than required, various mechanisms react to avoid an increase to toxic P concentrations. Schachtman et al. (1998) described these processes as (i) decline of P_i uptake from the soil solution, (ii) P_i losses by efflux (between 8-70% of the influx), and (iii) transformation of P_i into organic storage complexes. To preserve intracellular P_i homeostasis, all of these processes may be used.

1.6.3 Use of P fertilizer to enhance uptake

Phosphate fertilizers are used worldwide to increase crop yield (Stutter et al., 2015). In all regions of the world, P deficiency and low P solubility have been countered by the use of P fertilizers (Yang et al., 2012). Approximately, 42% of cultivated soils are affected by P deficiency worldwide; this is particularly common in developing countries (Yang et al., 2012). They reported that, in the agronomic revolution, P fertilizers derived from rock phosphate have largely governed P nutrition of crop plants.

The type of P fertilizers and application method can also have an effect on P uptake by plants. For example, Akhtar et al. (2016) investigated methods to improve P uptake by wheat cultivated in the calcareous soils of Pakistan. They found that P application methods affected grain yield and the efficiency of methods followed the order: phosphoric acid placed below the seed > phosphoric acid, DAP or TSP fertigation > DAP or TSP broadcast.

The efficiency of P fertilizers varies. Bhattacharyya et al. (2015) reported that only small amounts of applied P fertilizers are available and their efficiencies varied from 8% to 33% depending on the nature of soils and crops. Moreover, P fertilizer use efficiency is increased through combination with organic matter which enhances P availability in the soil (Garg and Bahl, 2008). The effects of P fertilizers also depend on the balance of other nutrients present in the soil (Maqsood et al., 2014, Grant et al., 2005).

In calcareous soils, plant P deficiency is especially common due to the presence of large amounts of CaCO_3 and high pH (Jalali and Ranjbar, 2010, Naeem et al., 2013). However, long-term application of P fertilizers causes an increase in P uptake by plants (Bhattacharyya et al., 2015) due to increased available P (Li et al., 2006). They reported that larger fertilizer applications containing phosphate should be applied to cultivated calcareous soils to achieve optimum plant yield. Long-term P fertilizer application in excess to that normally required by plants for optimum growth causes P accumulation in the surface horizon (0-30 cm). In such circumstances, P loss can increase eutrophication of surface water if the landscape is prone to erosion. For example, residual P in agricultural land in China was estimated as 4.8 kg ha^{-1} of P in the 1980s but increased to 37.7 kg ha^{-1} in the 1990s (Sheng-Li et al., 2008). Moreover, Mehlich³-extractable P build-up increased about 12% due to inorganic P with dairy manure application over a 10 year period in a sandy clay soil (Li et al., 2007). The potential for P loss clearly increases with soluble P in the soil (Pizzeghello et al., 2011, McDowell et al., 2003).

1.7 Phosphorus in humans and animals

Phosphorus plays an important role in human health and nutrition (Chen et al., 2016). In the human body, P is mainly acquired as organic P compounds found in food (Kiela et al., 2017). About 70-80% of dietary P is taken from milk products, meat, and grains (Kiela et al., 2017). It is one of the essential nutrients required by the body for energy

metabolism, gluconeogenesis, glycolysis, cellular signal transduction and skeleton mineralization processes for every system of animal and bacterial life on Earth (Suh and Yee, 2011, Lin et al., 2014). The structural role of hydroxyapatite in bone in the human body is the primary function of P (Bergman et al., 2009). The total P mass in an adult (70 kg) is about 650 g (Heaney and Graeff-Armas, 2018).

Ervin et al. (2004) reported that the daily intake of P by an age-stratified population in the USA during 1999-2000 (Table 1.1).

Table 1.1: Daily phosphorus intake depending on age during 1999-2000 in the USA.

Age (year)	P intake (mg d ⁻¹)
0-5	913
6-11	1078
12-19	1172
20-39	1251
40-59	1239
Over 60	1055

However, P daily dietary intake increased from 1233 mg day⁻¹ in the 1960s to 1421 mg day⁻¹ in 1995. While the usual range of P intake is between 775-1860 mg P d⁻¹, only about 60-80% of dietary P is absorbed (Favus et al., 2006). However, a significant percentage of absorbed P is from cereals because cereals are staple foods (Takeda et al., 2012).

The estimated P dietary intake differs depending on age and sex (Ervin et al., 2004). Recommended dietary allowances (RDA) and tolerable upper levels (UL) for P intakes are shown in Table 2. Since high intakes of P can cause health hazards, mean daily P consumptions should not exceed the UL (Gal and Dahl, 2013).

Table 1.2: Dietary intake reference and tolerable upper levels for P according to (Gal and Dahl, 2013; Kiela et al., 2017).

Age (years)	Recommended Daily Allowance	Upper Limit
	(mg d ⁻¹)	
Children		
1 to 3	460	3,000
4 to 8	500	3,000
9 to 18	1250	4,000
Adults		
19 up to 70	700	4,000
> 70	700	3,000
pregnant		
14 to 18	1250	3,000
19 to 50	700	3,000
lactating		
14 to 18	1250	4,000
19 to 50	700	4,000

1.7.1 The physiological function of P

In the human body, P is the second most abundant vital element after calcium (Raina et al., 2012); it is required by all cells for normal functions (Kalantar-Zadeh et al., 2010). Phosphorus constitutes about 0.65-1.1% of the adult body and 0.5% of the infant body (Kiela et al., 2017); 80-85 % of total P in the body is stored in bone and teeth as hydroxyapatite (Takeda et al., 2012, McDowell et al., 2003, Bergman et al., 2009). Approximately 14% to 15 % of P is in soft tissues and blood and less than 1% is in the extracellular fluids (Allen-Durrance, 2017, Heaney and Graeff-Armaz, 2018). Phosphorus is mostly present as phosphate (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) ions and P esters (Kiela et al., 2017). The level of phosphate esters (PO_4^{3-}), in serum, varies extensively depending on age, sex and dietary intake (Favus et al., 2006). Phosphorus is a vital constituent of adenosine triphosphate (ATP), nucleic acids and is essential for acid-base balance, a vital constituent of essentially all enzymes, carbohydrates, and cellular messengers such as the G-proteins (Takeda et al., 2014, Soetan et al., 2010). Phosphorus is needed to produce cyclic adenosine monophosphate, guanosine

triphosphate, and phosphocreatine (Allen-Durrance, 2017), which are needed to maintain energy stores, cellular membrane integrity and biochemical messenger systems (DiBartola and Willard, 2012). In addition, it is vital for regulating tissue oxygenation (2,3-diphosphoglycerate [2,3-DPG]), regulating many enzymes (eg, 1 α -hydroxylase and glutamines) (Soetan et al., 2010), and in maintaining bone and dental matrices (Heaney and Graeff-Armas, 2018).

Phosphorus is one of the key nutrients involved in several functions in living organisms (Puggaard et al., 2014) and sufficient P is required in the diet for normal skeleton growth (Mutucumarana et al., 2015), bone building (Neset et al., 2008), energy metabolism GTP (Ervin et al., 2004), glycolysis, gluconeogenesis (Takeda et al., 2012) and information coding in RNA and DNA (Heaney and Graeff-Armas, 2018). In body fluids, P is the main pH buffer (Kalantar-Zadeh et al., 2010). Phosphate has a vital role in managing peptide transporting activity through peptide transporter one (Pep T1) in the intestine (Furutani et al., 2013). Moreover, P has a major role in teeth maintenance, related to calcium utilization. Lin et al. (2014) reported that the ratio between P and Ca concentration in the diet is an important factor in demineralization and remineralization of enamel. They also reported that the P supply is related to the incidence of dental caries in young children.

1.7.2 Effect of P deficiency on humans

As already noted, P is a vital element for all life (Heaney and Graeff-Armas, 2018). The normal level of serum P should be between 25-35 mg L⁻¹ (Kiela et al., 2017). Phosphorus is one of the main factors in the preservation of bone health, and its deficiency causes clinical illness and bone pathology, such as osteomalacia and rickets (Chen et al., 2016). Moreover, low P levels cause bone pain, poor coordination, and muscle weakness while, in children, it causes poor and reduced tooth development and decreased growth rate (Gal and Dahl, 2013). Suboptimal Ca/P_i levels cause impaired

skeletal mineralization due to the low level of P in serum (Favus et al., 2006). Also, low dietary P_i significantly increases chronic kidney disease (Furutani et al., 2013).

Chen et al. (2017a) studied the effect of P deficiency in fish and reported that it caused weak bone mineralization, increased lipid content in the body, operculum and skeletal deformities. However, excessive intake of P is more widespread than inadequate. Excessive P intake through the diet causes deleterious effects on the health of heart, kidney, bone, and metabolism (Calvo and Uribarri, 2013, Takeda et al., 2012). They reported that the risk of cardiovascular disease in healthy people was increased due to an increase in serum P concentration because of a rise in medial calcification and chronic kidney disease.

1.7.3 Plant phytic acid content

Phytic acid (PA), the salt of (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate, InsP6) is the main form of stored P in plant seeds (Liu, 2005). Approximately, 50%- 80% of total P in plants is accumulated as PA (Steiner et al., 2007). Furthermore, it is about 1% to 5% weight of cereals, oilseeds, edible legumes and nuts used by humans and animals as a staple food (Okazaki and Katayama, 2014). In crops, rich in PA, such as grains, the bioavailability of P is between 10-30% due to the absence of the degradation phytase enzyme (Kalantar-Zadeh et al., 2010, Donate-Correa et al., 2012).

In humans, P deficiency may be caused by PA because PA cannot be utilized due to the lack of a digestive phytase enzyme in the intestines (Kumar et al., 2010, Kiela et al., 2017). Furthermore, the presence of high amounts of PA in foods has a negative result on human health due to chelation of dietary minerals such as Fe and Zn, which affects mineral-related deficiencies in humans, and decreases protein and lipid utilization (Kumar et al., 2010). They reported that the protection from various diseases, especially heart and cell activities can be increased by increasing the amount and

activity of the phytase enzyme in dietary foods which reduce PA during the processing of foods.

Recently, the beneficial role of PA in human health has been reported by some researchers. Undigested PA accumulates in the digestive area of monogastric animals such as humans and this has beneficial effects on gut microflora (Sekita et al., 2016). Furthermore, PA has additional benefits such as anticarcinogenic, antioxidant, and antidiabetic activity (Okazaki and Katayama, 2014). Phytate and myo-inositol encourage insulin sensitivity in adipocytes by improving glucose uptake, lipid storage capacity, and inhibiting lipolysis (Kim et al., 2014).

1.8 An overview of the Kurdistan region of Iraq

The Iraqi Kurdistan Region consists of three provinces: Erbil, Sulaymaniyah, and Duhok, with a population of 5.2 million. It covers an area of 40,640 km² and borders Iran to the east, Syria to the west, and Turkey to the north. The fertile land lies adjacent to the Zagros Mountains. It is crossed by the rivers Sirwan, Dukan, the Tigris and their branches and the large and small Zab River. There are several small lakes, such as Lake Sirwan and Lake Duhok.

The average height of the Kurdistan Region Mountains is about 2,400 m increasing to about 3,000–3,300m in the north. The Halgurd is the highest peak (3,660 m) in the region, close to the Turkey and Iranian borders. The Kurdistan region western and southern parts are not as mountainous as the eastern part.

The climate of the Kurdistan Region is semi-arid and conditions fluctuate with seasons: cold and dry in winter and hot and dry in summer. The other two seasons are moderate in the Kurdistan Region. Average temperatures range from 13-18 °C in March to 27-32 °C in May whereas temperatures reach 39-45 °C in July and August for the north and

south parts respectively. In winter high-temperatures reach 7-13 °C and low are 0-7 °C.

1.9 Objectives of the current project

Phosphorus and Zn are two essential elements required by humans and plants for healthy growth and development. Soil properties affect P and Zn bioavailability to plants and also the nature and extent of their interaction. Their bioavailability in calcareous soils is low due to various soil properties, principally a high calcium carbonate content and soil pH value and a low organic matter content.

The general aims of this current thesis are listed below:

1. Determine the chemical form, solubility and bioavailability of zinc (Svecnjaka et al.) and phosphorus (P) in the calcareous soils of Iraqi Kurdistan and the nature and extent of their interaction.
2. Assess the utilization of Zn and P by crop plants in Kurdistan and the viability for biofortification of Zn in the face of calcareous soils subject to long term large applications of P fertilizer.
3. Quantify the transfer of Zn and P into the Kurdistan diet and their possible interaction through Zn binding to phytate.

1.10 Hypothesis

In the calcareous soils of Kurdistan, the presence of high amounts of calcium carbonate and high soil pH values may not only impact on P and Zn availability and crop *yield* but may also have a negative impact on the *quality* of agriculture products. In particular, it is hypothesized that crops produced may contain low concentrations of Zn which may increase risk to human health, as well as restricting yield. Furthermore, in Kurdistan, farmers apply high amounts of P fertilizer to soils to offset the tendency of the calcareous soils to fix phosphate; the focus is, quite naturally, on maximising crop yield.

Thus Zn bioavailability to human populations may be decreased, both due to increased phytic acid (PA) concentration in crops and immobilization of Zn in soil by phosphate fertilizer and naturally occurring calcium carbonate. However, information about Zn deficiency in Kurdistan soils and the chemical forms of Zn and P in these soils is currently very limited.

2 Methods and Materials

This chapter gives details of methods and analytical procedures that have been used throughout the current study for soil and plant analysis. Methods relating to specific experiments are provided within the relevant chapters.

2.1 Soil sampling and preparation

Soil samples were collected using a stainless steel trowel and then sealed in plastic bags. About 1 kg of each soil was dried at room temperature in an aluminium tray, disaggregated, then sieved to obtain the <2 mm fraction. A representative sample of approximately 50 g of each sample was transferred to a plastic bag, labelled and transported to Nottingham University, UK. A 10 g portion of each sample was finely ground using an agate ball mill (Retsch, Model PM400), and then stored in a polyethene bag before further analysis.

2.2 Soil analysis

2.2.1 Soil pH

Soil pH was determined in a suspension of 5 g of 2 mm sieved air-dried soil suspended in 12.5 mL distilled water (ratio 1:2.5) after shaking at 40 rpm for 30 minutes on vertical shaker. A pH meter (model Hanna pH-209 with a combined glass electrode (Ag/AgCl; PHE 1400) calibrated using pH 4.01 and 7.0 buffer solutions was used for pH measurement after five minutes of stabilization.

2.2.2 Loss on Ignition (LOI %)

A known weight of <2 mm oven-dried soil was placed in a pre-weighed silica crucible and placed in a muffle furnace (Gallenkamp size 3, Weiss, Gallenkamp, UK) overnight at 550 °C to ignite the organic matter. After ignition, the crucible was cooled in a

desiccator before the weight loss was determined and expressed as a percentage of soil organic matter using equation 2.1.

$$\text{LOI \%} = \frac{(W_{\text{ODS}} - C_{\text{IS}}) * 100}{W_{\text{ODS}}} \quad \text{Eq. 2.1}$$

Where W_{ODS} is the weight of the oven dry soil and W_{IS} is the weight of the ignited soil.

2.2.3 Soil digestion

Approximately 200 mg of finely ground soil was digested in 2.5 ml HF (40% AR), 2.0 mL HNO_3 (Trace Analysis Grade: TAG), and 1 ml of HClO_4 (AR) in PFA digestion vessels using a 48-place block digester (Model A3, *Analysco* Ltd, UK). Digested samples were diluted to 50 ml of *Milli-Q* water and stored in universal sample bottles prior to elemental analysis. Samples were diluted 1-in-10 with *Milli-Q* water immediately before analysis.

2.2.4 Determination of Olsen-P

Approximately 2.0 g of air-dried soil was transferred into a 50 ml centrifuge tube with 30 ml of extracting solution (0.5 M NaHCO_3 , 5 ml of 5% polyacrylamide, pH 8.5), to achieve a 1:15 soil to extractant ratio. The sample was then shaken for 30 minutes before centrifugation for 15 mins at 3500 rpm. Phosphorus concentration in the supernatant was determined spectrophotometrically at 880 nm in a 1 cm cell using the ascorbic acid method of (Murphy and Riley, 1962). Available P in the sample was calculated from equation 2.2

$$P_{\text{Olsen}} = \frac{\left(P_{\text{soil}} \times \frac{V_{\text{ext}}}{V_{\text{soil-CD}}} \right) - \left(P_{\text{blank}} \times \frac{V_{\text{ext}}}{V_{\text{blank-CD}}} \right)}{W_s} \quad \text{Eq. 2.2}$$

Where P_{Olsen} is the available phosphate in the soil (mg P kg^{-1}), P_{soil} , and P_{blank} are the measured amounts of phosphate-P in the aliquots used for colour development ($\mu\text{g P}$), W_s is the weight of air-dried soil extracted (c.2.0 g), V_{ext} is the volume of sodium bicarbonate (c. 30 mL), and $V_{\text{soil-CD}}$ and $V_{\text{blank-CD}}$ are the volumes of the supernatant used for colour development.

2.2.5 Determination of DTPA-Zn

Approximately 2.0 g of air-dried soil was transferred to a 20 ml universal tube and 8 ml of diethylene triamine pentaacetic acid (DTPA)–Triethanolamine TEA buffer (0.005 M DTPA+ 0.01 M CaCl_2 + 0.1 M TEA) was added to achieve a 1:4 soil to extractant ratio. The suspension was shaken for 1 h at 30 rpm at room temperature before centrifugation for 10 min at 3000 rpm, filtration (Quevauviller, 1998). Samples (5 ml) for ICP-MS (Thermo Fisher scientific iCAP Q, Germany) analysis of Zn were preserved by the addition of 0.2 ml of 50% nitric acid. Zinc concentrations in each sample were converted to mg kg^{-1} using equation 2.3.

$$\text{Zn}_{\text{soil}} = \frac{(C_{\text{soil}} - C_{\text{blank}})V_{\text{sol}}}{W_{\text{Soil}}} \quad \text{Eq.2.3}$$

Where Zn_{soil} is the Zn concentration in soil samples (mg Kg^{-1}), C_{soil} and C_{blank} are Zn concentrations in the soil and blank digests ($\mu\text{g l}^{-1}$), V_{sol} is the diluted soil extraction volume (8 mL), and W_{soil} is the mass of soil sample used for extracting Zn (2.0 g).

2.2.6 Determination of soluble P and Zn

Approximately 2.0 g of air-dried soil was transferred to a 50 ml centrifuge tube with 20 ml of 0.01 M calcium nitrate and shaken for 48 h followed by centrifugation (2500 rpm for 15 min), filtration (0.22 μm) and acidification of 5 ml of the supernatant with 0.2 ml of 50% HNO_3 for preservation prior to analysis of P, Zn, Ca, Na, K, Mg, and S. A

separate aliquot was retained for pH determination and measurement of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and phosphate as described by Murphy and Riley (1962). Concentrations of soluble P in samples were calculated using equation 2.4.

$$\text{Soluble P} = \frac{(\text{Abs} \times \text{Slope})}{V_{\text{Ext.}}} \dots \dots \dots \text{Eq. 2.4}$$

In Eq. 3.1 Soluble P is the concentration of phosphate in the soil solution ($\mu\text{g mL}^{-1}$), Abs is the absorbance measured with a spectrophotometer at 880 nm, Slope is the slope of standard P solution, and V_{Ext} is the volume of the supernatant used in the colour development

2.2.7 Phosphate solubility

Double function parameters consisting of phosphate potential $\log \text{H}_2\text{PO}_4^- - \text{pH}$ and lime potential ($\log \text{Ca}^{2+} + 2\text{pH}$) were used to construct a solubility diagram for calcium phosphate minerals. It was assumed that the free ion activity of H_2PO_4^- was controlled by lime potential and pH based on published solubility product (K_{sp}). It was also assumed that the solubility of phosphate in calcareous soils is controlled by a solid phase of calcium phosphate minerals listed in Table 2.1 (Lindsay, 1979).

Table 2.1: Calcium phosphate phases, reactions and Ksp used in the construction of a phosphate solubility diagram.

Mineral	Formulae	Equation	Ksp
Monocalcium phosphate (MCP)	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O} \rightleftharpoons \text{Ca}^{2+} + 2\text{H}_2\text{PO}_4^- + \text{H}_2\text{O}$ * $2\text{H}^+ \rightleftharpoons 2\text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} = -0.575 - 0.5 (\text{Log Ca}^{2+} + 2\text{pH})$	-1.15
Dicalcium phosphate (DCP)	CaHPO_4	$\text{CaHPO}_4 + \text{H}^+ \rightleftharpoons \text{Ca}^{2+} + \text{H}_2\text{PO}_4^-$ $\text{H}^+ \rightleftharpoons \text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} \rightleftharpoons 0.3 - (\text{Log Ca}^{2+} + \text{pH})$	0.3
Brushite (DCPD)	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O} + \text{H}^+ \rightleftharpoons \text{Ca}^{2+} + \text{H}_2\text{PO}_4^- + 2\text{H}_2\text{O}$ $\text{H}^+ \rightleftharpoons \text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} = 0.63 - (\text{Log Ca}^{2+} + 2\text{pH})$	0.63
β -Tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	$\text{Ca}_3(\text{PO}_4)_2 + 4\text{H}^+ \rightleftharpoons 3\text{Ca}^{2+} + 2\text{H}_2\text{PO}_4^-$ $2\text{H}^+ \rightleftharpoons 2\text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} \rightleftharpoons 5.09 - 1.5 (\text{Log Ca}^{2+} + 2\text{pH})$	10.18
Octacalcium phosphate (OCP)	$\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 2.5\text{H}_2\text{O}$	$\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 2.5\text{H}_2\text{O} + 5\text{H}^+ \rightleftharpoons 4\text{Ca}^{2+} + 3\text{H}_2\text{PO}_4^- + 2.5\text{H}_2\text{O}$ $3\text{H}^+ \rightleftharpoons 3\text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} = -11.76 - (\text{Log Ca}^{2+} + 2\text{pH})$	11.76
Hydroxyapatite (HA)	$\text{Ca}_5(\text{PO}_4)_3\text{OH}$	$\text{Ca}_5(\text{PO}_4)_3\text{OH} + 7\text{H}^+ \rightleftharpoons 5\text{Ca}^{2+} + 3\text{H}_2\text{PO}_4^- + \text{H}_2\text{O}$ $3\text{H}^+ \rightleftharpoons 3\text{H}^+$ $\text{Log H}_2\text{PO}_4^- - \text{pH} = 4.82 - 1.66 (\text{Log Ca}^{2+} + 2\text{pH})$	14.46

* H^+ ion added to both side of equations in order to calculate lime potential (Lindsay, 1979).

2.2.8 Zn solubility

Log free ion activities of Zn^{2+} against hydrogen potential were used to construct a theoretical solubility diagram for Zn minerals. It was assumed that (Zn^{2+}) was controlled by pH based on published K_{sp} values for Zn minerals. It was assumed that the solubility of Zn in calcareous soils is controlled by solid phase of Zn minerals listed in Table 2.2 (Lindsay, 1979, Nazif et al., 2015, Urgun and Rimmer, 2000).

Table 2.2: Zinc minerals, reactions and Ksp used to draw a Zn solubility diagram.

Mineral	Formulae	Equation	Ksp
Smithsonite	ZnCO ₃	$\text{ZnCO}_3 + 2\text{H}^+ \rightleftharpoons \text{Zn}^{2+} + \text{CO}_2 + \text{H}_2\text{O}$ $\text{Log Zn}^{2+} = 7.71 - \text{PCO}_2 - 2\text{pH}$	7.91
Amorp	Zn(OH) ₂	$\text{Zn(OH)}_2 + 2\text{H}^+ \rightleftharpoons \text{Zn}^{2+} + 2\text{H}_2\text{O}$ $\text{Log Zn}^{2+} = 12.48 - 2\text{pH}$	12.48
Hydrozincite	Zn ₅ (OH) ₆ (CO ₃) ₂	$\text{Zn}_5(\text{OH})_6(\text{CO}_3)_2 + 10\text{H}^+ \rightleftharpoons 5\text{Zn}^{2+} + 2\text{CO}_2 + 8\text{H}_2\text{O}$ $\text{Log Zn}^{2+} = 9.2 - 0.4\text{pCO}_2 - 2\text{pH}$	46
Zincite	ZnO	$\text{ZnO} + 2\text{H}^+ \rightleftharpoons \text{Zn}^{2+} + \text{H}_2\text{O}$ $\text{Log Zn}^{2+} = 11.16 - 2\text{pH}$	11.16
Soil-Zn	Soil-Zn	$\text{Soil-Zn} + 2\text{H}^+ \rightleftharpoons \text{Zn}^{2+}$ $\text{Log Zn}^{2+} = 5.8 - 2\text{pH}$	5.8

Where the partial pressure of CO₂ = -1.52 for Smithsonite and hydrozincite

2.2.9 Phosphate Fractionation

The fractions of P in the soils were determined using a modified version of the procedure described by Audette et al. (2016) in Table 2.3.

Table 2.3: Modified sequential fractionation approach for phosphate fractionation.

Step	Inorganic P fraction	Extractant	pH	Shaking time
1 ^a	Ca ₂ -P type	0.25 M NaHCO ₃	8.0	1 h
2	Ca ₈ -P type	0.5 M NH ₄ Ac ^b	4.2	16 h stand, 1 h
3	Prevent re-adsorption	1.0 M MgCl ₂	8.0	2 h
4	Al-P	0.5 M NH ₄ F	8.2	1 h
5	Fe-P	0.1 M NaOH-Na ₂ CO ₃	12	2 h, 16 h stand, 2 h
6	Occluded	0.3 M CD ^c	13	16 h stand, 1 h
7	Ca ₁₀ -P type	0.25 M H ₂ SO ₄	1.0	1 h

a Ethanol wash after step 1, b Ammonium acetate, c 0.3 M Sodium citrate (20 mL)-dithionite (1.0 g)- 1.0 M sodium hydroxide (5 mL)

The extracts were stored at 5°C prior to analysis by ICP-MS for total P and colourimetrically using the molybdate blue method (Murphy and Riley, 1962) for inorganic P. Organic P concentrations were calculated by subtracting the amount of inorganic P from the total P.

2.2.10 WHAM speciation

The Windermere Humic Aqueous geochemical speciation model version 7 (WHAM (VII)) was used to estimate (H_2PO_4^-) and (Zn^{2+}) using the following input parameters: (a) [Na], [Mg], [K], [Ca], in mgL^{-1} and [Zn] in μgL^{-1} of suspensions, $[\text{PO}_4]$, $[\text{NO}_3]$, and $[\text{SO}_4]$ in mole L^{-1} (b) the suspension pH, (c) dissolved inorganic carbon (DIC), and (d) Fulvic acid (FA). The temperature was set to 22°C. It was assumed that DIC contained 50% C and that 50% of the DOC was organic in origin (Nazif et al., 2015).

2.3 Plant sampling and preparation

At harvest, plant samples were collected by hand. All samples were sealed in a polyethene bags to transport to soil laboratory of Agriculture Technical college of Halabja. At the laboratory, all samples oven dried at 45 °C to a constant weight for dry matter yield in a forced air oven (model LDO, 060E) and then recorded dry weight in an aluminium tray. A representative sample of approximately 10 g of each sample was transferred to plastic bags labelled and transported to the Nottingham University, UK. About 10 g portion of each sample was finely ground using a centrifugal mill (Retsch, Model ZM200), and then stored in a polyethene bag before further analysis.

2.4 Plant analysis

2.4.1 Plant digestion

Approximately 200 mg of milled plant material was digested under microwave heating (Anton Parr, Multiwave 3000) for about 45 mins at 2 MPa in 6.0 mL of 68% TAG HNO_3 for vegetables and 4.0 mL HNO_3 , 2mL H_2O_2 for grains. Digested samples were diluted to 20 mL with Milli-Q water and stored at room temperature prior to multi-element analysis by ICP-MS.

2.4.2 Determination of phytic acid (PA)

Phytic acid (PA) was determined using a Megazyme kit (K-PHYT, Megazyme, and Bray, Ireland). Approximately, 1 g of grain was extracted with 20 mL of hydrochloric acid (0.66 M), capped with aluminium foil and left to stand overnight at ambient laboratory temperature. An aliquot of the extract (1 ml) was centrifuged at 13000 rpm for 10 mins and neutralized with 0.5 mL of 0.75 M NaOH. Total P was estimated in 0.05 mL of extract to which 0.60 mL of distilled water was added followed by addition of 0.2 mL of 0.2 M sodium acetate buffer and 0.02 ml of phytase and mixing by vortex. After incubation at 40 °C for 10 min, 0.2 mL of 0.4 M glycine buffer and 0.02 ml of alkaline phosphatase (20 µl) were added. The reaction was stopped by the addition of 0.3 mL of 50% trichloroacetic acid. The contents were centrifuged at 13000 rpm for 10 min and to 1 ml of the solution 0.5 mL of colour forming reagent (10% ascorbic acid [5:1 ratio] 5% ammonium molybdate) was added and the optical density measured at 655 nm. At the same time, free phosphorus was determined by mixing 0.2 ml of sodium acetate buffer and 0.62 ml of distilled water to 0.05 ml of the mixed and neutralized extract, followed by incubation at 40 °C for 10 min. To this, 0.02 ml of distilled water and 0.2 mL of glycine buffer was added, mixed, and incubated at 40 °C for 10 min, followed by the addition of trichloroacetic acid to stop the reaction. The contents were centrifuged at 13000 rpm for 10 mins and to 1 mL of the aliquot, 0.5 ml of colour forming reagent added before the absorbance at 655 nm was measured. A calibration curve was generated using the standard solution supplied with the kit. The P and PA contents were calculated using equation 2.6.

$$C = \frac{(M \times 20 \times F)}{10000 \times W \times V} \times \Delta A(P) \quad \text{Eq. 2.6}$$

Where M is mean of phosphorus standard value, 20 is original sample extract volume (mL), F is dilution factor, 10000 is conversion from µg/g to g/100g, W is weight of

original material, v is sample volume used in colourimetric determination step (1 mL) and ΔA is the difference between total and free P (i.e. total P – free P). The concentration of PA was calculated using equation 2.7.

$$PA (g/100g) = \frac{P(g/100g)}{0.282} \quad \text{Eq.2.7}$$

Where 0.282 is a factor to convert P to PA

2.4.3 Determination of Phytic acid to Zn molar ratio (PA/Zn molar ratio)

The number of moles of PA and Zn was determined by dividing the weight of PA and Zn with its atomic weight (phytate: 660.8 g/mole and Zn: 65.38 g/mole). The molar ratio between PA and Zn was determined after dividing the number of mole of PA by the number of mole of Zn.

2.4.4 Determination of human Zn bioavailability

Estimated human bioavailability of Zn was calculated for an intake of 300 g of grain using a published model for Zn absorption as a function of dietary Zn and PA. The development of the model is described in detail by Miller et al., (2007):

$$TAZ = 0.5 \left(\frac{A_{MAX} + TDZ + K_R \times \left(1 + \frac{TDP}{K_p}\right) - \sqrt{\left(A_{MAX} + TDZ + K_R \times \left(1 + \frac{TDP}{K_p}\right)\right)^2 - 4 \times A_{MAX} \times TDZ}}{2} \right) \quad \text{Eq. 2.8}$$

The model has three predictor variables include of total daily Zn (TDZ), total daily PA (TDP) and the response variable is total absorbed Zn (Haynes et al.). All units are mmol d^{-1} . The model has three parameters maximal absorption (A_{MAX}), Zn-transporter

binding equilibrium dissociation constant (K_R) and a Zn-phytate binding equilibrium dissociation constant (Mitchikpe et al., 2008). The values of A_{MAX} , K_R , and K_P vary calculated for healthy adult depending on the reference Table 2.4.

Table 2.4: Reference values (A_{MAX} , K_R , and K_P) used for the calculation of human Zn bioavailability.

References	A_{MAX}	K_R	K_P
Miller et al. (2007)	0.13 (0.034 to 0.23)	0.1 (-0.073 to 0.28)	1.2 (-0.36 to 2.8)
Brown et al. (2010)	0.11	0.065	0.77
Imran et al. (2016)	0.091 ± 0.007	0.033 ± 0.22	0.68 ± 0.01

All values are mean $\pm$ SE. A_{MAX} , maximal absorption of Zn; K_P , Zn-phytate equilibrium constant; K_R , Zn- transport receptor equilibrium dissociation constant.

2.5 Elemental analysis using ICP-MS

The multi-element analysis was undertaken using ICP-MS (Thermo Fisher scientific ICAP Q, Germany) operated in “collision cell mode” using helium gas to reduce polyatomic interferences. Samples were introduced from an autosampler (Cetac ASX-520 with 4 x 60 place sample racks). Internal standards were introduced to the sample stream through a T-Piece and included Ge (100 ng mL^{-1}), Rh (20 ng mL^{-1}), and Ir (10 ng mL^{-1}) in 2% HNO_3 . External multi-element calibration standards (Claritas-PPT grade CLMS-2, Certiprep/Fisher) included As, Al, Ca, Cu, Co, Cs, Cr, Cd, Fe, K, Mo, Mg, Mn, Ni, Na, Rb, Se, Sr, U, V, and Zn, all in the range of 0-100 $\mu\text{g L}^{-1}$. Sample processing was undertaken using plasma lab software Qtegra intelligent data solution™ (ISDS) (Thermo-Fisher Scientific, UK). Measured concentrations were corrected using the average of three blank digestions. All elemental concentrations were converted to mg kg^{-1} using equation 2.9.

$$C_{\text{soil}} = \frac{(C_{\text{soil}} - C_{\text{blank}})V_{\text{sol}}}{W_{\text{Soil}}} \quad \text{Eq. 2.9}$$

Where C_{soil} is the element concentration in soil (mg kg^{-1}), C_{soln} and C_{blank} are the concentrations in the soil and blank digests ($\mu\text{g L}^{-1}$); V_{sol} is the digestion volume and W_{soil} the mass of finely ground soil digested.

2.6 Statistical analysis

For analysis of variance; one way and two way, person correlation coefficient were used for all samples by using SPSS statistics v. 24 and Minitab 17.2.1. Other statistical calculations e.g. standard deviation, standard error, mean, median and average values were calculated using Microsoft Excel 2013. Statistically significant differences were considered to have a P value <0.05 .

3 THE SOLUBILITY OF PHOSPHORUS AND ZINC IN THE CALCAREOUS SOILS OF KURDISTAN

3.1 Introduction

Phosphorus (P) and zinc (Zn) are two important nutrients in the biological system which are essential for plants and humans for optimal growth and development (Fekri et al., 2011, Maret and Sandstead, 2006). However, their deficiency is a worldwide nutritional problem that reduces the yield of many crops especially cereals (Drissi et al., 2015). In arid and semi-arid regions with calcareous soils, P and Zn solubility, and the processes that limit their solubility differ with soil properties (Catlett et al., 2002, Al-Tameemi et al., 2015). It is not always straightforward to differentiate between mechanisms (Wandruszka, 2006). Soil-applied Zn and P fertilizers react with the soil solid phase, causing reduced soluble P and Zn by forming secondary minerals. When applied to soil containing a large amount of calcium, typically soluble P is precipitated as dicalcium phosphate (DCP) or octacalcium phosphate (OCP) (Al-Tameemi et al., 2015). Soluble Zn may precipitate Zn as ZnCO_3 (Dahiya et al., 2005), Zn_2SiO_4 and $\text{Zn}(\text{OH})_2$ (Sadeghzadeh, 2013), or be adsorbed on soil colloids (Abid et al., 2013) and carbonates (Jalali and Zinli, 2012) at $\text{pH} < 8.4$ (Duffner et al., 2012).

Jalali and Tabar (2011) and Catlett et al. (2002) suggest that at low pH, adsorption may control P and Zn solubility while at high pH, precipitation appears to determine solubility. Duffner et al. (2012) reported that Zn deficiency frequently co-occurs with P inadequacy since the bioavailability of both P and Zn is reduced with increasing soil pH. In general, soil CaCO_3 content appears to have a substantial influence on the solubility of P and Zn. Al-Tameemi et al. (2015) and Jalali and Zinli (2012) reported that adsorption processes dominate at low P and Zn concentrations while precipitation reactions tend to occur at higher P and Zn concentrations.

In addition to CaCO_3 content, contact time and rate of P and Zn fertilizers are also important factors controlling P and Zn solubility (Gustafsson et al., 2012, Shen et al., 2011, Delgado et al., 2002, Halajnia et al., 2009). Numerous reports have demonstrated variations in P and Zn solubility with other soil properties. Soil type, soil cation exchange capacity (CEC), metal concentration, clay mineral type, soil pH, Fe, Mn and Al oxides and organic matter content are also important variables affecting solubility and bioavailability (Fernandez-Calvino et al., 2012, Bradl, 2004, Martinez and Motto, 2000, Catlett et al., 2002). It has been estimated that about 80% (Neset et al., 2008) and 90% (Abid et al., 2013) of applied P and Zn in soils is rendered unavailable for plant uptake.

To understand the adsorption reactions of P and Zn various models have been used (Sinegani and Sedri, 2011, McDowell et al., 2003, McDowella and Sharpley, 2003). These reports give some information about mechanisms governing P and Zn adsorption but have limited practical use (Samadi, 2006).

Knowledge about Zn inadequacy in Kurdistan soils is limited but, there are several papers concerning P status. The current chapter explores the impacts of incubation time, soil pH and CaCO_3 content on P and Zn solubility in Kurdistan calcareous soils. Thus, the purposes of current work were to (i) recognise mineral controls over P and Zn solubility, (ii) evaluate the influences of incubation time on P and Zn solubility and (iii) determine the minerals controlling P and Zn solubilities in the calcareous soils of Kurdistan and (iv) determine the solubility and availability of P and Zn with time in soils from areas used for vegetable cultivation.

3.2 Material and methods

3.2.1 Soil survey in Sulaimanyah Province

Twenty-one soil samples were taken from seven geographically distributed sites in Sulaimanyah province, Kurdistan region, Iraq in November 2011 from five main locations (Sulaimanyah, Halabja, Sirwan, Khormal, and Kalar) (Table 3.1; Fig. 3.1). Previous PhD student collects these samples. A number of reasons have been considered to select samples location including (i) agricultural activity, (ii) source of irrigation water and (iii) types of agricultural products. There are no consistent data on P and Zn status in agricultural soils in the selected sites. High applications of phosphate fertilizers as triple superphosphate and diammonium phosphate were observed when farmers were interviewed to determine the amount and types of phosphate fertilizer that they applied but Zn fertilizer application was not mentioned because farmers usually only apply NPK. Therefore, this is the first work investigating the P and Zn status of the areas and determining the solubility of selected elements for this province.

The soil at each site was collected from selected fields used to cultivate various types of crops and fruits and leafy and fruit vegetables for local use or commercial sale. The selected sites are described below. At each site, 3 three soil samples were collected depending on a variety of produced vegetables. The distance between samples varied between 200 m to more than 1000 m (Fig. 3.1).

Table 3.1: Soil sample locations and sites details.

Location	No. of farms	Site description	Code
Sulaimanyah	3	Tanjaro	STA
Halabja	3	Halabja vegetable area	HAL
	3	Halabja waste area	HWA
Sirwan	3	Sirwan vegetable field	SVF
Khormal	3	Khormal	KHO
Kalar	3	Grdagozina	KAG
	3	Sirwan	KAS

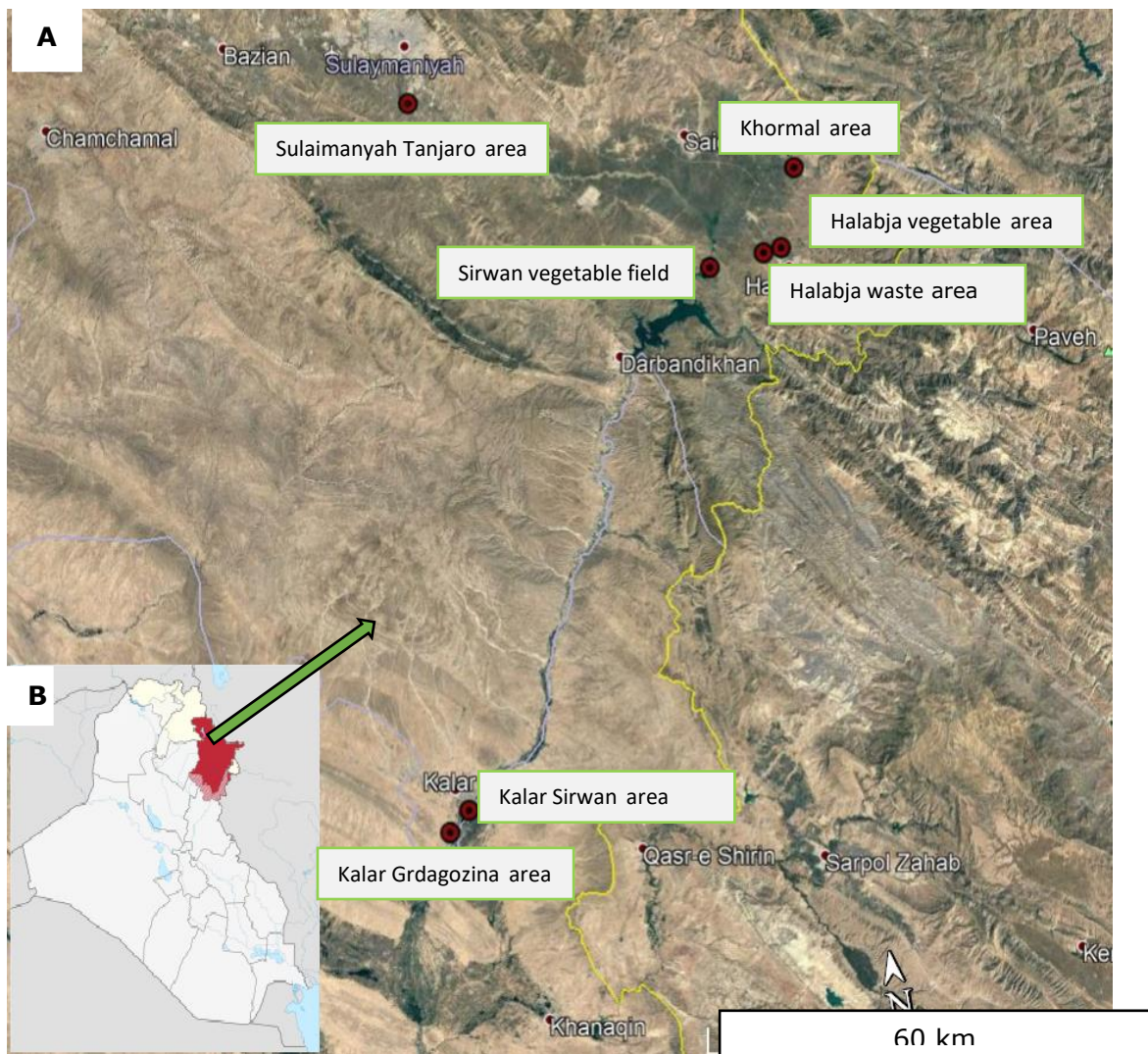


Fig. 3.1: Map of Sulaimanyah province showing soil collection sites. (A) Sulaimanyah province showing selected sites and (B) Iraqi map highlighting Sulaimanyah province.

3.2.1.1 Sulaimanyah Tanjaro

The Sulaimanyah Tanjaro site (STA) is located to the south of Sulaimanyah city, which is a peri-urban site ($45^{\circ}25'28.36''\text{E}$ and $35^{\circ}28'47.51''\text{N}$). Agricultural fields in this site are used for planting a range of vegetables and fruits. The main source of irrigation of water is the Tanjaro River, which contains of nutrients due to the inclusion sewage water from the city. Organic fertilizer, such as poultry manure and inorganic fertilizers are used as supplementary sources of nutrients-particularly NPK. Three sewage channels flow into the Tanjaro River; these were built to carry the household wastewater of the area. Beside this river, a range of agricultural activities is practised. Many types of fruit and vegetables are produced in this area for local consumption or commercial purposes. Close to the site, some places are used for city waste disposal, including metal workshop wastes, car scrap parts, and small-scale industries. Three soil samples were taken randomly from three different fields of waste located in this area (Fig. 3.2).



Fig. 3.2: Soil sampling around Tanjaro site in Sulaimanyah.

3.2.1.2 Halabja vegetable area

This site is located north-east of Halabja city ($45^{\circ}59'13.34''\text{E}$ and $35^{\circ}11'25.75''\text{N}$). Orchards, containing pomegranates and vineyards and many types of vegetable production are common including crops such as tomato, chard, radish, eggplant, okra, cowpea and beans. The main source of irrigation water is a natural spring which comes from Anab village located to the south-east of Halabja city and a well which has been made for agricultural purposes. A significant amount of fertilizer is used to increase fruit and vegetable production. The main markets for agricultural activity in this site are Halabja city and other cities in Kurdistan. Three soil samples were taken from this site randomly distributed across different fields (Fig.3.3).

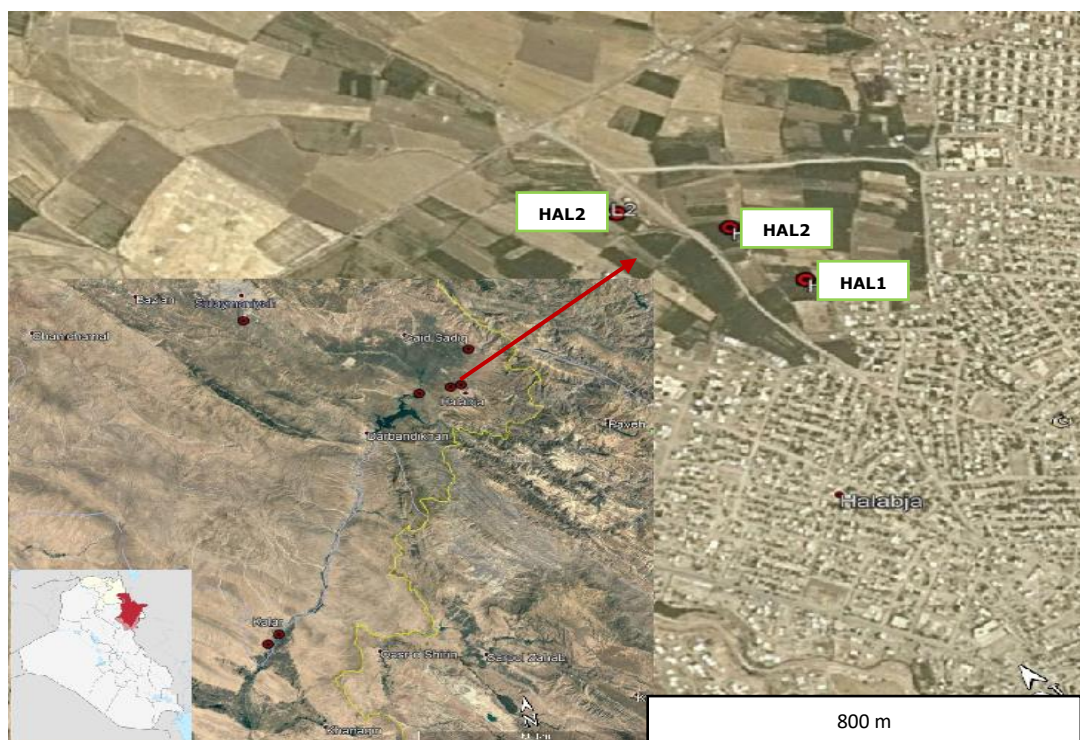


Fig. 3.3: Soil sampling location around Halabja vegetable site in Halabja location.

3.2.1.3 Halabja waste area

This site is located to the north-west of Halabja city in Zamaqi village a distance of approximately 3 miles from Halabja city ($45^{\circ}56'57.65''\text{E}$ and $35^{\circ}25'28.36''\text{N}$). Agricultural activity at this site commonly includes vegetable production. A natural spring located in the north of the area and a well located in the south are the main sources for irrigation in the area. A significant amount of fertilizer is used to increase fruit and vegetable production. As for the Halabja vegetable area, the main markets for agricultural activity in this site are Halabja city and other cities in Kurdistan. Three soil samples were taken in this area depending on the vegetable type being grown (Fig.3.4).

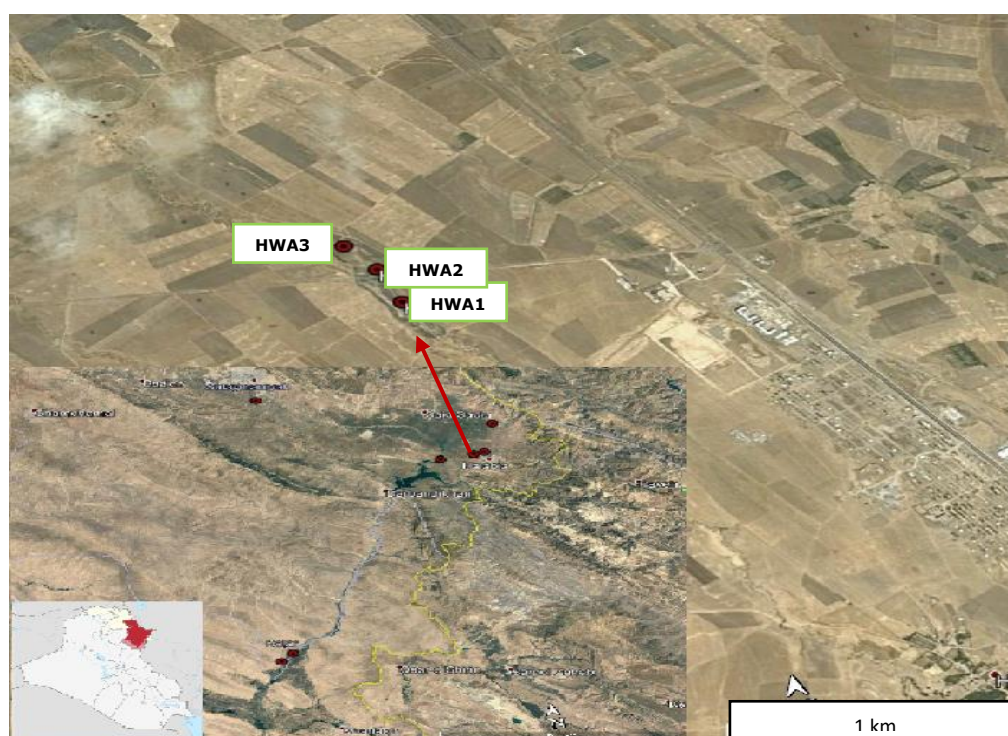


Fig.3.4: Soil sampling location around the waste site in Halabja.

3.2.1.4 Sirwan vegetable area

This site is located to the west of Halabja city in Sirwan district, 7 miles away from Halabja city close to rural areas near the Sirwan dams (45°51'31.72"E and 35°15'12.74"N). In this area both field and greenhouse agricultural activity is common and vegetables are widely produced. The main source of irrigation is an artesian well which has been made for agriculture purposes. Three soil samples were collected from different fields which were cultivated with many types of vegetable including cucumber, okra, eggplant, bean, cowpea, and tomato (Fig. 3.5).



Fig. 3.5: Soil sampling locations around the vegetable field site in the Sirwan location.

3.2.1.5 Khormal

This site was located to the west of Khormal district, approximately 2 miles from Khormal ($46^{\circ}1'29.88''\text{E}$ and $35^{\circ}18'15.83''\text{N}$). Agriculture activity in this area is commonly vegetable production including the use of greenhouses. The source of irrigation water is the Ahmed Awa River which is a large natural water source accessible to all fields in this area. This water is used by farmers with fields around the river as irrigation water to produce vegetables, field crops, and orchard fruit for local consumers or commercial outlets. Soil samples were taken in three different fields (Fig. 3.6).

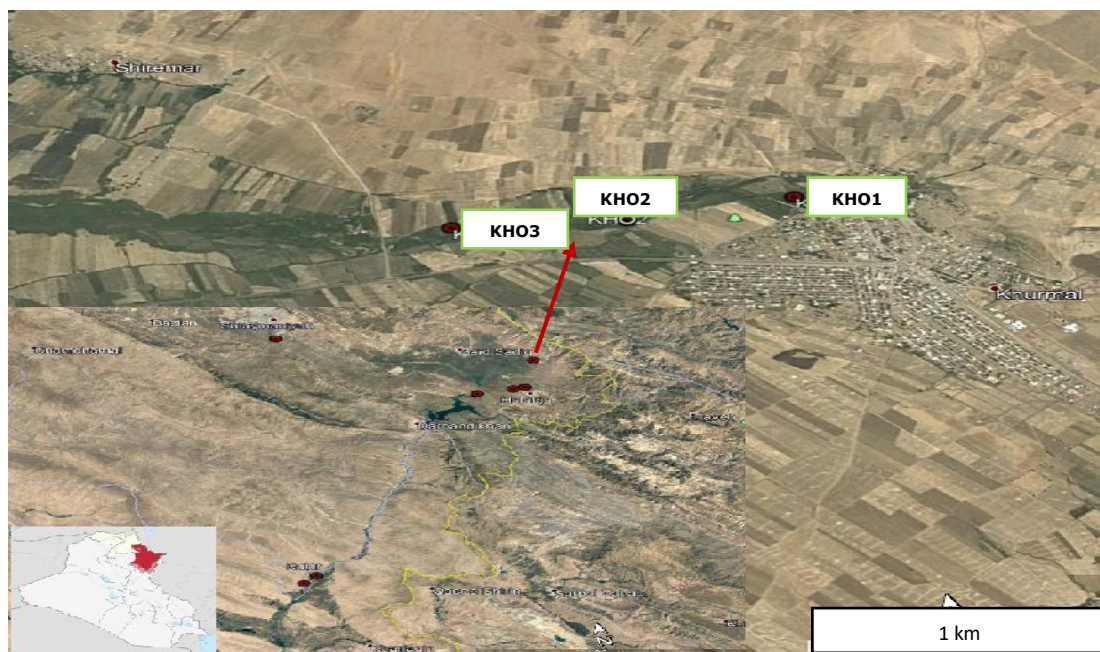


Fig.3.6: Soil sampling locations around the Khormal site.

3.2.1.6 Kalar Grdagozina area

This site was located to the south-west of Kalar city which is in the south-east of the Kurdistan region ($45^{\circ}16'55.18''\text{E}$ and $34^{\circ}35'02.44''\text{N}$). Grdagozina is rural land used mainly to product vegetables to sell in Kalar city. Sulaimanyah city normally depends on this site for its vegetable requirements because the area produces such a wide range of vegetables. Wells and water from the Sirwan River are the main sources of irrigation. To maximise production in this area large amounts of chemical fertilizer, especially NPK and organic manure, are used (Fig. 3.7). Three soil samples were taken of this site.

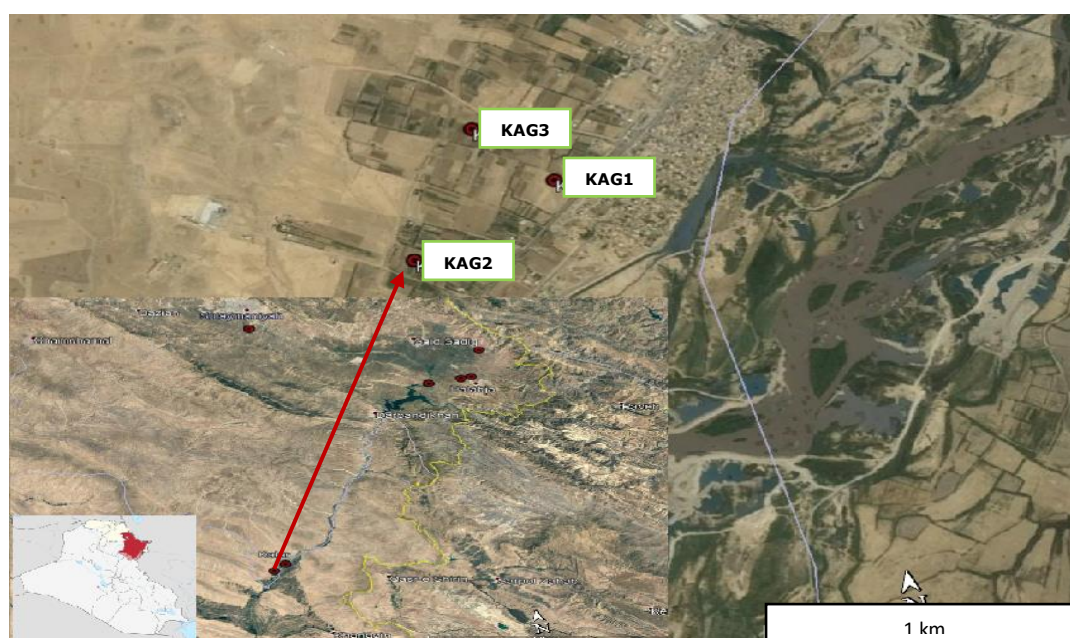


Fig. 3.7: Soil sampling locations around the Grdagozina site in Kalar.

3.2.1.7 Kalar Sirwan site

This site is located directly on the Sirwan River, south of Kalar city ($45^{\circ}19'04.63''\text{E}$ and $34^{\circ}36'21.63''\text{N}$). The Sirwan River is the main source of irrigation water in the area. A wide variety of leafy, root and fruit vegetables and crops are produced in this area, especially radish, cowpea, okra, tomato, and beans. In common with the Kalar Grdagozina area, farmers use a large amount of NPK and organic manures to increase production (Fig. 3.8).

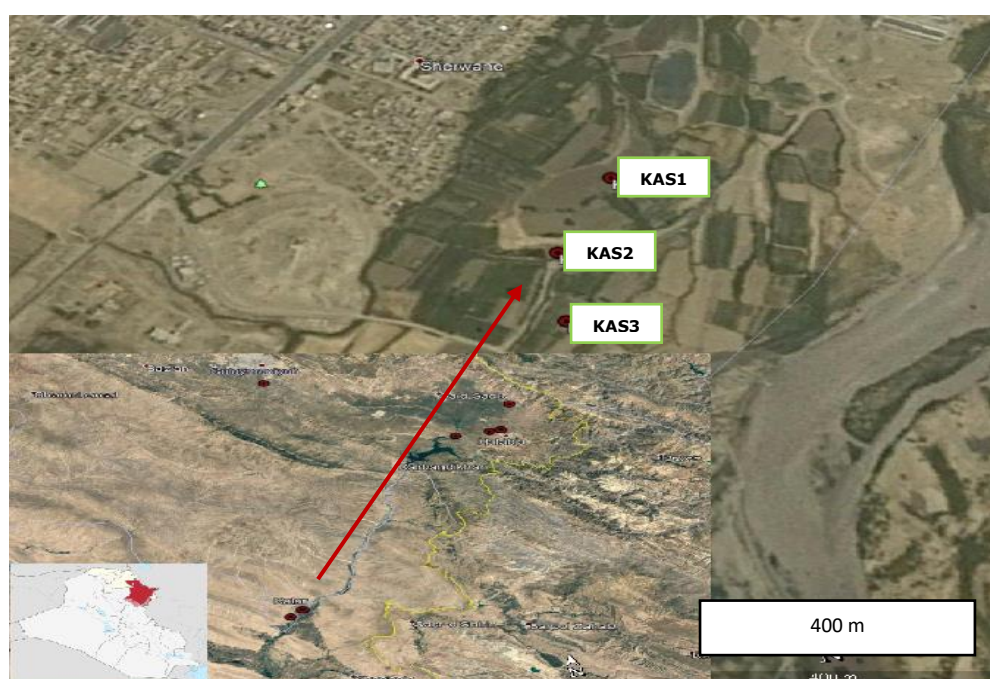


Fig. 3.8: Soil sampling locations around the Sirwan site in Kalar.

3.2.1.8 Soil sampling and preparation

Surface soil samples (0-30) cm were collected using a stainless steel trowel and then sealed in plastic bags for transport to a laboratory at the Technical Institute of Halabja. Approximately 1 kg of each soil was dried at room temperature in an aluminium tray, disaggregated, then sieved to obtain the <2 mm fraction. Approximately 25 g of each sample was then stored in plastic tubes, double labelled, and transported to Nottingham

University, UK. A portion of each sample was finely ground using an agate ball mill (Retsch, Model PM400), and then stored in polyethene bags.

3.2.2 Soil analysis

All the soils were analysed for soil chemical properties as shown in (Table 3.3).

Table 3.2: Sections and chapter used for soil analysis properties

Soil Analysis	Section	Chapter
Soil pH value	2.1.1	2
Loss on Ignition (LOI %)	2.1.2	2
Soil digestion	2.1.3	2
Elemental analysis	2.2.2	2
Determination of Olsen-P	2.1.4	2
Available Zn by DTAP	2.1.5	2

3.2.3 Soil inorganic carbon (SIC %)

About 50-100 mg air-dried soil was added to acid washed furnace ceramic boats and then, positioned in the Shimadzu solid sample unit instrument chamber (SSM-5000A). After 2.5 min. 0.7 mL 25% phosphoric acid (H_2PO_4^-) was added to the sample and positioned in the instrument again for measuring inorganic carbon content. Sodium bicarbonate and standard chalky soil (B2188) were used to produce a calibration curve for measuring soil inorganic carbon in the soil samples.

3.2.4 Phosphorus and zinc solubility experiment

The solubility of P and Zn were determined by incubating duplicate samples of 21 soils (c. 2 g, in total 220 samples incubated for five different period) at 50% water holding capacity (WHC %) at room temperature (20 ± 2) °C for up to eight weeks. Soil water-holding capacity was measured from each composite samples (one replicate) by soaking the soil samples in water for 2 h and then draining for 2 h with the method of Priha and

Smolander (1999). Periodically the solubility of nutrients was investigated by suspending 2 g soil in 20 ml of 0.0034 M calcium nitrate and shaking for 48 h followed by centrifugation (2500 rpm for 15 min), filtration (0.22 µm) and acidification of a 5 ml aliquot with 0.2 ml of 50% HNO₃ for analysis of P, Zn, Ca, Na, K, Mg, and S by ICPMS. A separate aliquot of prior to the un-acidified supernatant solution was retained for pH determination and measurement of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and phosphate concentration as described by Murphy and Riley (1962). Concentrations of soluble P (µg mL⁻¹) in samples were determined by using Equation 3.1.

$$\text{Soluble P} = \frac{(\text{Abs} \times \text{Slope})}{V_{\text{Ext.}}} \dots\dots\dots \text{Eq. 3.1}$$

In Eq. 3.1 Soluble P is the concentration of phosphate in the soil solution (µg mL⁻¹), Abs is the absorbance measured with a spectrophotometer at 880 nm, Slope is the slope of standard P solution, and V_{Ext} is the volume of the supernatant used in the colour development

3.2.5 Geochemical speciation using WHAM 7

The speciation of Ca and phosphate was undertaken using WHAM 7 according to Section 1.1.10 in Chapter 2.

3.2.6 Statistical analysis

Statistical analysis of data was performed using SPSS v. 24 to analyze multivariate relationships between soil properties and element concentrations. Soil parameters were subjected to analysis of variance (ANOVA) and means between sites were associated using an LSD (least significant difference) test ($p \leq 0.05$). Correlations were expressed as Pearson's correlation coefficient.

3.3 Results and discussion

3.3.1 Soil chemical characteristics

The measured values of soil pH, Loss on ignition (LOI %) and soil inorganic carbon (SIC %) in the soils are summarized in Table 3.3, for each field studied. The pH of soils ranged from 7.33 for STA1 to 8.27 for STA2 which reflects the calcareous soil characteristics with SIC (%) from 0.67-4.92. The loss on ignition ranged from 4.80% for the KAS2 field to 12.0% for the Hal1 field. There was a significant difference ($p < 0.05$) across sites for LOI and SIC but, no significant difference for soil pH due to the narrow range of pH values caused by buffering in all soils reducing from the universal presence of CaCO_3 . All pH values were in the range expected for calcareous soils (Alloway, 2009).

Table 3.3: Values of soil pH, %LOI and %SIC; (n=21). Data are organized by the field code.

Site	Field code	pH	LOI (%)	SIC (%)
HAL	HAL 1	7.85	12.0	1.86
	HAL 2	8.07	10.2	1.21
	HAL 3	8.17	11.8	1.15
HWA	HWA1	8.15	10.8	1.57
	HWA2	8.16	11.4	2.37
	HWA3	7.83	11.0	1.59
SVF	SVF1	8.13	9.00	0.67
	SVF2	8.17	8.40	3.97
	SVF3	8.12	8.00	3.08
KHO	KHO1	7.94	7.20	4.56
	KHO2	7.80	8.20	4.83
	KHO3	7.77	7.40	4.48
STA	STA1	7.33	10.0	4.92
	STA2	8.27	7.40	3.64
	STA3	8.23	10.0	4.01
KAG	KAG 1	8.13	8.00	3.71
	KAG 2	8.03	10.0	3.56
	KAG 3	8.04	8.80	3.71
KAS	KAS1	8.14	7.60	3.11
	KAS2	8.15	4.80	3.04
	KAS3	8.16	5.20	3.25
Average		8.03	8.90	3.06

All 21 soil samples were analyzed for total element concentrations by ICP-MS according to Section 2.2.2 in Chapter 2. The average soil P concentration (mg kg^{-1}) ranged from 622 for STA to 1210 for HWA respectively as shown in Fig. 3.9; a significant difference ($p < 0.05$) was found between sites. The P concentrations in the studied soils fell in the worldwide range reported by Lindsay (1979); the reported world average is 600 mg kg^{-1} with a range from 200 to 5000 mg kg^{-1} . The difference in P concentration between the sample sites may reflect the influence of the parent material and environmental conditions but probably mainly reflects the phosphate fertilizer application history by individual farmers because these areas are used to produce a range of vegetables, crops, and fruits. Farmers in these areas have traditionally applied large rates of fertilizer-especially NPK. Farmers apply NPK every month, especially in the case of leafy, root and fruit crop production. Song et al. (2017) studied the long-term application of organic manures and inorganic phosphate fertilizers on soil inorganic phosphorus in calcareous soil. They found, using a P fractionation approach that continued application of inorganic phosphate fertilizers caused accumulation of DCP, OCP and Al-P in the soil which increased the risk of pollution.

Regression analysis indicated that total P concentration was negatively related to soil pH ($r = 0.74$, $p < 0.05$) (Fig. 3.10) but not significantly correlated with %LOI.

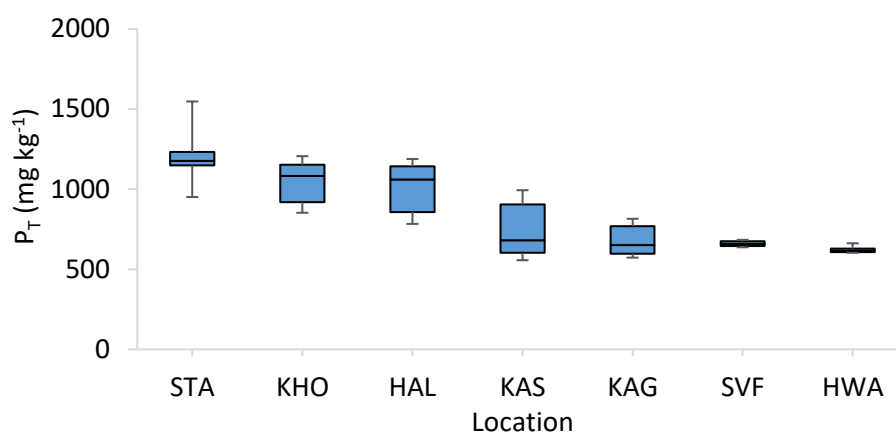


Fig. 3.9: The average values of total P concentration in studied soils collected at seven different locations in Sulaimanyah province. The source of irrigation water in STA, KHO and HAL was water waste from cities, in KAS and KAG was leake and in SVF and HWA was well water (clean water).

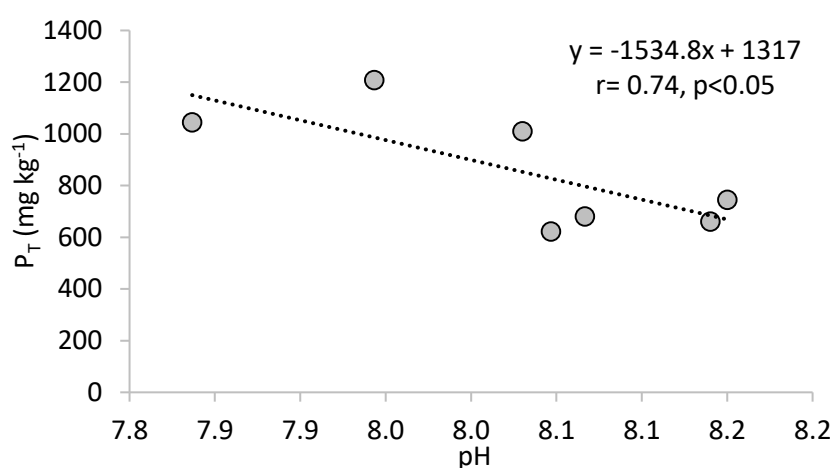


Fig. 3.10: The relationship between total P and soil pH in studied soils collected in seven different sites in the agricultural activity fields.

The average values of Zn_T ($mg\ kg^{-1}$) ranged from 48.8 ± 4.65 to 151 ± 58.2 for KAG and STA sites respectively as shown in (Fig. 3.11). A significant difference ($p < 0.05$) was found between sites. Similar Zn concentrations (15.2 to $277\ mg\ kg^{-1}$) have been reported in urban soils (Romic and Romic, 2003) and Zn_T in all the studied soils were in the worldwide range reported by Preetha and Stalin (2014) and Noulas et al. (2018) average = $50\ mg\ kg^{-1}$; range = 10 - $300\ mg\ kg^{-1}$. According to Kabata-Pendias and

Mukherjee (2007a), the range of Zn in calcareous soils varies from 50 to 100 mg kg⁻¹. The difference in Zn concentration between the sample sites may reflect the influence of the parent material and environmental conditions. Thus, Chowaniak and Gondek (2009) found that soil properties have more effect than quantities of fertilizer applied on Zn_T. However, variation in Zn_T may also reflect the use of sewage water for irrigation by farmers. The sample sites are used to produce a range of vegetables, crops, and fruits and farmers in these areas use sewage water as a source of irrigation water. Kharche et al. (2011) studied the long-term application of sewage water on soil Zn content. They found that Zn_T in the soils irrigated with sewage water was four times higher than in the soils irrigated with (clean) well water.

A number of parameters may control metal solubility and bioavailability in the soil, including metal concentration and speciation, soil type, soil pH, and contact time (Bradl, 2004). In general, greater metal retention and lower solubility occur at high soil pH and low organic carbon content and at low metal concentrations. In the current study, STA site had much higher Zn_T compared with other studied sites. This is may be because this site used by farmers for longer time than others and higher applications of organic matter and sewage water were used. Rattan et al. (2005) studied effect of long-term using waste water on heavy metal content in soils, they found that irrigation soil with sewage increased Zn concentration by 113%. The regression analysis showed that Zn_T was only related to LOI% ($R^2=0.3349$, $p<0.006$) (Fig. 3.12). No significant correlation was observed between Zn_T and pH suggesting that the ability of the soil to retain Zn was not a factor in determining the total Zn content.

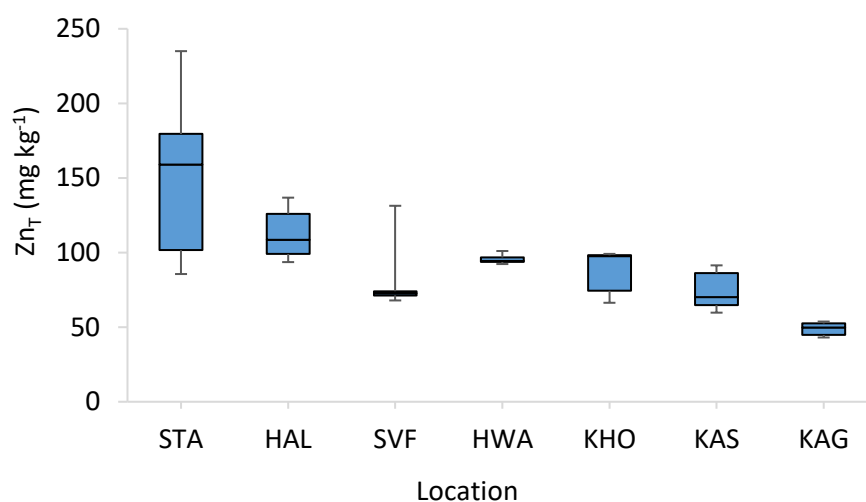


Fig. 3.11: The average total concentration of Zn_T in 21 soils from seven locations (Table 3.3). The source of irrigation water in STA, KHO and HAL was water waste from cities, in KAS and KAG was leake and in SVF and HWA was well water (clean water).

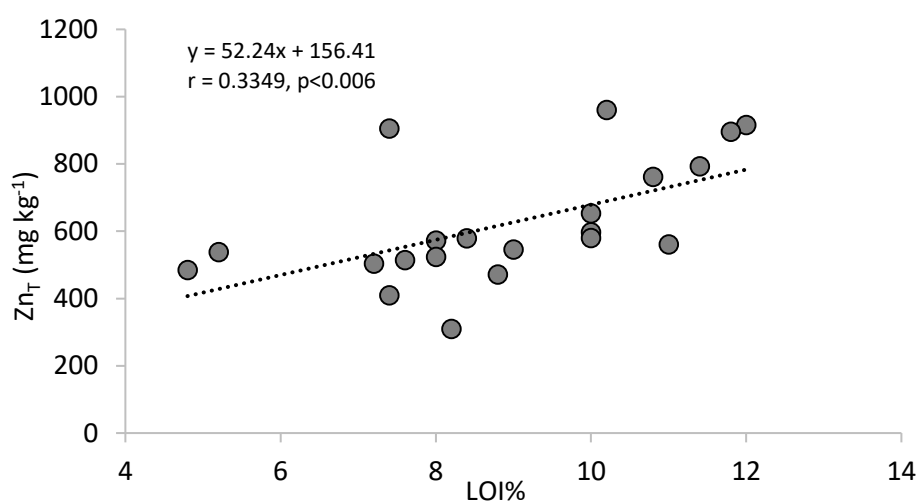


Fig. 3.12: Correlation between loss on ignition and Zn_T in 21 soils from seven locations (Table 3.3).

3.3.2 Soils phosphate

3.3.2.1 Phosphorus balance under phosphate fertilization

Average values of total P (P_T), Olsen-P (P_{Olsen}) and extractable P by (0.034 M Ca (NO_3)₂) (P_{Sol}) in all sites are summarised in Table 3.3. Large concentrations of P_T , P_{Olsen} and P_{Sol} were present in selected areas, probably due to long-term P fertilization. Values of P_{Olsen}

(mg kg⁻¹) and P_{Sol} (mg L⁻¹) varied from 24.2±6.14 mg kg⁻¹ and 0.04±0.01 mg L⁻¹ for the HWA site to 122.4±16.7 mg kg⁻¹ and 1.46±0.37 mg L⁻¹ for the STA site respectively. The mean concentrations for P_{Olsen} and P_{Sol} across all the studied soils were 65±2.33 mg kg⁻¹ and 0.59±0.03 mg L⁻¹ respectively (Table 3.4). The concentrations of P_{Olsen} and P_{Sol} forms were significantly greater in the STA and KAG sites compared to other sites and differences between the mean values of all sites were also significant.

The P_{Olsen} and P_{Sol} concentrations increased with P_T. Shaheen et al. (2007) and Garg and Bahl (2008) reported that P_{Olsen} strongly depended on the inorganic P fractions in the soil. Values of P_{Sol} also increased with P_{Olsen} especially above a value of approximately 60 mg kg⁻¹ of P_{Olsen}. A similar result was reported by Motavalli and Miles (2002); they found that the long-term application of inorganic P fertilizers was the main determinant of soluble P due to the accumulation of P in the surface soil. P_{Sol} may be regarded as the primary indicator of P accumulation.

The concentrations of P_{Olsen} accounted for only about 4-15% of P_T; values of P_{Sol} were approximately 0.10-0.20 % of the P_T. Then only about 3.70-1.21% of P_{Olsen} was present as P_{Sol}. Regression suggested that P_{Olsen} and P_{Sol} were linearly correlated to P_T (r=0.54, p<0.01, r=0.42, p<0.05) (Figs 3.13a and b respectively), but not significantly correlated with soil pH, LOI and SIC contents. Similar results were reported by Devau et al. (2011). The results show a significant difference between soils in their chemical P forms possibly an indicator of the effect of environment, soil type and the amount of applied P fertilizer. McDowell et al. (2003) indicated that the kinetics of P release differ between soil types and may control the availability of P in the soil environment. Shariatmadari et al. (2006) suggested that the availability of P in the soil is a function of the quantity and intensity of P rather than specific soil properties. Poor correlations were found between P_T and P_{Olsen} with LOI% and SIC%.

Table 3.4: The average values of P_T , P_{Olsen} in (mg kg^{-1}) and P_{Sol} (mg L^{-1}) in studied sites.

Location	No. of farms	P_T	P_{Olsen} (mg kg^{-1})	P_{Sol} (mg L^{-1})
HAL	3	1010±180c	61.7±9.33bc	1.00±0.10bc
HWA	3	622.0±22.6c	24.2±1.73c	0.89±0.02c
SVF	3	660.0±19.6c	31.1±3.05c	0.88±0.02c
KHO	3	1040±151bc	57.1±10.7bc	1.30±0.50abc
STA	3	1210±195a	122±29.5a	1.40±0.66a
KAS	3	745.0±195c	57.7±10.1bc	1.00±0.19abc
KAG	3	680.0±107c	101±15ab	1.40±0.30ab

* Different letters show significant differences among soil sites

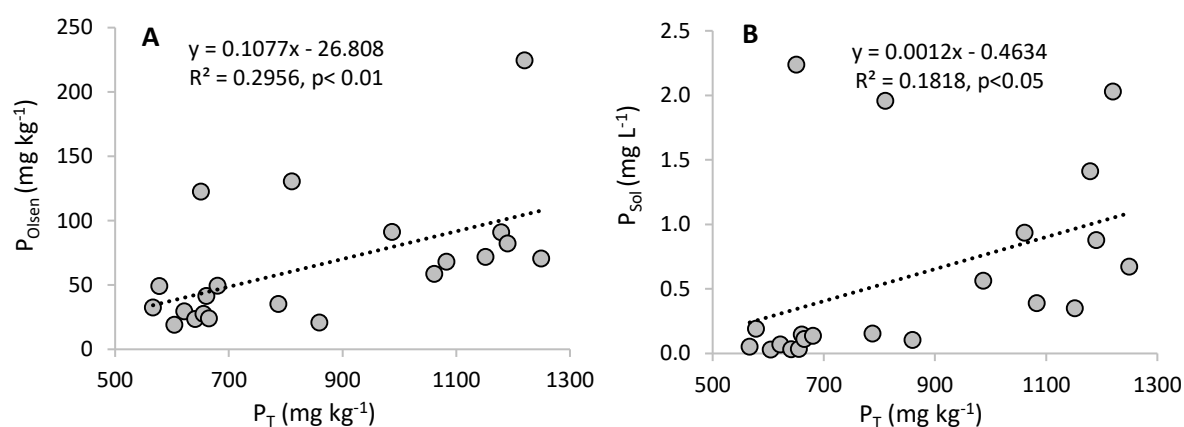


Fig. 3.13: Correlations between (A) P_T and P_{Olsen} and (B) P_{Olsen} and P_{Sol} in soils from seven study locations (Table 3.3).

3.3.2.2 Soluble P related to Olsen-P and incubation time

Various studies showed that long-term phosphate fertilizer application increases P mobility and environmental risk (LI Yu-yuan et al., 2013). Wand et al. (2015) reported that in calcareous soils, continuous phosphate fertilizer application leads to an increase in potential P losses. Wang et al. (2015a) reported that, in calcareous soil, long-term phosphate fertilizer application can cause a build-up Ca-P minerals as stable solid phases. Jalali and Jalali (2017) reported that 0.2-0.3 mg P L⁻¹ is adequate for maximum crop growth in the calcareous soils but levels above this can cause an increase in the

surface water contamination risk. When P concentration in the soil solution exceeds the solubility product of solid phase, P will tend to precipitate.

All the studied soil samples were analyzed for P_{Sol} , ranged from 2.24 ± 0.28 to $0.03 \pm 0.005 \text{ mg L}^{-1}$ for SVF3 and KHO1, KAG3 and HAL1 fields respectively (Table 3.5). A significant difference ($p < 0.05$) was found in P_{Sol} between soil sites. In 62% of soil samples, the P_{Sol} value was more than 0.3 mg L^{-1} , which increases the risk of P losses. This unexpectedly high level of P is probably due to long-term phosphate fertilizer application by farmers.

Table 3.5: Values of P_{Olsen} and P_{Sol} in studied soils; mean values with standard deviations.

Site	Farm/site	P_{Olsen} (mg kg^{-1})	SD	P_{Sol} (mg L^{-1})	SD
STA	STA1	58.6	1.25	0.94	0.025
	STA2	35.3	5.70	0.15	0.015
	STA3	91.1	0.39	1.41	0.049
KHO	KHO1	19.2	1.46	0.03	0.000
	KHO2	29.6	4.77	0.07	0.002
	KHO3	23.7	2.16	0.03	0.000
HAL	HAL1	27.7	0.25	0.03	0.005
	HAL2	41.5	1.01	0.15	0.003
	HAL3	24.1	4.35	0.11	0.000
KAS	KAS1	20.8	0.24	0.10	0.003
	KAS2	82.3	3.96	0.88	0.005
	KAS3	68.3	2.33	0.39	0.011
KAG	KAG1	71.9	0.04	0.35	0.001
	KAG2	70.6	2.68	0.67	0.012
	KAG3	224	1.00	2.03	0.247
SVF	SVF1	49.2	1.81	0.19	0.007
	SVF2	131	3.40	1.96	0.042
	SVF3	123	15.7	2.24	0.280
HWA	HWA1	91.4	0.87	0.56	0.005
	HWA2	49.6	3.18	0.14	0.006
	HWA3	32.6	0.21	0.05	0.000

Concentrations of P_{Sol} from incubated soils are shown in (Fig. 3.14); although the data were slightly affected by time of incubation the changes were not significant. During the experiment, the amount of P_{Sol} remained stable over time from the first day until

the end of the experiment. Exceptionally, in KAG2, P_{Sol} decreased from 28 days to the end of the incubation while in STA3 and KAS1 P_{Sol} increased slightly over time. The stability of P_{Sol} after 14 days of incubation suggests equilibrium was achieved.

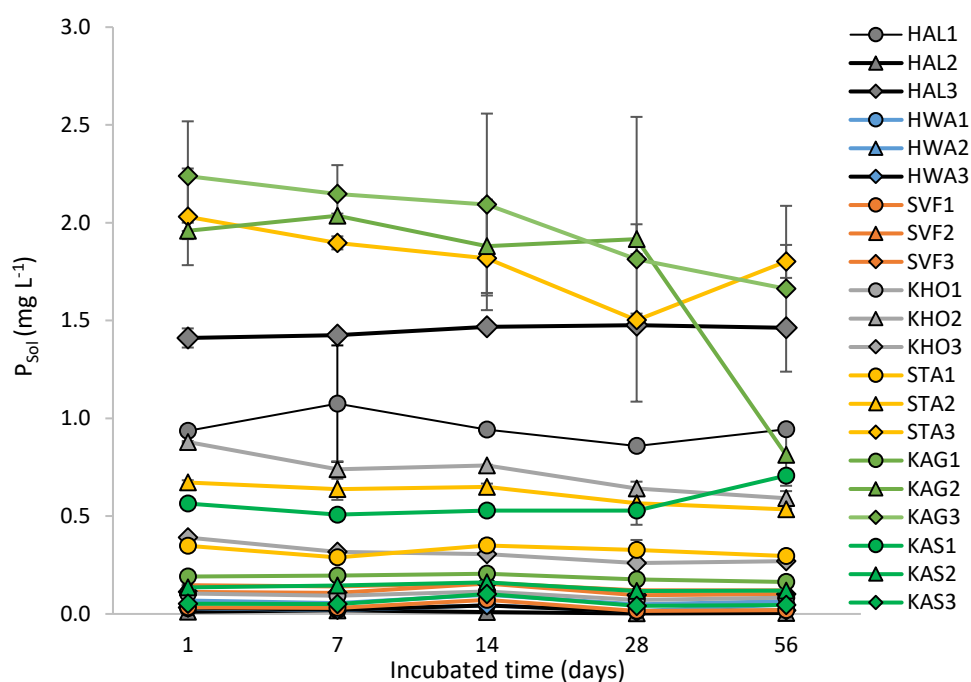


Fig. 3.14: Mean change in P_{Sol} (mg L^{-1}) over time after incubation for 1, 7, 14, 28 and 56 days.

A strong significant correlation ($R^2 = 0.84$, <0.001) was found between P_{Olsen} and P_{Sol} (Fig. 3.15), but no correlations were found between P_{Sol} with P_T , soil pH and LOI. Jalali and Tabar (2011) and Mohasses (2015) reported a similar correlation between P_{Olsen} and P_{Sol} in calcareous soils from Hamedan and Qazvin, Iran. Figure 3.16 shows the correlation between P 'intensity' (HPO_4^{2-} and H_2PO_4^- activities in the soil solution following speciation using WHAM-7, mg L^{-1}) and P 'quantity' (mg kg^{-1}) for all studied soil with respect to time of incubation. The P intensity with time of incubation showed a strong correlation between P quantity and P intensity for both anion species.

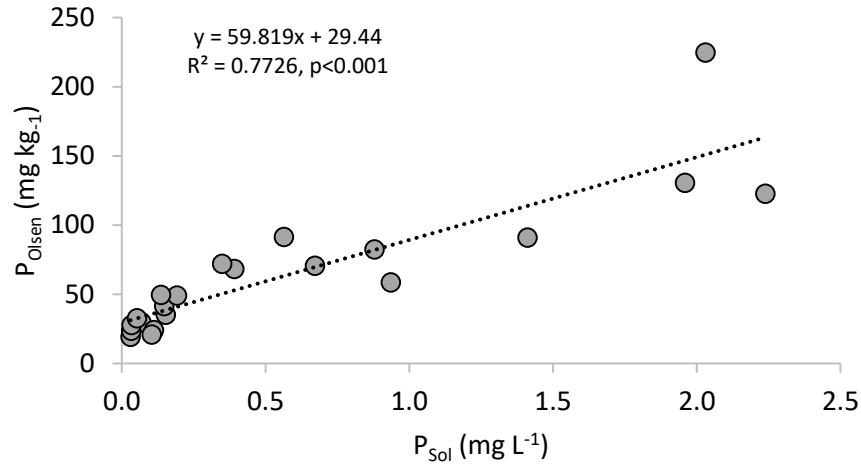


Fig. 3.15: Correlation between P_{Olsen} and P_{Sol} in studied soils before incubation.

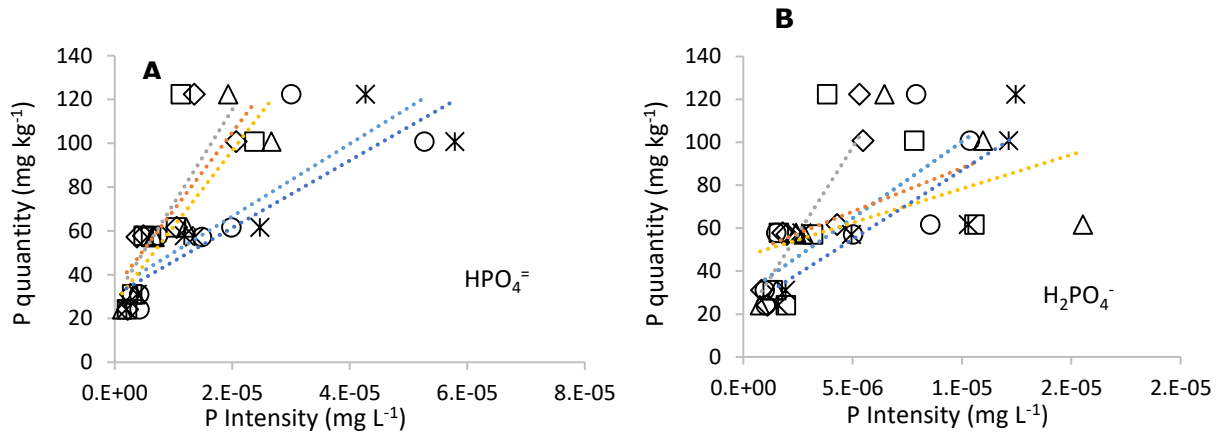


Fig. 3.16: The average of P Quantity-intensity relationship for studied soils, expressing P intensity concentration (X-axis) (A) HPO_4^{2-} and (B) H_2PO_4^- as a function of P quantity 'Olsen-P'. The studied soils from Kurdistan were incubated for (○) 1 day, (□) 7 days, (◇) 14 days, (△) 28 days and (×) 56 days.

3.3.2.3 Phosphate solid $\rightleftharpoons$ liquid partition coefficient ($\text{Log } K_d P$)

The solid-liquid partition coefficient ($\log K_d$) is widely used in risk assessments as a guide to the environmental mobility of elements, pesticides etc. (Chen et al., 2009). In the studied soils, the value of $\log K_d P$ was estimated for P adsorption ($C_{\text{solid}}/C_{\text{aqueous}}$), where C_{solid} (mg kg^{-1}) and C_{aqueous} (mg L^{-1}) refer to the P_{Olsen} content and H_2PO_4^- ion

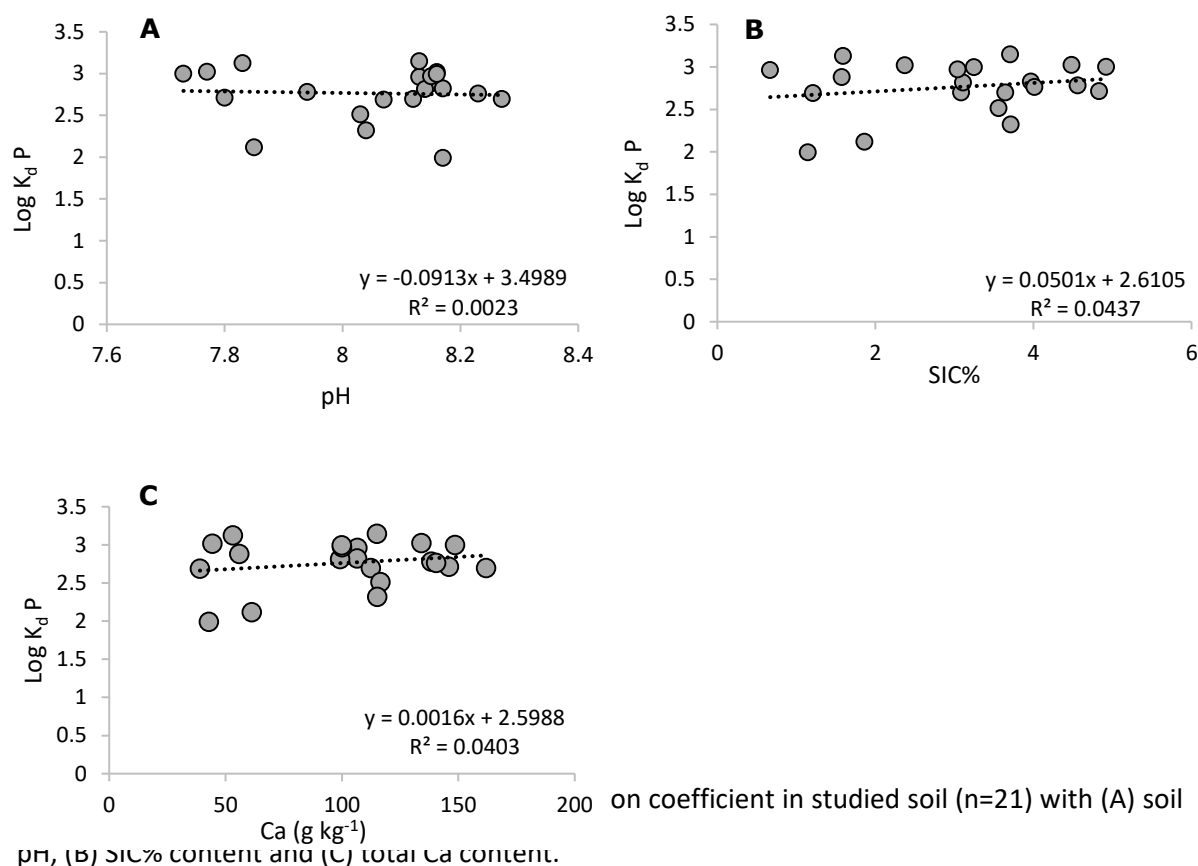
concentration in the solution determined following speciation using the geochemical model WHAM-7. The partition coefficients suggest a generally low soil adsorption strength for labile P; log K_d P ranged from 2.27 ± 0.37 for HWA sites to 3.01 ± 0.12 for HAL sites respectively (Table 3.5).

The soil properties likely to affect log K_d P values include soil pH and the concentrations of CaCO_3 , soil organic matter and Fe, Al and Mn oxyhydroxides. However, log K_d P values were very conservative and, unexpectedly, were not related to the value of soil pH due probably to the small range of soil pH values (Fig. 3.17a). Values of log K_d P increased slightly with Ca concentration and the %SIC (Figs 3.17b and c) but again variation was restricted due to similar Ca and SIC contents in all the studied soils. Correlation between k_d P and SIC was reported by Shaheen et al. (2013) and Wandruszka (2006).

Table 3.6: The average values of Log K_d for H_2PO_4^- in studied sites.

	Soil sites						
	HAL	HWA	SVF	KHO	STA	KAG	KAS
Log K_d P	2.27	3.01	2.83	2.84	2.82	2.66	2.93
SD	0.37	0.12	0.13	0.16	0.16	0.43	0.10

*SD= standard deviation



3.3.2.4 Phosphate solubility

Phosphate solubility is generally low in calcareous soils because of rapid immobilization by calcium carbonate and slow re-crystallisation as poorly soluble calcium phosphate minerals (Pizzeghello et al., 2011, Braschi et al., 2003). The initial mineral phosphate formed in calcareous soils is dicalcium phosphate, from the original mono-calcium phosphate in TSP, which is then converted into octacalcium phosphate and more basic hydroxyapatite. A 'predominance' or 'stability' diagram for calcium phosphate equilibria was developed by plotting 'phosphate potential' ($\log H_2PO_4^- - pH$) against lime potential ($\log Ca^{2+} + 2pH$). The activities of Ca^{2+} , $H_2PO_4^-$ and H^+ (pH) used to develop this diagram, were determined using measured values of pH and Ca and P concentration in solution followed by speciation using WHAM-7. This approach can indicate the likelihood of specific phosphate minerals being present in soils at different pH values (Zhang et al., 2014).

Figure 3.18 presents the soil solution composition (phosphate and lime potential) in relation to common Ca phosphate minerals in the studied soils. The Ca and phosphate data fell between slightly supersaturated with respect to dicalcium phosphate (DCP) and undersaturated with respect to β -tricalcium phosphate (β -TCP) or hydroxyapatite across all the studied soils and in all incubation periods (weeks 1-8). The results in Fig. 3.16 also suggest that the soils that have more available phosphate (Indices 6 and 8) were saturated with DCP while the soils that have less available P (Indices 2 and 3) were undersaturated with respect to OCP, indicating a clear link between available P and indicated Ca phosphate minerals. A similar result was reported by (McDowell et al., 2003). The results of the current study indicate that OCP and β -TCP minerals may be stable forms of P mineral in all the studied soils. This is agreement with the finding of Zhang et al. (2014) who found that the solubility of phosphate in farmed soils was controlled by β -TCP while, in unfarmed soils it was controlled by HP. They also reported that in farmed calcareous soils, after several years, HA became the controlling Ca phosphate mineral despite historically large applications of phosphate fertilizers.

The solubility of phosphate minerals appeared to decrease over time with small fluctuation from the first day to 56 days of incubation for all studied soils. Thus the Ca-phosphate minerals gradually transformed to more stable forms in the presence of a large excess of CaCO_3 in the calcareous soils.

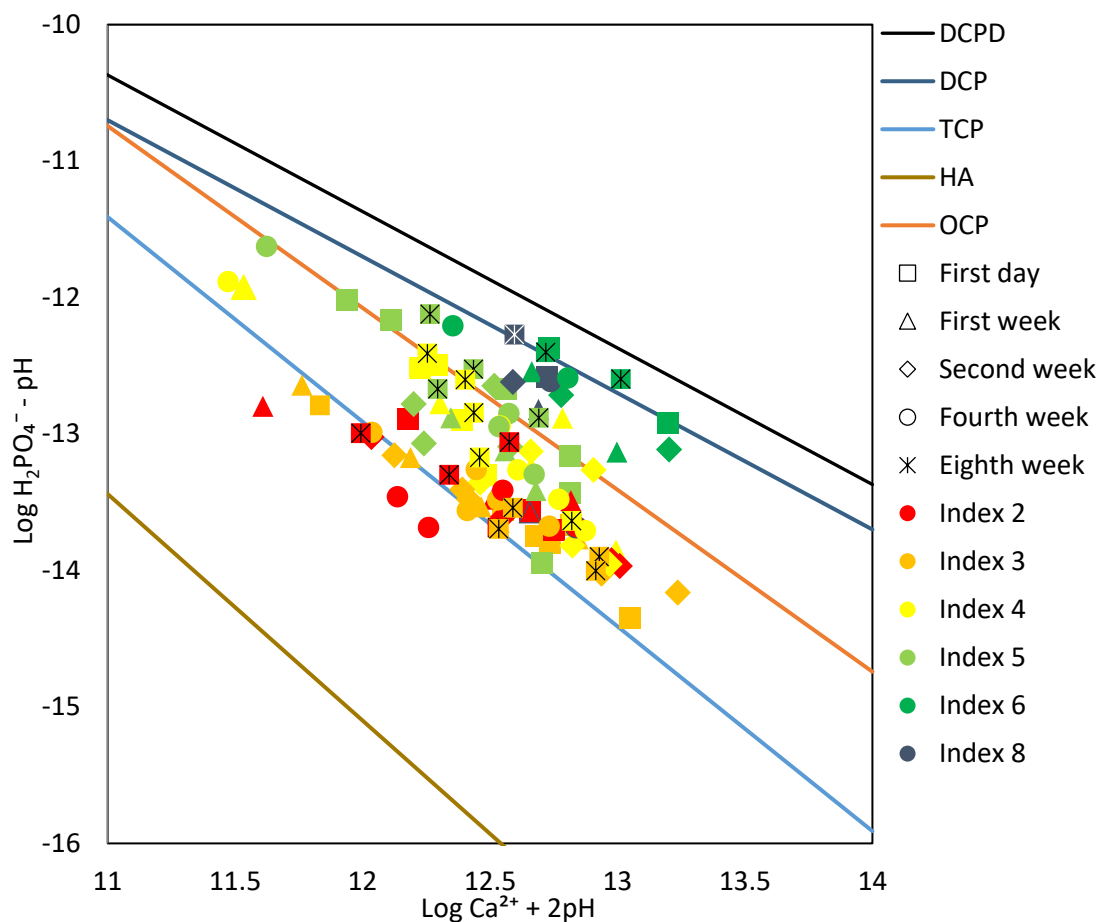


Fig. 3.18: Phosphate solubility in the studied soils in relation to crystalline Ca–P minerals: monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; β -tricalcium phosphate, β -TCP; and hydroxyapatite, HAP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by Lindsay (1979). The points in the diagram separated with different color depending on its index (amount of available P (mg kg^{-1}) as P_{Olsen} in the soil); including index 2 (10-20), index3 (20-30), index4 (30-40), index5 (40-50), index6 (50-60) and index8 (>80) mg kg^{-1} P_{Olsen} in the soil.

3.3.3 Soils zinc

3.3.3.1 Zinc fractionation in the absence of Zn fertilization

The average values of Zn_T , Zn_{DTPA} and Zn_{Sol} at all sites are summarised in Table 3.8. There was variation in all three measures of soil Zn status (Zn_T , Zn_{DTPA} and Zn_{Sol}). HAL and KHO had the highest and lowest Zn_{DTPA} respectively but STA and SVF had the highest and lowest Zn_{Sol} concentrations. It is known that farmers in the area use sewage

water for irrigation, although the amounts and composition of this water are not recorded. It is also recognised that use of sewage water can increase Zn_T (Jalali and Khanlari, 2006) and Zn_{DTPA} (Kidd et al., 2007). The data from the current study shows that Zn_{Sol} increased with Zn_{DTPA} – possibly suggesting a solid-solution equilibrium within the ‘available’ Zn pool; a similar result was reported by Motavalli and Miles (2002).

Values of Zn_{Sol} were under the critical value for optimum plant growth in all the studied soils; a similar result for calcareous soils was reported by Zeng et al. (2011); they found a negative correlation between soil pH and extractable Zn in paddy soils. Kabata-Pendias and Mukherjee (2007a) reported that only about 1% of Zn_T was present as Zn_{DTPA} . In the current data, Zn_{DTPA} was slightly greater and accounted for 0.48-8.57% of Zn_T ; Zn_{Sol} was 0.008-0.034% of Zn_T and 0.24-1.81% of Zn_{DTPA} . Regression against soil characteristics suggested that Zn_{DTPA} and Zn_{Sol} were linearly correlated to Zn_T ($r=0.52$, $p<0.004$; $r=0.47$, $p<0.03$) (Fig 3.19a and) respectively, suggesting broadly similar controls on solubility and availability in the range of soils surveyed.

Table 3.7: Average values of Zn_T , Zn_{DTPA} and Zn_{Sol} in 21 soils collected from seven different locations.

Location	No. of farms	Zn_T	Zn_{DTPA}	Zn_{Sol}
			($mg\ kg^{-1}$)	($mg\ L^{-1}$)
HAL	3	113±18.0b	9.65±6.69a	0.009±0.004bc
HWA	3	95.5±3.20bc	0.45±0.17c	0.006±0.002c
SVF	3	81.7±24.4bc	1.31±0.18c	0.005±0.00c
KHO	3	87.6±16.3bc	1.23±0.19c	0.013±0.008ab
STA	3	151±58.2a	3.01±1.41b	0.016±0.009a
KAG	3	48.8±4.65d	3.01±1.41bc	0.008±0.004bc
KAS	3	74.3±13.8cd	2.99±1.78bc	0.007±0.002c

Note: different letters show significant difference among soil areas at ($p<0.05$)

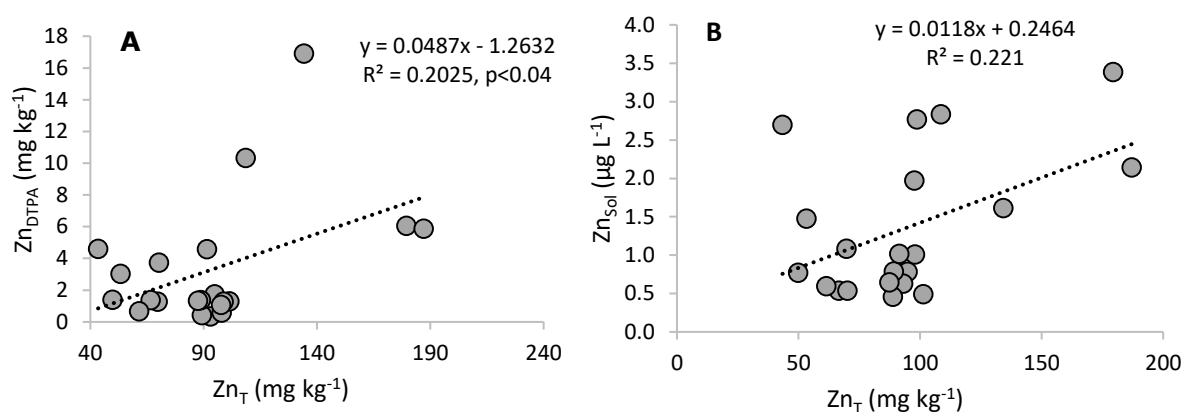


Fig. 3.19: Correlation between (A) Zn_T and Zn_{DTPA} , (B) Zn_T and Zn_{Sol} , and (C) Zn_{DTPA} and Zn_{Sol} in the studied soils collected at seven different sites in the agricultural activity fields.

3.3.3.2 Soluble Zn related to DTPA-Zn and incubation time

The 21 soil samples were analyzed for extractable Zn_{Sol} by equilibration in 0.034 M $Ca(NO_3)_2$; STA1 and SVF1 fields had the highest and lowest values of Zn_{Sol} (Table 3.9). A significant difference (≤ 0.05) was found in Zn_{Sol} between soil sites. Values of Zn_{Sol} were lower than the critical value for optimum plant growth 4-270 $\mu g L^{-1}$ (Alloway, 2009), again reflecting the calcareous nature of soils, high pH, low amount of organic matter and possibly high application of phosphate fertilizers (Noulas et al., 2018). Singh et al. (2017) reported that in calcareous soils, long-term phosphate fertilizer application decreases soluble Zn due to change in soil physiochemical properties or formation of Zn-P compounds. No correlations were found for Zn_{Sol} with Zn_T , soil pH or LOI%.

Table 3.8: The values of Zn_{DTPA} and Zn_{Sol} and standard deviation in 21 soils sampled.

Site	Farm	Zn_{DTPA} ($mg\ kg^{-1}$)	SD	Zn_{Sol} ($\mu g\ L^{-1}$)	SD
HAL	HAL1	10.2	0.04	2.84	1.48
	HAL2	1.69	0.10	0.79	0.24
	HAL3	15.6	0.74	1.61	0.04
HWA	HWA1	0.36	0.06	0.63	0.26
	HWA2	0.44	0.00	1.01	0.31
	HWA3	0.42	0.02	0.79	0.32
SVF	SVF1	1.23	0.14	0.46	0.03
	SVF2	1.34	0.16	1.09	0.74
	SVF3	1.38	0.07	0.49	0.02
KHO	KHO1	1.47	0.16	0.54	0.05
	KHO2	1.36	0.07	2.77	0.09
	KHO3	1.04	0.02	1.97	0.23
STA	STA1	6.52	0.26	3.39	1.12
	STA2	5.55	0.28	2.14	0.06
	STA3	1.28	0.06	0.65	0.04
KAG1	KAG1	1.38	0.01	0.77	0.13
	KAG2	3.07	0.07	1.48	0.51
	KAG3	4.31	0.26	2.70	0.61
KAS	KAS1	4.47	0.04	1.02	0.41
	KAS2	3.60	0.10	0.54	0.04
	KAS3	0.66	0.02	0.60	0.07

The concentrations of Zn_{Sol} in incubated soils are shown in Fig.3.20; there was a slight effect of incubation time but this was not significant. During the first 7 days of incubation, a decrease in Zn_{Sol} was observed but values did not change between 14-28 days of incubation. The similar result obtained by Ma and Uren (1997) who found that extractable Zn in calcareous soils decreased as a time of incubation increased. There was a weak but positive linear correlation ($R^2 = 0.24$, $p < 0.023$) between Zn_{DTPA} and Zn_{Sol} (Fig. 3.21) suggesting similar labile Zn adsorption behaviour and active buffering of the labile Zn pool (Zn_{DTPA} adsorbed and Zn_{Sol} in solution).

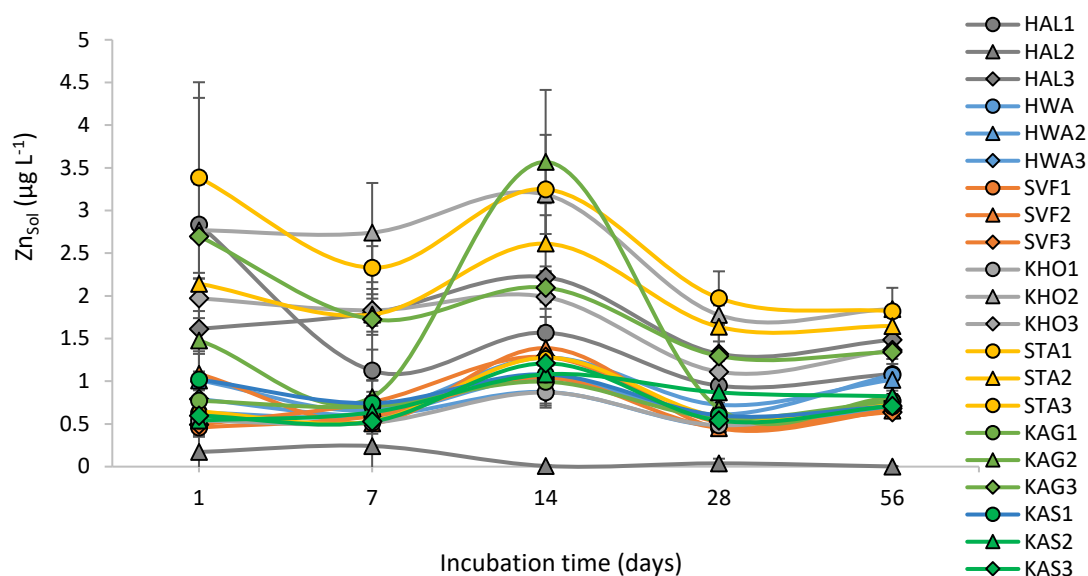


Fig. 3.20: Mean change in Zn_{Sol} ($\mu g L^{-1}$) over time following incubation for 1, 7, 14, 28 and 56 days.

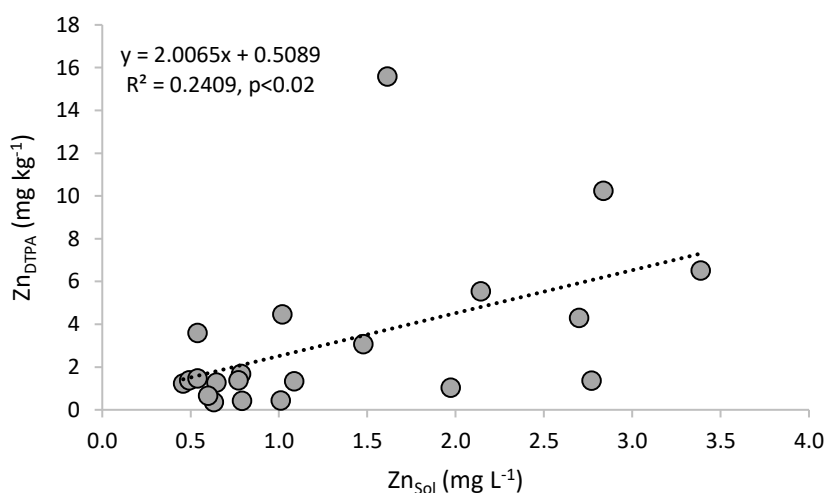


Fig. 3.21: Correlation between Zn_{DTPA} and Zn_{Sol} in studied soils.

Figure 3.22 shows the correlation between Zn intensity (Zn^{2+} ion activity in the soil solution following speciation of Zn_{Sol} using WHAM-7, $\mu g L^{-1}$) and Zn quantity (Zn_{DTPA} ; $mg kg^{-1}$) for all studied soil with respect to time of incubation. The results indicated that intensity of Zn^{2+} increased with increasing the quantity of Zn in all the studied soils,

possibly due to high Zn quantity values. The Zn intensity with time of incubation showed a strong correlation with Zn quantity.

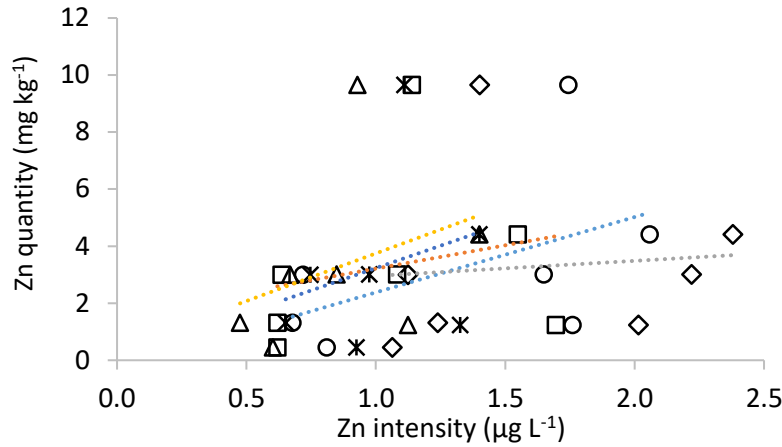


Fig. 3.22: The average Zn Quantity-intensity relationship for studied soils, expressing Zn intensity concentration (Zn_{Sol} , X-axis) as a function of Zn quantity Zn_{DTPA} . The studied soils from Kurdistan were incubated for (○) 1 day, (□) 7 days, (△) 14 days, (◇) 28 days and (x) 56 days.

3.3.3.3 Zinc solid $\rightleftharpoons$ liquid partition coefficient ($\log K_d Zn$)

The quantity-intensity relationship has been used for the study of many nutrients such as Mg^{2+} , NH_4^+ , and K^+ (Pasricha et al., 1987). Zinc has not been studied in this way, partly due to the analytical challenge of determining the extremely low concentrations of Zn in the solution phase of calcareous soils. In the current study, the Zn quantity-intensity relationship was determined, using ICP-MS, to examine the conditions arising in Kurdistan soils from the absence of Zn fertilizer and reported application of sewage irrigation water.

In the studied soils, the value of $\log K_d Zn$ was estimated for Zn adsorption in all the studied soils ($C_{Solid}/C_{Aqueous}$), where C_{Solid} ($mg\ kg^{-1}$) and $C_{Aqueous}$ ($mg\ L^{-1}$) refer to Zn_{DTPA} and Zn^{2+} ion concentration in solution following speciation of Zn_{Sol} data using the geochemical speciation program WHAM-7. The soil KAS had the highest $\log K_d Zn$ value and STA had the lowest value, but all $K_d Zn$ values fell within one log unit across the

range of soil locations Table 3.10. The variation in $\log K_d \text{ Zn}$ with soil pH, Ca content and SIC content is shown in Fig. 3.23 (a, b and c). A slightly positive correlation was observed between $\log K_d \text{ Zn}$ and soil pH as might be expected notwithstanding the narrow range of soil pH values, but a slight negative correlation was found for $\log K_d \text{ Zn}$ with Ca and SIC contents in all the studied soils. This is may be due to competitive adsorption between Zn and Ca for carbonate adsorption sites as suggested by Degryse et al. (2011) and by Merrikhpour and Jalali (2015). However, the correlations were very weak which again reflects the similarity of the soils in terms of their Zn dynamics. The relatively high value of $\log K_d \text{ Zn}$, compared to $\log K_d \text{ P}$, reflects a greater attraction of the soil solid constituent for Zn and may reflect greater strength of adsorption generally at low concentrations (Merrikhpour and Jalali, 2015, Sastre et al., 2002).

Table 3.9: The average and standard deviation of $\log K_d \text{ Zn}$ values for studies sites at Sulaimanyah province.

	HAL	HWA	SVF	KHO	STA	KAG	KAS
Log K_d	3.45	3.49	2.91	2.96	2.90	2.91	3.95
SD	0.39	0.94	0.25	0.42	0.35	0.33	0.15

*SD= standard deviation

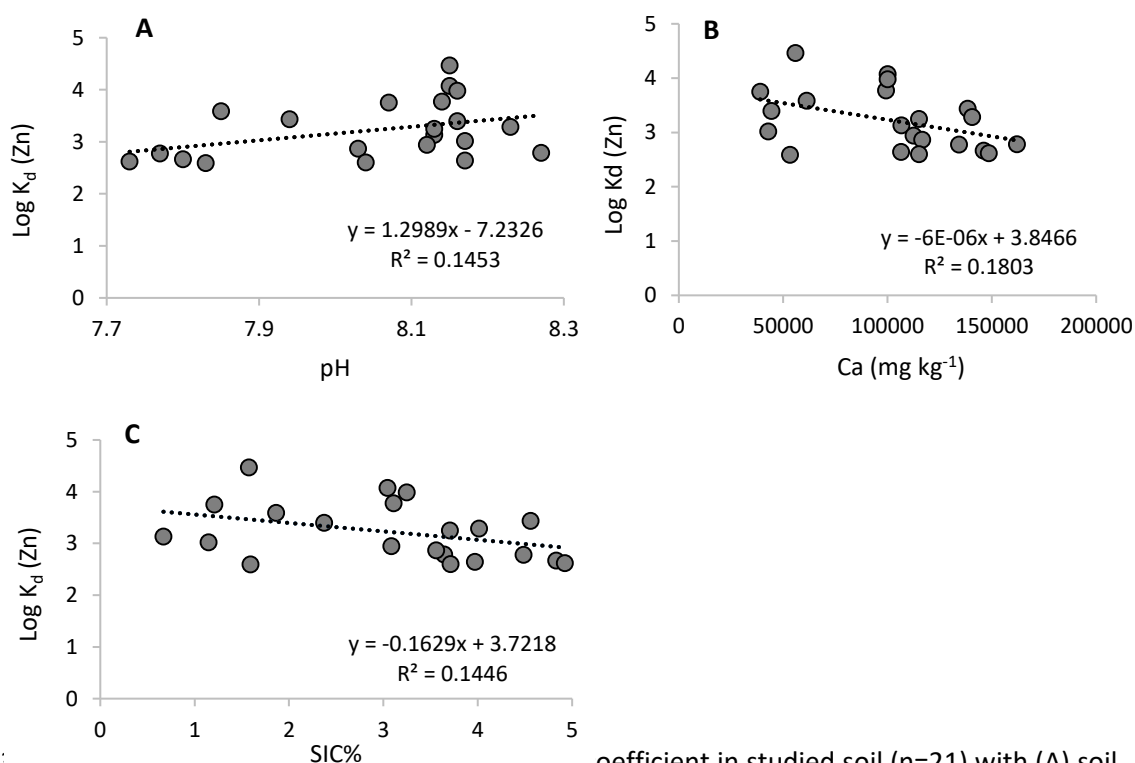


Fig. 3.23. Relationship between $\text{Log } K_d (\text{Zn})$ and soil properties in studied soil (n=21) with (A) soil pH, (B) soil Ca content and (C) soil SIC% in studied soils (n=21).

3.3.3.4 Zn solubility

Speciated values of Zn_{Sol} were used to determine the solid phase of Zn minerals which potentially control the solubility of Zn in the studied soils. A solubility diagram for Zn minerals was developed by plotting 'logarithm activity of Zn' ($\log \text{Zn}^{2+}$) against soil pH. The activity of Zn^{2+} , pH and partial pressure (CO_2) used to develop this diagram, were determined using measured values of pH and Zn concentration in solution with speciation using WHAM-7. This approach has been used to indicate the likelihood of specific Zn minerals being present in soils at different pH values (Lindsay, 1979, Nazif et al., 2015, Urgan and Rimmer, 2000).

Figure 3.24 presents a 'predominance' or 'stability' diagram for Zn^{2+} in relation to possible common Zn minerals in the studied soils. The Zn data fell between slightly supersaturated with respect to a hypothetical solid phase suggested by

Lindsay (1979) called 'Soil-Zn' and undersaturated with respect to Smithsonite (ZnCO_3) across all the studied soils and in all incubation periods (week 1-8). Similar results were reported by Urgan and Rimmer (2000) who found that, in calcareous soil, the solubility of Zn^{2+} was controlled by Smithsonite and Sachdev et al. (1992) who found that the solubility of Zn in the calcareous soils controlled by Lindsay's hypothetical 'Soil-Zn'.

The solubility of Zn minerals appeared to decrease slightly over time with small fluctuations from the first day to 56 days of incubation for all studied soils. Moreover, the solubility of Zn in studied soils decreased with increasing soil pH. There was also a general trend towards solubility control by Smithsonite as pH increased. At the same time it is difficult to distinguish between changes in solubility control and adsorption reactions stated by Alloway (2009) and Ashworth and Alloway (2004) that the ability of soil solid surfaces to absorb trace cations is pH-dependent because of changes in negative surface charge, hydrolysis of Zn species and chemi-sorption on Fe oxides and calcite.

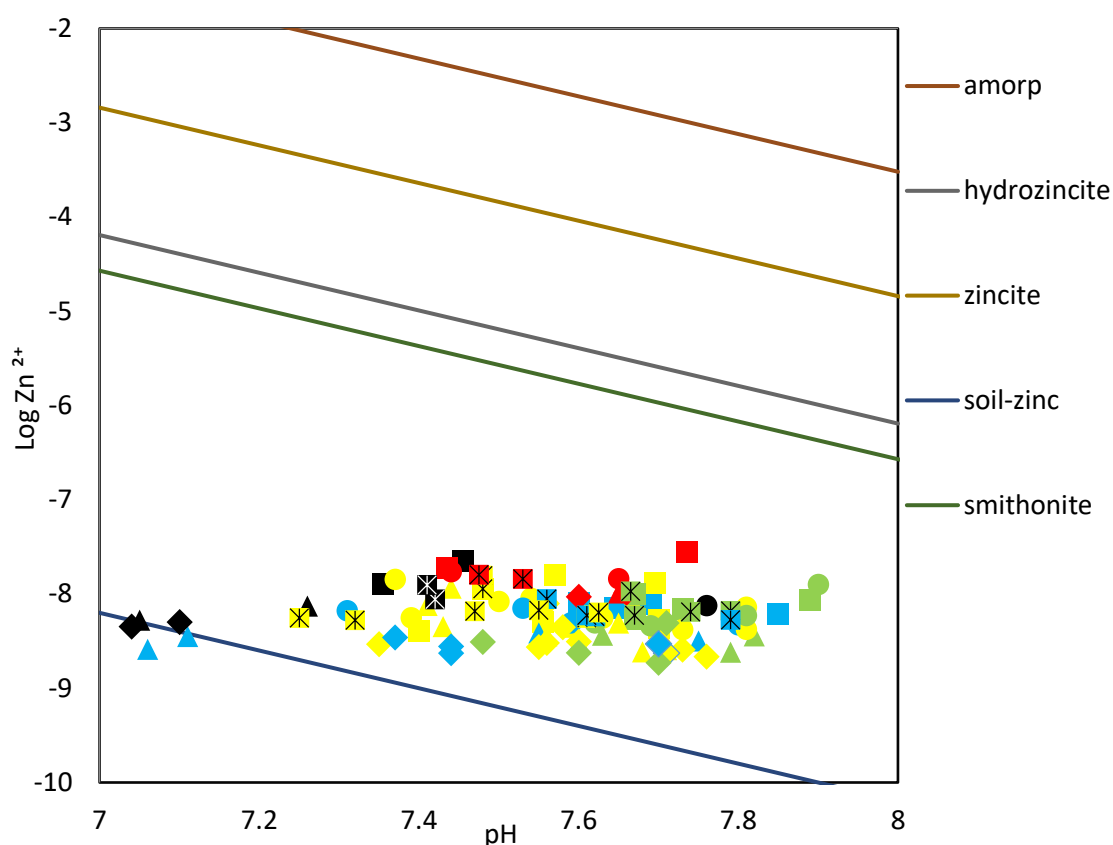


Fig. 3.24: Stability or predominance diagram for soil data and the Zn minerals: amorphous Zn carbonate, hydrozincite, zincite, smithsonite and 'soil-Zn'. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Lindsay, 1979, Nazif et al., 2015, Urgun and Rimmer, 200). The points in the diagram separated with different color depending on its index (amount of available Zn (mg kg^{-1}) as Zn_{DTPA} in the soil); including index 1 (0-1), index2 (2-3), index3 (3-4), index4 (4-5), and index8 (>5) mg kg^{-1} Zn_{DTPA} in the soil.

3.3.4 Multivariate statistical analysis

3.3.4.1 Correlation matrix of soil elemental analysis and other soil properties

For all the studied soils ($n = 21$), Pearson correlation coefficients were calculated for soil pH, LOI%, and SIC%, 5 macroelements and 10 microelements to examine relationships between soil chemical properties (Table 3.11). Among the soil properties, only %LOI was significantly correlated ($r=0.47$) with SIC% at ($p<0.05$). %LOI was significantly correlated ($r= 0.60, 0.47, 0.57, 0.43, 0.45, 0.68, 0.43$ and 0.47) with Na,

Ca, Mn, Fe, Co, As, Cd, Pb and U at ($p < 0.05$). Soil pH was significantly correlated (0.55 and 0.51) with Mg and K at ($p < 0.05$). SIC% was significantly correlated ($r = 0.71, 0.71, 0.57, 0.63, 0.54, 0.5, 0.45, 0.46$ and 0.66) with Co, Ni, Mo and Cd at ($p < 0.05$) and with K, Ca, Mn, Fe and U at ($p < 0.001$). Phosphorus was significantly correlated (0.67, 0.57) with Zn at ($p < 0.001$). Zn was significantly correlated ($r = 0.47, 0.42, 0.57, 0.49, 0.79, 0.58$ and 0.83) with Mn, Fe, Co, Cu at ($p < 0.05$) and Ni, Cd and Pb at ($p < 0.001$). No correlations were found between P_T and Zn_T with selected soil properties.

Table 3.10: Correlation matrix presenting significant positive and negative relationships (r values) among soil pH, %LOI, %SIC, macro and micro elements in the studied soils (n=21).

Parameters	LOI%	pH	SIC%	Na	Mg	P	K	Ca	Mn	Fe	Co	Ni	Cu	Zn	As	Mo	Cd	Pb
%LOI	1																	
pH	-0.177	1																
%SIC	-0.477*	-0.359	1															
Na	-0.601*	0.343	-0.09	1														
Mg	0.025	0.557*	-0.324	0.324	1													
P	0.219	-0.339	0.206	-0.34	-0.218	1												
K	0.403	0.515*	-0.710**	0.123	0.621*	-0.155 1	1											
Ca	-0.478*	-0.3	0.713**	-0.014	-0.204	0.371	-0.779**	1										
Mn	0.579**	0.272	-0.578**	-0.148	0.453*	0.145	0.856**	-0.603**	1									
Fe	0.433*	0.412	-0.633**	-0.122	0.571**	-0.031	0.921**	-0.700**	0.871**	1								
Co	0.458*	0.36	-0.548*	-0.209	0.613**	0.059	0.855**	-0.563**	0.897**	0.968**	1							
Ni	0.406	0.396	-0.509*	-0.115	0.749**	0.023	0.814**	-0.485*	0.829**	0.895**	0.96**	1						
Cu	0.411	0.142	-0.343	-0.256	0.549*	0.383	0.679**	-0.257	0.809**	0.823**	0.905**	0.886**	1					
Zn	0.254	-0.311	-0.008	-0.255	0.193	0.676**	0.234	0.129	0.471*	0.423*	0.522*	0.494**	0.793*	1				
As	0.423*	-0.073	-0.33	-0.478*	-0.399	0.113	0.327	-0.530*	0.448*	0.438*	0.341	0.089	0.192	0.086	1			
Mo	0.414	-0.129	-0.453*	-0.187	-0.275	-0.054	0.305	-0.733**	0.321	0.31	0.229	0.16	0.031	-0.11	0.570**	1		
Cd	0.687**	-0.065	-0.462*	-0.539*	0.024	0.414	0.596**	-0.537*	0.806**	0.736**	0.751**	0.626**	0.744**	0.581**	0.680**	0.551*	1	
Pb	0.431*	-0.233	-0.251	-0.231	0.304	0.576**	0.479*	-0.242	0.595**	0.593**	0.644**	0.639**	0.805**	0.836**	0.169	0.229	0.691*	
U	0.479*	0.371	-0.668**	-0.031	0.571**	0.028	0.937**	-0.702**	0.892**	0.906**	0.867**	0.818**	0.751**	0.351	0.376	0.337	0.721**	0.585**

*correlation significant at ($p \leq 0.05$), **correlation is significant at ($p \leq 0.01$)

3.3.4.2 Hierarchical clustering analysis

A dendrogram of soil properties (Fig. 6.25) showed that four coherent groups could be identified with similarity levels greater than 65%. From left to right in Fig. 3.25, Group I (LOI, Cd and Mo (weak)), might arise from Cd and Mo association with humus. Group II represents the presence of an oxide mineralogy (Al, V, Fe, Mn, Co, Ni and Cu). Group III, includes P, Zn, Pb and Ag which might indicate a common source of soil contamination such as phosphate fertilizers and wastewater. Group IV includes pH and some base cations (Mg, Na and Ca) reflecting the calcareous nature of soils with soil pH values around 8.

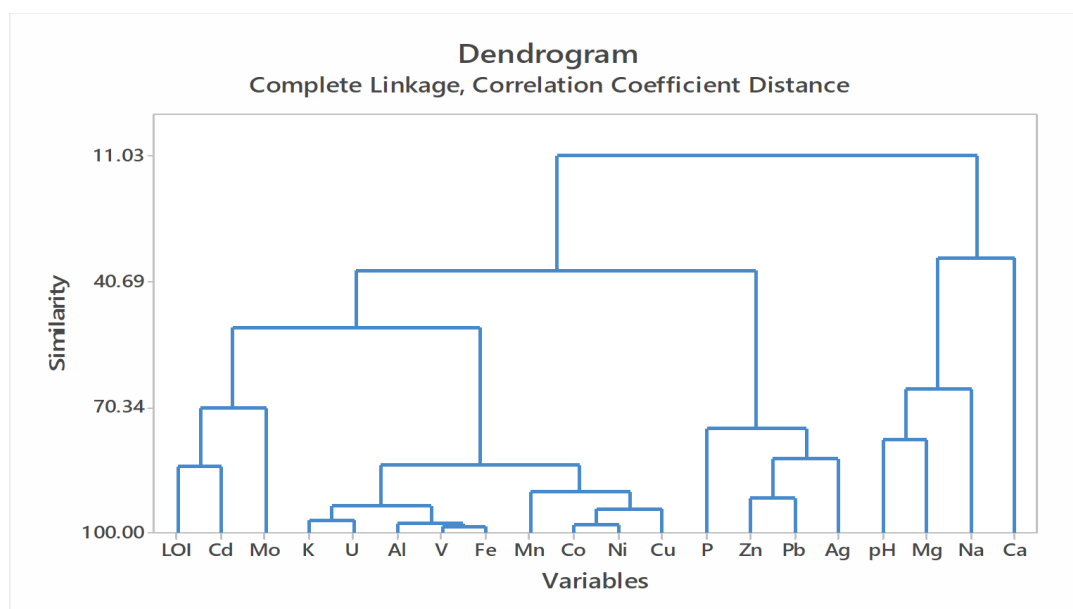


Fig. 3.25: Hierarchical cluster analysis of elemental analysis data of soils (n=21) collected in Sulaimanyah province, Kurdistan at seven different sites.

3.4 Conclusions

In this study, the mean concentrations of P_T and Zn_T were 842 ± 247 and 93.5 ± 36.8 mg kg^{-1} which differed between sites. In all soil samples, P_{Olsen} was high and in about 67% of samples P_{Sol} was higher than the critical value for optimum plant growth. In approximately 85% of samples, Zn_{DTPA} was lower than the critical value while P_{Sol} in all samples was lower than the critical value for plants optimum plant growth. The high amount of available P may be due to the high application of NPK fertilizer, organic matter and the irrigation of plants with sewage sludge, while low Zn_{Sol} values may be due to high adsorption of Zn in soil due to high $CaCO_3$ contents and the absence of Zn fertilizer use. Log K_d values between 2.98-3.65 for Zn, expressed as Zn_{DTPA}/Zn_{Sol} , were 1-2 orders of magnitude greater than similar values (1.74-2.91 (P_{Olsen}/P_{Sol})) for P. This appears to reflect the strong adsorption of low concentrations of Zn in these soils in contrast to the effects of large annual phosphate fertilizer additions which create a supply of available P despite the calcareous conditions.

The results of the incubation experiment showed that P and Zn solubilities differed between soils depending on $CaCO_3$, pH and the amount of P_{Olsen} and Zn_{DTPA} . An attempt was made to determine possible P and Zn mineral control over solubility. Solubility decreased with increasing $CaCO_3$ content, time and pH. OCP and β -TCP were apparently the stable calcium phosphate minerals and Smithsonite or 'Soil-Zn' were the stable forms of Zn minerals in the Kurdistan arable soils. Overall, the most outstanding characteristics of the soils surveyed was their similarity. All measures of Zn and P status suggested a conservative range of values – almost certainly reflecting the calcareous nature of all the soils and a common agricultural policy of high phosphate applications and the absence of Zn fertilizer use.

4 TIME-DEPENDENT SORPTION OF PHOSPHATE AND ZINC BY CALCAREOUS SOILS OF KURDISTAN

Abstract

Adsorption reactions regulate nutrient availability in soil. However, establishing relationships between adsorbed and dissolved nutrient concentrations is not straightforward because of the time-dependent transformation of nutrient ions. Precipitation and absorption reactions can be slow and persistent following initial contact between fertilizer and soil adsorption surfaces. To evaluate time-dependent changes in P and Zn adsorption in Kurdistan soils, an experiment was conducted with two calcareous soils from Sulaimanyah province, Kurdistan region, Iraqi. Triplicate samples of the soils (c. 30 g) were treated with a range of P (KH_2PO_4) concentrations (0, 50, 100, 200, and 300 mg kg^{-1}) and $^{70}\text{Zn}^{2+}$ isotope concentrations (0, 2, 5, 10, and 20 mg kg^{-1}). The soil samples were incubated at 20 ± 2 °C, and 50% WHC, and sub-sampled for analysis after 1, 7, 14, 28, 56, and 84 days. The 'available' P and ^{70}Zn were determined by extraction with 0.5 M NaHCO_3 and 0.005 M DTPA respectively. In addition, the solubility of P and ^{70}Zn in 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution were also measured at each sampling time. From the determination of the isotopic ratio $^{70}\text{Zn}/^{66}\text{Zn}$ in solution it was possible to determine the pool of soil Zn with which the spiked isotope (^{70}Zn) had mixed – this is referred to as the 'apparent' E-value (Zn_E). With increasing soil contact time the proportions of P and Zn adsorbed and the apparent Zn_E values significantly increased while available P and Zn decreased in both soils. Initial adsorption was very rapid. The Langmuir-Freundlich model described P adsorption well for both studied soils. The Residual Standard Deviation (RSD; mg kg^{-1}) values for the Langmuir-Freundlich model applied to soils 1 and 2 were lowest at 1.10 and 10.4 compared to 1.46 and 10.8 for Langmuir and 1.75 and 10.7 for Freundlich models respectively. However, the Freundlich model described Zn adsorption best for both studied soils. The RSD values (mg kg^{-1}) for the Freundlich model applied to soils 1 and 2 were 0.026 and 0.073

compared to 0.027 and 0.096 for Langmuir and 0.034 and 0.075 for the Langmuir-Freundlich models respectively. The results suggested that the most important period of incubation is the first day. In this time, across the range of P and Zn concentrations applied, the soils lost almost 65.7, 61.3, 53.3 and 57.7% and 20.8, 21.6, 30.6 and 34.5% of added P and 91.6, 92.7, 88 and 95.9% and 98.4, 98.6, 99.1, 99.5 of added ^{70}Zn for soil 1 and 2 respectively. A solubility analysis, comparing ion activity products with solubility products, showed that P in control soils fell between hydroxyapatite and tri-calcium phosphate but the further addition of phosphate showed progressive movement towards di-calcium phosphate. Similarly, Zn solubility moved from being under-saturated with respect to ZnCO_3 (smithsonite), by approximately two orders of magnitude in (Zn^{2+}) activity, towards the smithsonite solubility line with increased Zn addition.

4.1 Introduction

Phosphorus and Zn are important nutrients for plant health (Ashraf et al., 2008, Sheng-Li et al., 2008, Hararah et al., 2011, Bai et al., 2017) and crop production (Heidari et al., 2017, Zhu et al., 2018). However, calcareous soils have a large capacity for P and Zn adsorption and fixation (Jalali and Zinli, 2012) and so deficiency of these two elements in soils containing CaCO_3 is common (Alloway, 2009, Zhu et al., 2001b). The application of P and Zn as solid or liquid fertilizers may also lead to a reaction between these two nutrients, and with various soil components, which causes the removal of P and Zn from the soil solution phase and conversion to progressively less soluble P and Zn forms with time. The mechanisms whereby P and Zn fertilizers in soils are changed to less soluble forms are generically referred to as 'fixation' and may include precipitation, adsorption and absorption processes (Graouer-Bacart et al., 2015, Samadi, 2006, Jalali, 2007, Dandanmozd and Hosseinpur, 2010, Baranimotlagh and Gholami, 2013).

The application of P fertilizer to calcareous soils will cause the formation of more stable phosphate minerals with a higher Ca:P ratio because of the high Ca^{2+} concentration in the soil solution supported through equilibrium with CaCO_3 . Thus Al-Tameemi et al. (2015) reported that in calcareous soils, P is mostly bound to the adsorption surface at low concentrations of orthophosphate in solution but precipitation of Ca phosphates occurs at higher concentrations of P. Soluble Zn also reacts with soil geocolloids which reduce Zn availability to plants at low Zn concentrations (Nystrom, 2012) but adsorption probably remains the dominant process controlling Zn availability even at high Zn concentrations (Jalali and Zinli, 2012).

The relationships between Zn and P-sorption capacity, availability to plants and the nature of soil constituents in calcareous soil have been studied in other countries. It is recognised that some soil parameters strongly influence adsorption and distribution

between the soil solid and solution phases (Guttieri et al., 2015), including soil texture, Fe oxide and CaCO_3 content, soil pH and cation exchange capacity (CEC). In addition, experimental conditions such as solid: solution ratio, analyte concentration and contact time also influence the apparent behaviour of P and Zn in soil adsorption experiments (Bradl, 2004, Jalali and Zinli, 2012). Zhou and Li (2001) reported that P sorption in soils is primarily associated with Fe oxides and that CaCO_3 plays a less important role. Samadi (2006) found that soil clay content significantly correlated with P adsorption while CaCO_3 has less effect. However, Jalali and Moharrami (2007) and Zhu et al. (2018) reported that in calcareous soil the adsorption of P was significantly correlated with CaCO_3 content. Jalali and Tabar (2011) found that soil CEC, pH and CaCO_3 content significantly correlated with Zn sorption. Duffner et al. (2012) reported that in calcareous soils P and Zn deficiency occurred at high soil pH values because the bioavailability of both elements decreases with increasing pH.

Following the reaction of nutrients added to soil through time is compromised by the presence of soil-derived nutrients. One solution to this problem is to employ enriched stable isotopes of the nutrient which allows discrimination between fertilizer- and soil-derived species. Thus, for example, an enriched stable isotope of Zn can be used to determine the solid $\rightleftharpoons$ solution equilibrium of added Zn and also, from isotopic ratios in solution, the extent to which the added Zn has mixed with the native soil Zn pools (Buekers et al., 2007, Degryse et al., 2011, Nazif et al., 2015). Furthermore, the 'isotopically exchangeable' Zn (E-value) can be determined from isotope ratios in a labile phase (e.g. the solution phase of a soil suspension) following spiking with an enriched stable isotope such as ^{70}Zn . The E-value can then be used to provide an estimate of free Zn^{2+} activity in the soil solution when used as an input variable in geochemical 'assemblage' models that predict solid $\rightleftharpoons$ solution equilibrium (Marzouk et al., 2013). However, there are some practical problems when applying a low concentration of a Zn isotope to calcareous soils. Sufficient isotope has to be added to

enable measurement in the solution phase after equilibration. However, too large an addition might result in precipitation of the Zn isotope which would then be misinterpreted as a large soil pool of 'reactive' Zn with which the isotope had apparently mixed (Nazif et al., 2015). In the current study, isotope exchange measurements using a ^{70}Zn addition (spike) was used to follow the time-dependent inclusion of Zn into two representative calcareous soils from Kurdistan. *Apparent* Zn lability (the E-value) was determined from measurements of the isotopic ratio $^{70}\text{Zn}/^{68}\text{Zn}$ in DTPA extractions of the soils. In following time-dependent fixation of Zn it is important to appreciate that an apparent E-value measured over long isotope contact times simply represent the pool of soil Zn with which the isotope has mixed; it is not indicative of the 'labile' or 'reactive' Zn but rather of the extent of the ^{70}Zn spike fixation. At the same time, measurements made after one or two days may be taken as representative of a 'traditional' E-value and would provide a reasonable estimate of the labile Zn pool. The assumption has to be made that the ^{70}Zn spike has only mixed with reactive Zn within the 1 – 3 day period of equilibration. In addition, using DTPA as the soil extractant permits a contrast between estimates of labile Zn from (i) the total soil-derived Zn and added ^{70}Zn extracted by DTPA and (ii) the isotopically exchangeable Zn determined after a short period of isotopic equilibrium (1 – 3 days).

The overall aims of the current study were to: (i) evaluate the time-dependent reaction of calcareous Kurdistan soils with P and Zn; (ii) identify possible mineral solubility control over P and Zn solubility from applied fertilizers; (iii) determine isotopically exchangeable Zn using a ^{70}Zn isotopic exchange method (E-value); (iv) assess the validity of using DTPA and Olsen-P as measures of 'reactive' fractions during progressive fixation of Zn and P respectively by determining the consistency of adsorption isotherms.

4.2 Methods and Materials

4.2.1 Soil sampling

The calcareous soils used in the present experiment were collected from the top 0-30 cm layer in two different locations selected to represent contrasting soil properties. Site 1 was close to Kalar city belonging to the order of Aridisol (calcareous) (34°41'52"N, 45°12'41"E); Site 2 was a Mollisol from Halabja city (35°17'46"N, 45°59'55"E). Due to the prevailing climatic conditions, no organic horizon existed in either of these two soils. Subsamples from the surface horizon were combined to provide one sample of about 2 kg in both cases. All visible roots, macrofauna, and fresh litter were removed from the samples and the soil material was homogenized, air dried for several days, and the aggregates crushed to pass through a 2-mm sieve.

4.2.2 Soil analysis

The ground air-dried soil samples were digested in a $\text{HClO}_4\text{-HNO}_3\text{-HF}$ mixture to determine total soil P and Zn contents (Bai et al., 2017). The digested soil solutions were analyzed by ICP-MS (Model X-Series^{II}, Thermo-Fisher Scientific, Bremen, Germany) operated in 'collision cell mode' (7% hydrogen in helium) to reduce polyatomic interferences. Available P (Olsen-P) was extracted using 0.5 M sodium bicarbonate (NaHCO_3) solution and subsequently determined colorimetrically (Olsen et al., 1954, Murphy and Riley, 1962a). For available Zn (including ^{70}Zn), 2.0 g of soil was suspended in 8 mL of 0.005 M DTPA (Quevauviller, 1998) and shaken end-over-end in polypropylene centrifuge tubes, for 2 h. Suspensions were then centrifuged at 2500 rpm for 15 min and the supernatant was filtered (0.22 μm , Millex® Millipore) and acidified to produce 2% HNO_3 prior to multi-element analysis by ICP-MS. Soluble P and Zn were determined by shaking (end-over-end) 2.0 g soil with 20 ml of 0.01 M calcium nitrate for 48 h prior to centrifuging at 2500 rpm for 15 min and filtering the supernatant (<0.22 μm syringe filters, Millex® Millipore). The isolated supernatant solution was

acidified to produce 2% HNO₃ prior to multi-element analysis (including ⁷⁰Zn) by ICP-MS.

Soil pH was determined in soil: water suspensions (1:2.5 ratio). For soil organic carbon, a known weight of <2 mm oven-dried soil (105°C) was placed in a pre-weighed silica crucible and ignited in a muffle furnace (Gallenkamp size 3, Weiss- Gallenkamp, UK) overnight at 550 °C (Heiri et al., 2001). Exchangeable cations and cation exchange capacity (CEC) were determined by extraction with ammonium nitrate (NH₄HNO₃). Soil samples (c. 2.0 g) were suspended in 20 mL of 1M NH₄HNO₃ and shaken end-over-end in polypropylene centrifuge tubes, for 2 h. Suspensions were then centrifuged at 3500 rpm for 30 min and the supernatant was filtered (<0.22 µm syringe filters, Millex® Millipore) and acidified to produce 2% HNO₃ prior to Na, K, Mg, Ca, Zn and ⁷⁰Zn analysis by ICP-MS. Total organic and inorganic carbon (TOC and TIC) were determined using a Total Organic Carbon Analyser (Shimadzu TOC-V CPH). Soil texture determined by hydrometer method according to (Rowell, 1994).

4.2.3 Adsorption isotherms

Time-dependent adsorption isotherm experiments were performed using three replicates from each of the two soils equilibrated under laboratory conditions to investigate P and Zn adoption. Approximately 30 g soil samples of each soil were placed into screw-cap plastic jars (60 mL) and then adjusted to half the water holding capacity (WHC, %) including P and Zn standard solutions as part of the added solution. The P and Zn were added to separate incubations as KH₂PO₄ and ⁷⁰Zn(NO₃)₂ solutions at various concentrations: 0, 50, 100, 200, 300 mg P kg⁻¹ and 0, 2, 5, 10 and 20 mg ⁷⁰Zn kg⁻¹. The samples in plastic bottles were then incubated at room temperature 20±2°C for up to 84 days. Periodically, Olsen-P was determined by shaking subsamples of 2.0 g soil with 30 ml of 0.5 M NaHCO₃ for 30 min followed by centrifugation (3500 rpm for 15 min), and then available P determined colorimetrically as described by (Murphy and

Riley, 1962b). For DTPA-Zn, approximately 2.0 g soil subsamples were transferred into 20 ml centrifuge tubes with 8 ml of DTPA-TEA buffer (0.005 M DTPA+ 0.01 M CaCl₂ + 0.1 M TEA) added to achieve a 1:4 soil to extractant ratio. Soil suspensions were then shaken end-over-end for 2 h at 30 rpm at room temperature. The suspension was then centrifuged for 15 min at 2500 rpm, filtered, and stored in a polyethene container at 4 °C prior to analysis (Quevauviller, 1998). ICP-MS (Thermo Fisher scientific iCAP Q, Germany) was used to measure Zn isotopes (⁶⁶Zn and ⁷⁰Zn) in solution after dilution 1 to 2 (1:2 dilution ratio). Finally, 2.0 g of subsamples were extracted by addition of 20 ml of 0.01 M calcium nitrate (Ca(NO₃)₂) with shaking for 48 h followed by centrifugation (2500 rpm for 15 min), filtration (< 0.22 µm) and acidification of 5 ml with 0.2 ml of 50% HNO₃ for analysis of soluble P, Zn, Ca, Na, K, Mg, and S by ICP-MS. A separate aliquot was retained for pH determination and measurement of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and determination of the amount of soluble P as described by (Murphy and Riley, 1962b) .

The percentage of adsorbed P and Zn were determined by the difference between the added amount of P and Zn and the measured amount of the extracted solution by soil.

Eq. 4.1.

$$Q = \frac{(C_o - C_t)}{C_o} \times 100 \quad \text{..... Eq. 4.1}$$

Where Q is the percentage of P or Zn adsorbed (mg kg⁻¹), C_o is the initial P or Zn applied (mg kg⁻¹), C_t is the equilibrium P or Zn concentration (mg kg⁻¹).

4.2.4 Adsorption isotherm models

Adsorption isotherms show how elements are distributed between the solid phase and the liquid phase when the adsorption process achieves equilibrium. The analysis of the isotherm data by fitting them to different isotherm models is an important step to find a suitable model that can be used for predictive purpose. For Zn and P isotherm data

were fitted to three well known models: the Langmuir, Freundlich and Langmuir-Freundlich isotherms. Phosphorus and Zn adsorption were determined by subtracting the equilibrium Zn or P concentration in solution (0.01 M Ca(NO₃)₂ suspensions) from either the added Zn or P, or from the measurement of available Zn (DTPA) and P (Olsen et al.) at each sampling interval. It is possible to present adsorption isotherms as a relationship between solution concentration (X-axis) and either (i) the total adsorbed P and Zn (including reactive and fixed forms) or (ii) the 'reactive' adsorbed P and Zn alone, as determined by Olsen-P and DTPA-extractable Zn. The time-dependency of the fixation process can be quantified by comparison of these two approaches as the relationship between 'reactive' and solution P or Zn would be expected to be constant with time – whereas the relationship between 'total adsorbed' and solution P and Zn would be expected to change as fixation proceeds. The general form of Langmuir, Freundlich and Langmuir-Freundlich equations are presented in Equations 4.2, 4.3 and 4.4.

4.2.4.1 Freundlich Model

$$q_{ads.} = K_F (C_{Sol.})^n \dots \dots \dots Eq. 4.2$$

Where

q_{ads} = Amount of P and Zn adsorbed per unit weight of soil

C_{Sol} = Equilibrium P and Zn concentration in soil solution (mg L⁻¹)

K_F and n = Freundlich constants; K_F reflects the strength of adsorption by the soil and n gives an indication of how variable the adsorption strength is across the range of the dataset.

4.2.4.2 Langmuir Model

$$q_{ads} = \frac{K_L * N_s * C_{Sol}}{1 + K_L * C_{Sol}} \dots \dots \dots Eq. 4.3$$

Where

q_{ads} = Amount of P and Zn adsorbed per unit weight of soil (mg kg⁻¹)

C_{Sol} = Equilibrium P and Zn concentration in soil solution (mg L⁻¹)

K_L and N_s = Langmuir constants; K_L is related to bonding energy of P and Zn to the soil and N_s is the maximum adsorption capacity of the soil (mg kg⁻¹).

4.2.4.3 Langmuir-Freundlich Model

$$q_{ads} = \frac{N_s (K C_{Sol})^n}{1 + (K C_{Sol})^n} \dots \dots \dots Eq. 4.4$$

Where

q_{ads} = Amount of P and Zn adsorbed per unit weight of soil (mg kg⁻¹)

C_{Sol} = Equilibrium P and Zn concentration in soil solution (mg L⁻¹)

K , N_s and n = Langmuir-Freundlich constants – essentially as described above.

4.2.5 Calculation of E_{value}

The enriched stable isotope ⁷⁰Zn (95.47%) was purchased from ISOFLEX USA in form of a metal foil. HNO₃ was used to dissolve the isotope foil initially and the solution was diluted with Milli-Q water to give a ⁷⁰Zn isotope stock solution with a concentration of 238.7 µg L⁻¹ in 5% HNO₃. Working spike solutions for the isotope adsorption experiment were made up freshly from this stock solution with concentrations of 0, 2, 5, 10 and 20 mg ⁷⁰Zn kg⁻¹ to be added to 30 g samples of the two test soils and incubated in plastic

pots. The nitric acid (HNO₃) concentration in the solutions added to the soils was less than 5%.

The apparent E-value (Zn_E) was measured periodically. Approximately 2.0 g of incubated soil sample was weighed into a 20 mL universal tube and equilibrated with 8 mL of 0.005 M DTPA+0.01 M CaCl₂+0.1 M TEA and shaken for 2 h followed by centrifugation (2500 rpm for 15 min) and filtration (0.22 µm, Millex®). The filtered solution of 5 ml was acidified with 5 ml of 2% HNO₃ for analysis of ⁷⁰Zn and ⁶⁶Zn isotopes by ICP-MS as described by (Nazif et al., 2015). The E-values were measured from the ratio of the main spike isotope (⁷⁰Zn) to one of the major background isotopes (⁶⁶Zn). The isotope ratio ⁷⁰Zn:⁶⁶Zn was then used to determine Zn_E from

$$Zn_E = \left(\frac{Am_{M_{soil}}}{W} \right) \left(\frac{C_{spike} V_{spike}}{Am_{M_{spike}}} \right) \left(\frac{{}^{70}Zn IA_{spike} - {}^{66}Zn IA_{spike} R_{ss}}{{}^{66}Zn IA_{soil} R_{ss} - {}^{70}Zn IA_{soil}} \right) \dots \dots \dots \text{Eq. 4.5}$$

Where Am_{Msoil} is the average atomic mass of the Zn in the soil, W is the weight of the soil used (g), C_{spike} is the concentration (mg L⁻¹) of the metal spike, V_{spike} is the volume of spike added (L), IA is the isotopic abundance (mole basis) and R_{ss} is the ratio of isotopic abundances for ⁷⁰Zn:⁶⁶Zn determined in the spiked soils. The Zn isotope ratio analysis was undertaken using ICP-MS as described by (Marzouq et al. 2013, Nazif et al. 2015).

4.2.6 Geochemical speciation using the WHAM-VII program

The geochemical speciation model (WHAM VII: Windermere Humic Aqueous Model, version 7) was used to estimate the free ion activities of the orthophosphate anion (H₂PO₄⁻) and the divalent form of Zn (Zn²⁺) using the following input parameters: (a) solution concentration of [Na], [Mg], [K], [Ca], in mg L⁻¹ and [Zn] in µg L⁻¹ of suspensions, and [PO₄], [NO₃], and [SO₄] in mol L⁻¹; (b) the suspension pH; (c) dissolved inorganic carbon (DIC); and (d) the Fulvic acid (FA) concentration. The

temperature was set to 22°C. It was assumed that fulvic acid contained 50% C and also that only 50% of the DOC was fulvic in origin (Nazif et al., 2015).

4.2.7 Statistical analysis

Three replicates were used for all analysis. Statistical analysis was performed on all datasets using SPSS (SPSS, version 22). The least significant differences (LSD) was used to investigate correlations. To determine differences and significance, the level of probability ($p \leq 0.05$) was used.

4.3 Results and discussion

4.3.1 General soil characteristics

Selected soil characteristics are summarised in Table 4.1. The soil pH values and carbonate content indicate the calcareous nature of both Kurdistan soils. The soil textures were silty clay for soil 1 and silty clay loam for and 2. Values of P_T and Zn_T were in the ranges reported globally. The values of P_{Olsen} and Zn_{DTPA} were below recommended values of 10 and 0.5 mg kg for P and Zn respectively for optimum plant growth (Ahmed, 2013, Imran et al., 2016). Alvarez and Maria (2003) reported that a value of Zn_{DTPA} below 0.5 mg kg⁻¹ is deficient in alkaline soils. Alloway (2009) reported values of Zn_{DTPA} between 0.10-8.70 for Iraqi soils. The cation exchange capacity (CEC) and soil organic carbon concentration (as %LOI) were higher in Soil 1 than Soil 2, indicating the effect of parent material, environment and geographical differences. Free oxide Fe and Al concentrations were considered due to the effect of both elements on soil P and Zn adsorption properties.

Table 4.1: Soil characteristics of the two selected calcareous soils used in the experiment.

Parameters	Unite	Soil 1	Soil 2
pH		8.1	8.24
CEC	(cmol kg ⁻¹)	30.9	10.1
LOI	%	12.2	2.45
Sand	%	11.4	14.4
Silt	%	45.6	54.2
Clay	%	43	31.4
Soil inorganic carbon	%	6.13	41
P_{Olsen}	(mg kg ⁻¹)	1.45	0.92
Zn_{DTPA}	(mg kg ⁻¹)	0.26	0.14
P_T	(mg kg ⁻¹)	583	418
Zn_T	(mg kg ⁻¹)	87.3	38.7
Fe	(g kg ⁻¹)	39.3	19.5
Al	(g kg ⁻¹)	46.9	24.7

4.3.2 Phosphorus behaviour in two calcareous soils from Kurdistan

4.3.2.1 Time-dependence of phosphorus adsorption (%) in studied soils

Figure 4.1 shows that phosphate fertilizer application rates and time of incubation increased P adsorption significantly ($p < 0.05$) in the two selected soils. The results indicate that P adsorption onto the soils was comparatively rapid in the first day and continued until reaching an apparent equilibrium. From the results of the study we can see that the adsorption of P in Soils 1 and 2 increased from 65.7% and 20.8% on day one to 80.2% and 63.4% on day 84 for the 50 mg P kg⁻¹ treatment.

The results in (Fig.4.1) indicate rapid adsorption of applied P on the first day increased the adsorbed P concentration in both soils. Jalali and Zinli (2012) reported that P adsorption is very fast in the early stages after application but continued slowly with an increase in the incubation time. Equilibrium had not been reached in the incubated soils by the end of the experiment. Mallikarjun and Shashikant (2013) also found that P adsorption increased with an increase in time of incubation. Moreover, the high adsorption capacity of the studied soils may be due to their calcareous nature (high CaCO₃ and pH). Shaheen et al. (2009) studied P sorption in acid, neutral and alkaline soils and found the highest sorption was in alkaline soils (90%).

The results in (Fig. 4.2) show that total P adsorption is higher in Soil 1 than Soil 2, possibly due to the greater soil clay content, and Fe and Al concentrations in Soil 1. Uddin et al. (2014) studied the effect of time of incubation on P adsorption in four different calcareous soils of Pakistan and found that adsorption differed between soils due to physio-chemical properties such as organic matter content, nature and amount of clay content, and pH value. Rashmi et al. (2016) studied P sorption parameters related to soil properties in four different types of Indian soils, they found a significant correlation between P sorption with concentration of clay ($r^2=0.70$), Fe ($r^2=0.81$) and Al ($r^2=0.73$). Devau et al. (2011) found that in soils with neutral-to-alkaline conditions, clay minerals appeared to be the principal

determinant of P-fixation – presumably a reflection of the density of adsorption sites rather than specifically indicating phosphate adsorption on aluminosilicate clays. Thus it may be also expected that P adsorption is significantly correlated with soil CEC because the values of CEC in soils were 30.9 and 10.1 $\text{cmol}_c \text{kg}^{-1}$ in Soils 1 and 2 respectively.

The mineralogy of the clay content of both soils was not determined but it is known that the majority of the soils in Kurdistan contain montmorillonite in various amounts. However, it is clear from the results that these calcareous soils have a high capacity for P adsorption/fixation - probably due to a high CaCO_3 content, high pH and relatively high Fe and Al concentrations.

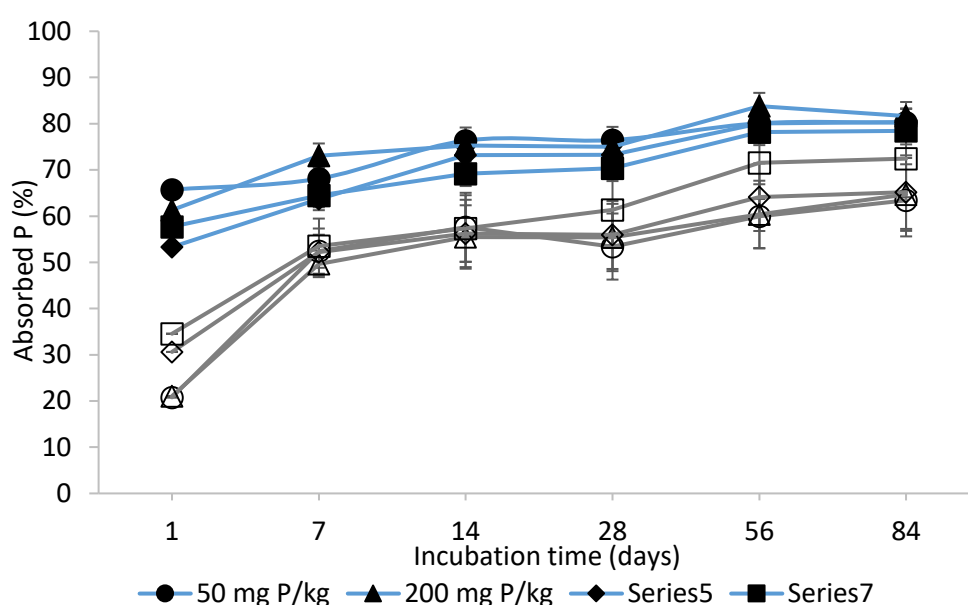


Fig. 4.1: The percentage of adsorbed phosphorus in the two calcareous soils collected from Sulaimanyah province, Kurdistan region, Iraq: Soil 1 (filled symbols) and Soil 2 (open symbols). At ($p \leq 0.05$) significant differences were found between soils and day one when compared with other incubated days.

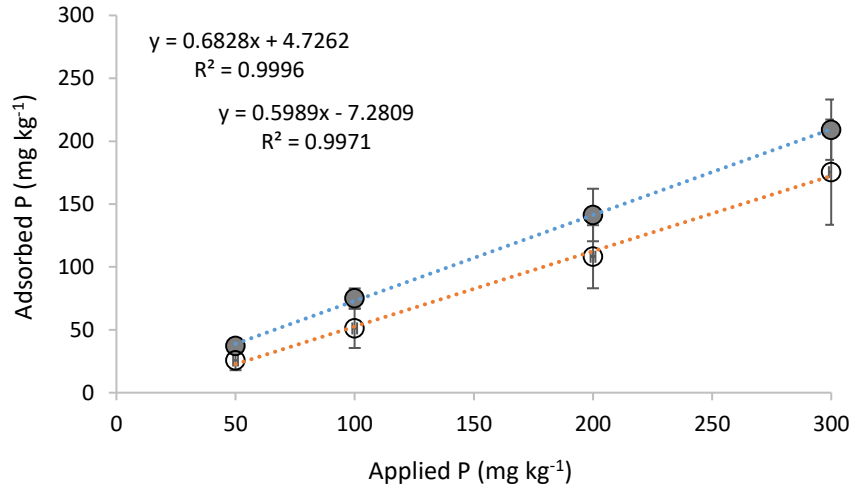


Fig. 4.2: Effect of phosphate fertilizer application rates on adsorption of P in the two calcareous soils collected from Sulaimanyah province, Kurdistan region, Iraq: Soil 1 (filled symbols) and Soil 2 (open symbols).

4.3.2.2 Phosphorus recovery (%) extracted by NaHCO_3 (Olsen-P, mg kg^{-1})

Understanding the main factors affecting the recovery of applied P is important and may be facilitated with knowledge of soil properties and the fractionation of P in the soil (Samadi, 2006). Table 4.2 presents the proportion (%) of P in a reactive state in the two studied soils - measured as P_{Olsen} . In the P-treated soils recovery of P (as Olsen-P) increased with increasing P application rates but decreased with time of incubation ($p < 0.05$). Audette et al. (2016) studied the kinetics of P transformation applied to calcareous soil and found that application of 225 mg P kg^{-1} soil as KH_2PO_4 , increased labile P extracted by NaHCO_3 . However, more stable P compounds formed slowly during 126 days of incubation time in a calcareous sandy loam soil containing a much lower clay content 5% compared to the soils used in this study 43% (Soil 1) and 31% (Soil 2).

The results in Table 4.2 also indicate that the effects of P application rate were greater in the period between 1-7 days. From the first day to 7 days of incubation, the percentage of P recovery (as Olsen P) decreased for Soils 1 and 2 and increased with P

application rate with the greatest proportional change occurring within the first day. However, from 14 days to the end of the incubation period (84 days), the proportion of Olsen-P decreased steadily and did not reach an equilibrium. (Al-Rohily et al., 2013) studied the effect of time of incubation (up to 16 weeks) on inorganic P fertilizer in calcareous soils of Iran and concluded that the most critical time period affecting P availability was the first week. The decrease of P recovery in the studied soils from the first day to the end of the experiment (1-84 days) suggests that there are slow time-dependent reactions, perhaps including precipitation or progressive absorption of applied phosphate into mineral structures. The concentration of Olsen-P remained greater in Soil 2 which had a lower CEC (3-fold), clay content, and Fe and Al concentrations than Soil 1. Halajnia et al. (2009) also found a negative relationship between P_{Olsen} and soil CEC. Samadi and Gilkes (1999) recovered about $40 \pm 9\%$ of applied P as $\text{NaHCO}_3\text{-P}$ in 14 calcareous soils containing similar clay contents 20-48% as the soils used in this study after incubation for 160 days.

Regression analysis showed that P_{Sol} was positively correlated with P_{Olsen} in the case of P rates and incubation times, Table 4.3; Jalali (2007) reported that application of inorganic P fertilizer caused an increase in the dissolved P in the soil solution.

Table 4.2: The proportion (%) of phosphorus recovery ($\pm$ SD) as NaHCO_3 -extractable P (P_{Olsen}) in two soils used in the study.

Soil	P added (mg kg^{-1})	Incubation time (days)					
		1	7	14	28	56	84
1	50	34.3 $\pm$ 2.17	31.9 $\pm$ 4.25	23.7 $\pm$ 2.65	23.5 $\pm$ 0.59	19.9 $\pm$ 1.66	19.8 $\pm$ 0.93
	100	38.7 $\pm$ 3.45	26.9 $\pm$ 2.38	24.7 $\pm$ 1.52	25.0 $\pm$ 0.56	16.2 $\pm$ 2.93	18.3 $\pm$ 1.51
	200	46.7 $\pm$ 1.44	36.3 $\pm$ 1.75	26.8 $\pm$ 0.79	26.7 $\pm$ 3.44	19.9 $\pm$ 2.05	19.6 $\pm$ 0.28
	300	42.3 $\pm$ 1.21	35.5 $\pm$ 4.98	30.8 $\pm$ 0.88	29.7 $\pm$ 1.95	21.8 $\pm$ 0.52	21.5 $\pm$ 0.84
2	50	79.2 $\pm$ 7.74	47.6 $\pm$ 2.81	42.4 $\pm$ 6.24	46.4 $\pm$ 4.54	40.0 $\pm$ 1.04	36.6 $\pm$ 1.56
	100	78.5 $\pm$ 1.99	50.3 $\pm$ 3.63	44.5 $\pm$ 2.60	44.6 $\pm$ 2.16	39.6 $\pm$ 1.74	35.3 $\pm$ 1.40
	200	69.4 $\pm$ 4.19	47.8 $\pm$ 1.69	43.7 $\pm$ 2.09	44.1 $\pm$ 2.09	35.9 $\pm$ 1.03	34.8 $\pm$ 1.10
	300	65.5 $\pm$ 3.09	46.5 $\pm$ 1.61	42.7 $\pm$ 4.83	38.6 $\pm$ 0.83	28.5 $\pm$ 1.82	27.6 $\pm$ 0.79

Table 4.3: Correlations between P_{Olsen} and P_{Sol} in the two studied soils incubated up to 84 days.

		Incubation time (days)					
		1	7	14	28	56	84
Soil 1	p-value	0.002	0.004	0.003	0.001	0.001	0.002
	r	0.98	0.98	0.99	0.99	0.99	0.98
Soil 2	p-value	0.002	0.004	0.001	0.03	0.05	0.01
	r	0.98	0.97	0.97	0.91	0.86	0.78

4.3.2.3 Phosphorus adsorption isotherm

Adsorption isotherms were modelled by fitting data to the nonlinear forms of three equations, the Langmuir, Freundlich and Langmuir-Freundlich models. Adsorbed P was assessed both as P_{Olsen} (Fig. 4.3 A and C) and as total adsorbed P (Fig. 4.3 B and D). The continuing adsorption of phosphate through time (due to P fixation) should produce a progressive change in the slope of the adsorption isotherm. By contrast, if P_{Olsen} really represents a 'reactive P' pool then there should be a single constant adsorption isotherm with all data falling on a single trendline regardless of incubation (P contact) time. This would reflect the existence of a true equilibrium between P_{Olsen} and P_{Sol} . A comparison of Fig. 4.3 A and B and Fig 4.3 C and D clearly illustrates the fixation process through time and also verifies that P_{Olsen} and P_{Sol} are in a robust equilibrium regardless of incubation time. This justifies the use of P_{Olsen} in these soils, not just as a measurement of available P reserves but also as an indicator of immediately available P in the soil solution.

Overall, the Langmuir-Freundlich model described the P_{Olsen} data best for both soils although there was little difference in the fitting of all three models. For Soils 1 and 2 respectively, the residual standard deviation (RSD) values for the fit of the Langmuir-Freundlich model were lowest (1.10 and 10.4 mg kg⁻¹) compared to 1.46 and 10.8 for Langmuir and 1.75 and 10.7 for Freundlich models .

Soil 1 demonstrated stronger P adsorption than Soil 2 at the start of the experiment – again reflecting the greater clay, Fe and Al oxides content and CEC value. Rashmi et al. (2016) studied phosphate adsorption parameters in relation to agricultural soils of India, they found that P adsorption maxima were significantly related to clay ($r^2=0.70$), Fe ($r^2=0.81$) and Al ($r^2=0.73$). However, it can also be seen that at the end of the incubation period, Soil 2 had greater P adsorption (at 84 days) – this may be due to a higher carbonate content and a greater pH value in Soil 2. Hadgu et al. (2014) studied phosphorus adsorption its relation to soils properties, they found that P adsorption increased with increase in CaCO_3 content. Tunesi et al. (1999) found that the formation of Ca-P mineral phases increased with an increase in the reaction time. Mallikarjun and Shashikant (2013) conducted a batch experiment to study P adsorption characteristics in different soils and found that an increase in soil pH from 8.0 to 8.5 slightly increased P adsorption from 62.5 to 64%.

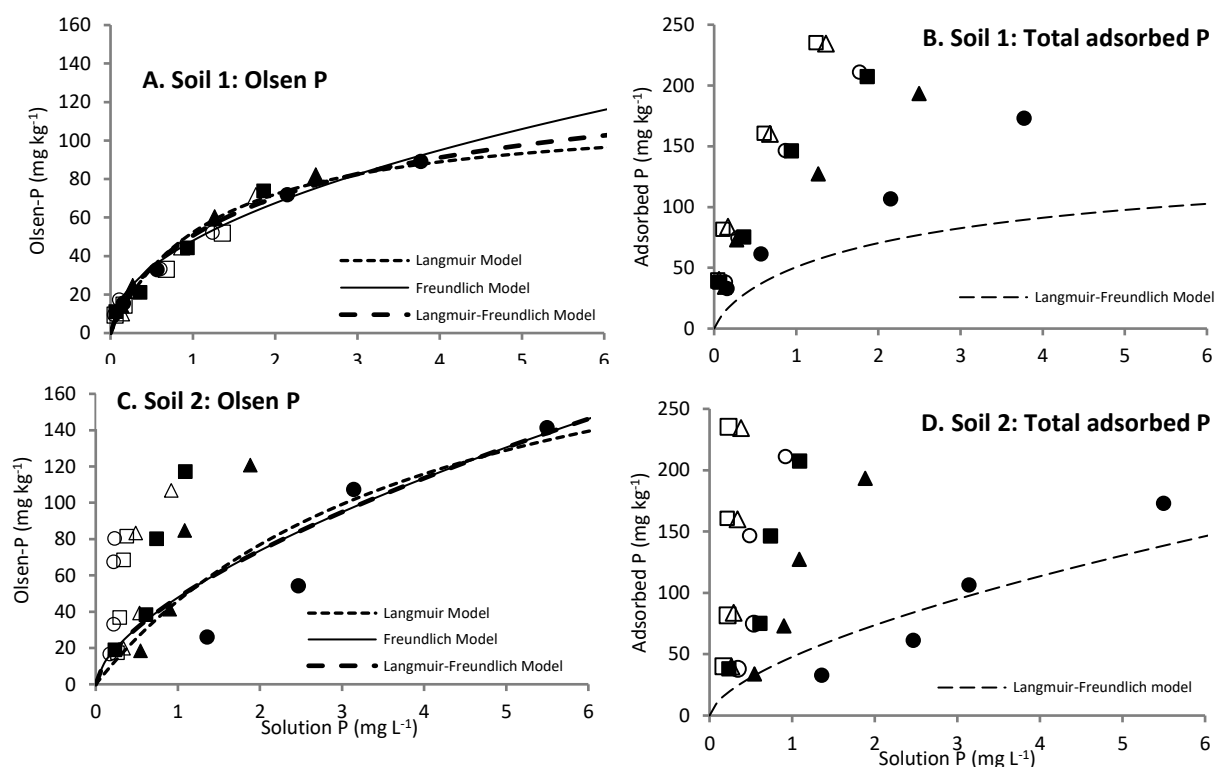


Fig. 4.3 Phosphate adsorption isotherms for Soils 1 and 2, expressing solution P concentration (X-axis) as a function of 'adsorbed Olsen-P' (A and C) and 'total adsorbed P' (B and D). The two calcareous soils from Kurdistan were incubated with phosphate for (●) 1 day, (▲) 7 days, (■) 14 days, (○) 28 days, (△) 56 days and (□) 84 days respectively. In graphs A and C three models are fitted to the entire 'reactive phosphate' dataset across all incubation times. In graphs B and D the best-fit model line achieved for the Olsen P data is shown to illustrate the progressive adsorption (fixation) reaction against a consistent relationship between solution P and reactive adsorbed P.

4.3.2.4 Solubility of phosphate minerals in studied soils

In calcareous soils, phosphate is expected to form a range of Ca phosphate minerals (MCP, DCPD, OCP, TCP and HA) with progressively decreasing solubility in a Ca-rich environment (Al-Tameemi et al., 2015). Figure 4.4 presents the soil solution composition (phosphate potential and lime potential) in relation to common Ca phosphate minerals for the two studied soils. The Ca and phosphate data fell between undersaturated with respect to DCPD and supersaturated with respect to HA, OCP and β -TCP across the two studied soils and across all incubation periods (day 1-84). This result is in agreement with results reported by (AL-Tameemi et al., 2015) who applied 0, 60 and 120 mg P kg as superphosphate to two calcareous soils from southern Iraq and incubated the soils for one week at 30 °C and at 1.5 and 0.5 of their field capacity. They found that the applied P had been converted to OCP and HA by the end of the experiment. Wadu (2015a) found that Ca plays a major role in causing P precipitation and decreasing its solubility; Jalali and Jalali (2017) suggested that Ca-phosphates control P solubility calcareous soils.

The results in (Fig. 4.4) also suggest that in control versions of both soils the P solubility is probably controlled by HA which therefore probably represents the stable Ca phosphate mineralogy for those soils. Jalali and Jalali (2017) studied P solubility in long-term cultivated calcareous soils of Iran and found that HA is the most stable and least soluble of the Ca-P minerals found in aerobic calcareous soils. Where P has been added,

the solubility of the phosphate minerals appeared to systematically decrease with increasing time of incubation. Increased applied P rates also increased the solubility of P and actually produced supersaturated conditions with respect to DCP on day one. By contrast, progressive re-crystallisation (possibly) led eventually to undersaturated conditions with respect to OCP and β -TCP by day 84. Notwithstanding the suggestion of HA in control soils, the results of the current study indicate that OCP and β -TCP minerals are the stable forms of P mineral in both soils where P has been recently added. This is agreement with the finding of Zhang et al. (2014) who found that the solubility of phosphate in farmed soils was controlled by β -TCP while, in unfarmed soils it was controlled by HA. Zhang et al. (2014) reported that in farmed calcareous soils, after several years, HA gradually develops even in the presence of high application of phosphate fertilizers.

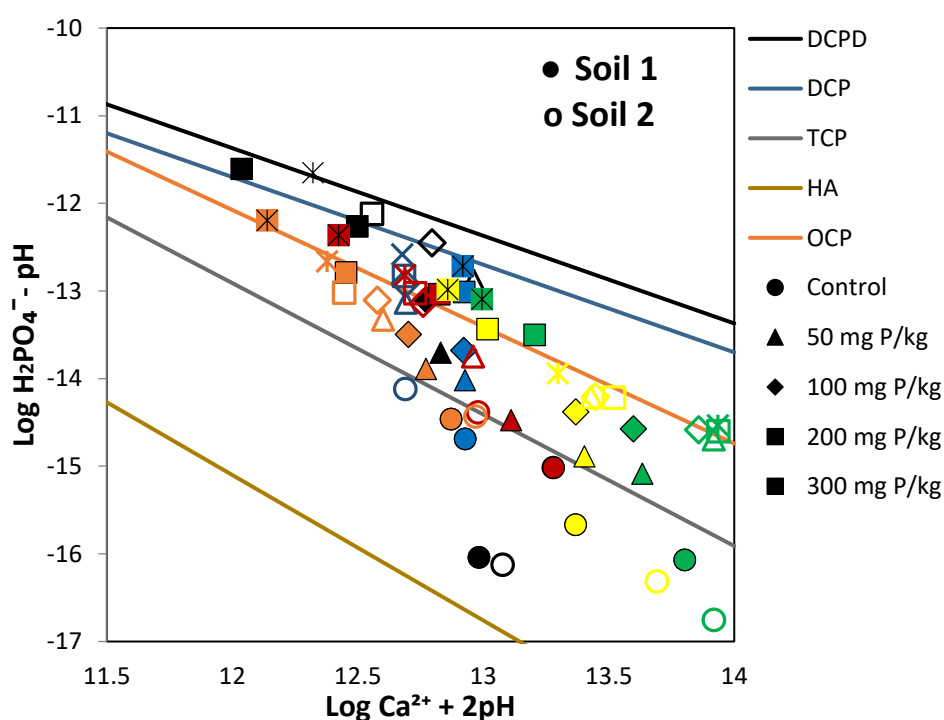


Fig. 4.4: Phosphate solubility in the studied soils in relation to crystalline Ca–P minerals; monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; β -tricalcium phosphate, β -TCP; and hydroxyapatite; HAP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by Lindsay

(1979). The two calcareous soils from Kurdistan were incubated with phosphate for (black) 1 day, (blue) 7 days, (red) 14 days, (orange) 28 days, (yellow) 56 days and (green) 84 days respectively.

4.3.3 Zinc behaviour in two calcareous soils from Kurdistan

4.3.3.1 Time-dependence of zinc adsorption in the studied soils (%)

Figure 4.5 shows that ^{70}Zn applied rates and time of incubation increased Zn adsorption significantly under ($p < 0.05$) in the two selected soils. The results indicate that Zn adsorption onto the soils was comparatively rapid in the first day and continued until approximating an apparent equilibrium. The proportional change in adsorbed Zn was quite minor across the duration of the trial. For example, adsorption of Zn in the two soils increased from 93.4 and 92.2% on day one to 98.7 and 97.4% on day 84 for the 2 mg ^{70}Zn kg $^{-1}$ treatment for Soil 1 and 2 respectively. Results are broadly in agreement with those reported by Ashraf et al. (2008) who studied Zn adsorption in calcareous soils.

Figure 4.6 suggests that the two studied soils had similar Zn adsorption capacities although adsorption in Soil 2 was slightly higher, possibly reflecting the higher soil carbonate-C content (41%), low organic matter content as LOI 2.45% and a high pH (8.24) compared to corresponding values of 6.13%, 12.2% and 8.20 in Soil 1. Ashraf et al. (2008) and Kamali et al. (2010) studied Zn adsorption behaviour in a range of calcareous soils and found that increasing soil CaCO_3 content increased Zn adsorption. Giriya et al. (2013) also reported that pH value had the main role in Zn absorption, at high soil pH values (>7.7). Abdu and Mohammed, (2016) studied Zn adsorption in a Savanna soil and found maximal Zn adsorption in soil with a pH value above 7. Al-Tamimi (2006) studied Zn adsorption in a Torrifluvents soil of the Sub-Saharan region in the south of Libya and found a negative correlation between Zn adsorption and soil organic matter content. Given the proportion of Zn adsorbed, the results suggest that

application of Zn to the calcareous soils of Kurdistan is probably not a viable method of increasing the available Zn for plants (Fig. 4.5).

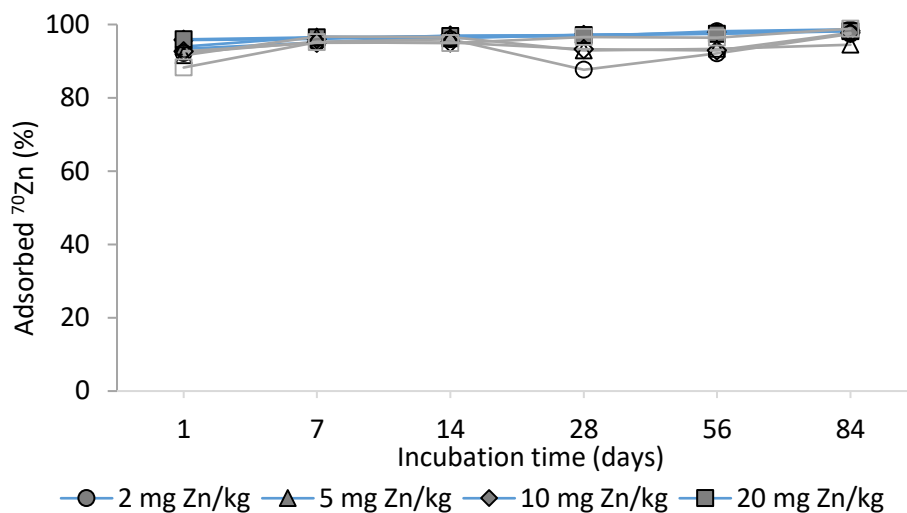


Fig. 4.5: The percentage of ^{70}Zn adsorbed by two calcareous soils, (●) Soil 1 and (o) Soil 2, from Sulaimanyah province, Kurdistan region, Iraq. Adsorption was determined from the difference between ^{70}Zn added and the solubility measured in 0.01 M $\text{Ca}(\text{NO}_3)_2$ suspension.

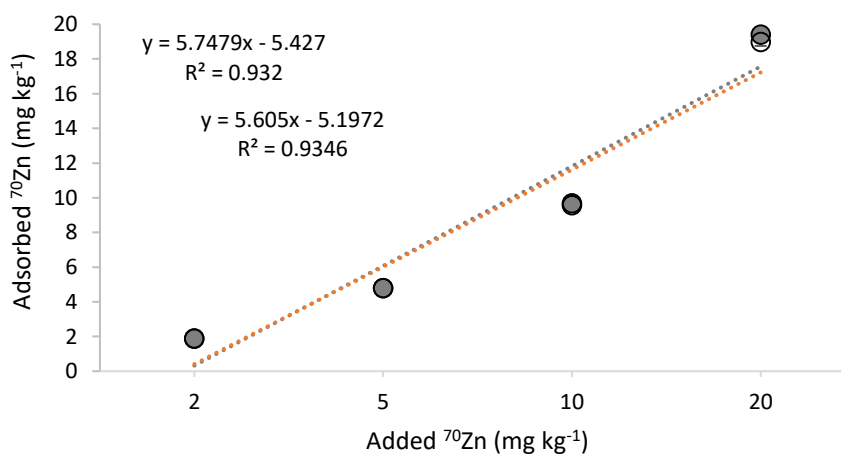


Fig. 4.6: Effect of ^{70}Zn isotope application rates on the amount of adsorbed Zn in Soil 1 (●) and Soil 2 (o).

4.3.3.2 ^{70}Zn recovery (%) extracted by DTPA (available, mg kg^{-1})

Table 4.4 presents the percentage of ^{70}Zn extractable as Zn_{DTPA} . The results indicate that the recovery of Zn increased with increase in the applied Zn rates but decreased with time of incubation significantly ($p < 0.05$). Similarly, Kamali et al. (2010) studied Zn adsorption in calcareous soil and found that increasing time of incubation significantly decreased exchangeable and soluble Zn. The results indicate that the effect of ^{70}Zn application rate was greatest in the first day and higher rates caused a greater proportion of Zn to remain in a plant-available form as Zn_{DTPA} for a longer period of time. From the first day to 7 days of incubation, the % Zn recovered decreased for soil 1 and 2 and increased with Zn rate, suggesting that the critical time for Zn application is the first day. However, from 14 days to the end of the incubation period (84 days), the value of % Zn recovered continued to decrease and did not reach an equilibrium. Alvarez and Maria (2003) reported that incubation time is an important factor in the process of decreasing available Zn in calcareous soils. They found that incubation of calcareous soils in columns with 10 and 20 $\text{mg Zn-EDTA kg}^{-1}$ for 60 days, decreased Zn_{DTPA} by 72 and 75% respectively. The difference in Zn recovery between the results of the current study and those reported may be due to the type of Zn fertilizer and soils.

The amount of Zn_{DTPA} data in Soil 1 were lower than in Soil 2 from day one to day 14, while from day 28 to end of the experiment the trend was reversed. This may reflect differences in key soil properties such as carbonate content, soil pH and soil organic matter content. According to Kamali et al. (2010), a high amount of applied Zn reacts rapidly with carbonate present in the soil. Ramzan et al. (2014) study the fractionation of Zn and their association with soil properties in the soil of the Kashmir Himalayas. They found a negative relationship between exchangeable Zn and pH value and a positive correlation with SOM content.

Table 4.4: The recovery of $^{70}\text{Zn}_{\text{DTPA}}$ in (mg kg^{-1}) in the two soils used in the study, which were incubated for days and at laboratory condition with different applied amount of ^{70}Zn .

Soil	Zn added (mg kg^{-1})	Incubation time (days)					
		1	7	14	28	56	84
1	2	6.64±2.45	3.23±0.46	3.23±1.77	3.24±0.39	1.89±0.39	1.32±0.24
	5	6.02±0.66	3.35±0.14	3.73±0.53	2.82±1.00	2.42±0.33	1.51±0.18
	10	4.23±0.10	3.90±0.74	3.04±0.36	2.91±0.07	2.30±0.25	1.74±0.60
	20	4.06±0.85	3.50±0.29	3.15±0.56	2.56±0.42	2.56±0.21	1.82±0.13
2	2	7.77±1.89	4.69±2.05	4.00±2.11	2.88±1.13	2.15±0.79	1.25±1.05
	5	8.39±3.11	3.29±1.08	3.42±0.19	4.94±1.77	3.13±1.27	1.03±0.24
	10	7.32±0.11	5.00±2.02	4.87±1.49	3.54±0.52	3.34±0.53	1.84±1.60
	20	11.7±2.61	4.79±1.97	5.04±0.64	3.38±0.32	3.55±0.65	1.14±0.28

4.3.3.3 Measurement of isotopically exchangeable Zn in soils (E -value; Zn_E)

The effects of incubation time and applied Zn rates on measurement of Zn 'lability' and fixation were studied by determination of isotopically exchangeable Zn as an apparent E -value (Zn_E). It is important to recognise that Zn_E was determined following progressively longer contact times between the tracer isotope (^{70}Zn) and the soil and so the values of Zn_E presented do not represent 'labile' Zn except perhaps for the earliest measurements. Rather the values of Zn_E represent the progressive mixing of the ^{70}Zn isotope with the native soil Zn and so effectively quantify a fixation process. Generally, increasing ^{70}Zn application rates and time of incubation significantly increased the values of Zn_E in the two soils ($p \leq 0.05$) as shown in Table 4.5. Sinaj et al. (2004) studied soil isotopically exchangeable Zn in the batch experiment and found that the (apparently) isotopically exchangeable Zn increased with time. They also found that about 52% of the total Zn extracted by DTPA was from the pool of isotopically exchangeable Zn at the end of an incubation experiment (109 days). The Zn_E values were 1.04 ± 0.08 and 0.76 ± 0.18 for Soils 1 and 2. This difference was probably due to clay content, CEC value, and Al and Fe concentrations which are higher in Soil 1. The

fluctuation of Zn_E values at the end of the experiment (day 84) might be due to other processes affecting the distribution of ^{70}Zn isotope in the studied soils with time.

The percentage of isotopically exchangeable Zn in the two selected soils ($\%Zn_E$), generally increased with applied ^{70}Zn rates Table 4.6, a significant correlation was found between $\%Zn_E$ with applied rates ($R^2 = 0.42$ and 0.47 , $p < 0.05$) for soil 1 and 2 respectively when the full dataset was considered. However, there was no correlation found between $\%Zn_E$ and time of incubation. This has been detected before for soils treated with soluble Zn salts (Degryse et al., 2011).

Table 4.5: Effect of time of incubation and Zn application rates on Zn E-values ($\pm$ standard deviation) for the two soils.

Soil	^{70}Zn Added ($mg\ kg^{-1}$)	Zn_E ($mg\ kg^{-1}$)					
		Incubation time (days)					
		1	7	14	28	56	84
1	2	0.33 $\pm$ 0.11	0.37 $\pm$ 0.03	0.62 $\pm$ 0.42	0.57 $\pm$ 0.08	0.90 $\pm$ 0.16	1.03 $\pm$ 0.18
	5	0.38 $\pm$ 0.03	0.57 $\pm$ 0.01	0.50 $\pm$ 0.05	0.78 $\pm$ 0.22	0.84 $\pm$ 0.22	0.97 $\pm$ 0.09
	10	0.56 $\pm$ 0.01	0.63 $\pm$ 0.09	0.69 $\pm$ 0.06	0.77 $\pm$ 0.03	0.91 $\pm$ 0.04	1.06 $\pm$ 0.35
	20	0.75 $\pm$ 0.11	0.80 $\pm$ 0.01	0.86 $\pm$ 0.12	1.37 $\pm$ 0.15	1.01 $\pm$ 0.02	1.12 $\pm$ 0.03
2	2	0.17 $\pm$ 0.03	0.29 $\pm$ 0.09	0.27 $\pm$ 0.10	0.41 $\pm$ 0.13	0.60 $\pm$ 0.29	1.31 $\pm$ 0.23
	5	0.24 $\pm$ 0.14	0.40 $\pm$ 0.10	0.30 $\pm$ 0.01	0.31 $\pm$ 0.10	0.40 $\pm$ 0.11	0.86 $\pm$ 0.17
	10	0.25 $\pm$ 0.01	0.35 $\pm$ 0.14	0.33 $\pm$ 0.07	0.40 $\pm$ 0.05	0.38 $\pm$ 0.12	0.48 $\pm$ 0.11
	20	0.23 $\pm$ 0.02	0.45 $\pm$ 0.11	0.40 $\pm$ 0.02	0.52 $\pm$ 0.03	0.51 $\pm$ 0.05	1.01 $\pm$ 0.22

Table 4.6 presents lability (Zn_E) as proportion of total ^{70}Zn in the two selected soils. About 70.4 and 90.5% Zn_E and 13.8 and 8.86% of total applied Zn were recovered when the whole dataset was considered in soil 1 and 2 respectively. In general, the recovered $\%Zn_E$ from soil increased with increase in the applied Zn rates (R^2 ranged between 0.32-0.91 and 0.41-0.86, $p < 0.001$) but decreased with incubation time, (R^2 ranged between 0.17-0.52 and 0.26-0.42, $p < 0.01$) for soil 1 and 2 respectively. These high values of $\%Zn_E$ in relatively fertilized soils suggest that the majority of the available soil Zn is from applied Zn. Ayoub et al. (2003) also observed a high $\%Zn_E$, about 65%

in soil from Great Billings (UK) amended with sewage sludge. They calculated %Zn_E in 4 treated soils and attributed high %Zn_E values ranging between 70-90% for most treatments. In contrast, Nazif et al. (2015) measured low values of %Zn_E, 0.24-6.24% extracted by 0.01 M Ca(NO₃)₂ in 36 uncontaminated calcareous soils from Peshawar, Pakistan. Thus, increasing %Zn_E in the studied soils is due to the application of Zn.

Table 4.6: The proportion of Zn_E of total added ⁷⁰Zn extracted by DTPA in the two studied soils which were incubated for 84 days with different level of applied ⁷⁰Zn isotope.

Soil	⁷⁰ Zn added (mg kg ⁻¹)	Lability (%Zn _E) extracted by 0.005M DTPA					
		Incubation time (days)					
		1	7	14	28	56	84
1	2	56.5	41.5	27.6	72.1	44.5	32.8
	5	52.4	28.8	32.6	66.0	62.0	39.2
	10	37.0	34.1	26.6	69.2	57.8	45.8
	20	35.6	30.7	27.7	70.4	65.6	48.8
2	2	68.0	40.9	34.9	68.6	54.0	38.7
	5	73.8	28.9	30.1	68.9	79.8	27.3
	10	64.5	44.0	42.9	86.4	82.1	64.5
	20	90.5	42.2	44.5	84.3	94.0	31.3

4.3.3.4 Zinc adsorption isotherms

The adsorption isotherms were assessed by fitting nonlinear forms of the three adsorption equations (the Langmuir, Freundlich and Langmuir-Freundlich models). The 'adsorbed' data was either Zn_{DTPA} or total adsorbed Zn (both in mg kg⁻¹) for the two studied soils; these were plotted against equilibrium Zn concentrations (mg L⁻¹) measured in 0.01 M Ca(NO₃)₂ (Fig. 4.7). Overall, the Freundlich model described the Zn_{DTPA} data well for both studied soils; the RSD values (mg kg⁻¹) were 0.026 and 0.073 for Soils 1 and 2, compared to 0.027 and 0.096 for the Langmuir model and 0.034 and 0.075 for the Langmuir-Freundlich model. Hararah et al. (2011) studied Zn adsorption in some calcareous soils of Iran and also found that Zn sorption was described by the Freundlich isotherm. There have been many studies of heavy metal retention in which

the adsorption characteristics appear to follow the Freundlich model (Antoniadis et al., 2017, Dandanmozd and Hosseinpour, 2010, Hararah et al., 2011). The Freundlich equation is an empirical equation but equates broadly to a distribution coefficient (K_F) qualified by an affinity (power) term (n) which represents a progressive reduction in adsorption strength with site coverage. In this way the Freundlich model accommodates heterogeneity of adsorption sites in soils. Ashraf et al. (2008) suggested that the success of the Freundlich isotherm is that while it supposes unlimited adsorption sites it includes provision for variation in soil adsorption site chemical and physical properties.

The values of the Freundlich constants, K_F and n , for Zn_{DTPA} adsorption were 2.28 and 0.56 for Soil 1 and 9.59 and 1.18 for Soil 2. According to Selim (2017), n values greater than 1 could suggest precipitation reaction. Adsorption reactions should produce n values less than 1.0 as adsorption weakens with progressive site coverage. However, a rising trend ($n > 1$) in the adsorption isotherm could suggest precipitation. Thus the value of n for Soil 2 (1.18) may be because Soil 2 contains a greater amount of $CaCO_3$ and a higher pH value than Soil 1; and so the applied Zn may react with $CaCO_3$ to form surface precipitates as $ZnCO_3$ or mixed Zn, $CaCO_3$ particles. Dandanmozd and Hosseinpour (2010) studied the thermodynamic parameters of Zn sorption in some calcareous soils of Iran and found that the sorption maxima correlated to the soils' calcium carbonate contents which again is reflected in the current comparison of Soils 1 and 2. Reyhanitabar and Gilkes (2010) studied the characteristics of Zn sorption in some calcareous soils of Iran and reported a highly significant relationship ($p \leq 0.01$) between the Freundlich K_F constant and calcium carbonate contents in soils and between the Freundlich n coefficient and soil pH value.

Surprisingly, Soil 1 had greater adsorbed Zn at the end of the experiment (84 days) than Soil 2 (Fig.4.7b and c). This may reflect the greater clay content and Fe and Al

oxide content and CEC value in Soil 1. Antoniadis et al. (2017) studied the effect of removal of carbonates and hydrous oxides on Zn sorption in different soils. They found that Zn sorption decreased from 98% to 83% with the remove of CaCO_3 and from 99% to 85% with the remove of Fe and Al oxides in all soil samples. They also found that in the CaCO_3 -removed soils, Fe and Al oxides were the main soil components affecting Zn sorption. (Antoniadis et al., 2017) also found that Fe and Al oxides become functional when CaCO_3 was removed.

Figure 4.7 B and D also clearly show the effect of time on Zn adsorption. The single model trend representing Zn_{DTPA} adsorption is far lower than the total Zn adsorption data and represents the relatively constant relationship between reactive adsorbed Zn (Zn_{DTPA}) and solution Zn concentration. By contrast, the total adsorbed Zn data shows a clear time-dependency suggesting (i) there is clear evidence of progressive Zn fixation and (ii) the majority of the sorbed Zn is indeed fixed and unreactive. Even after just one day the recovery of Zn by extraction with DTPA is very small and further fixation proceeds beyond day 1 up to the time of the final measurement after 84 days of incubation. It is clear from the trend in the data that the half-life of the reactive Zn would be < 1 day suggesting extremely rapid fixation. The trends in Fig 4.7 B and D clearly underline that it would be pointless adding soluble Zn fertilizer to these calcareous soils as the vast majority would be rendered unavailable to plants long before any benefit to plant health could be realised.

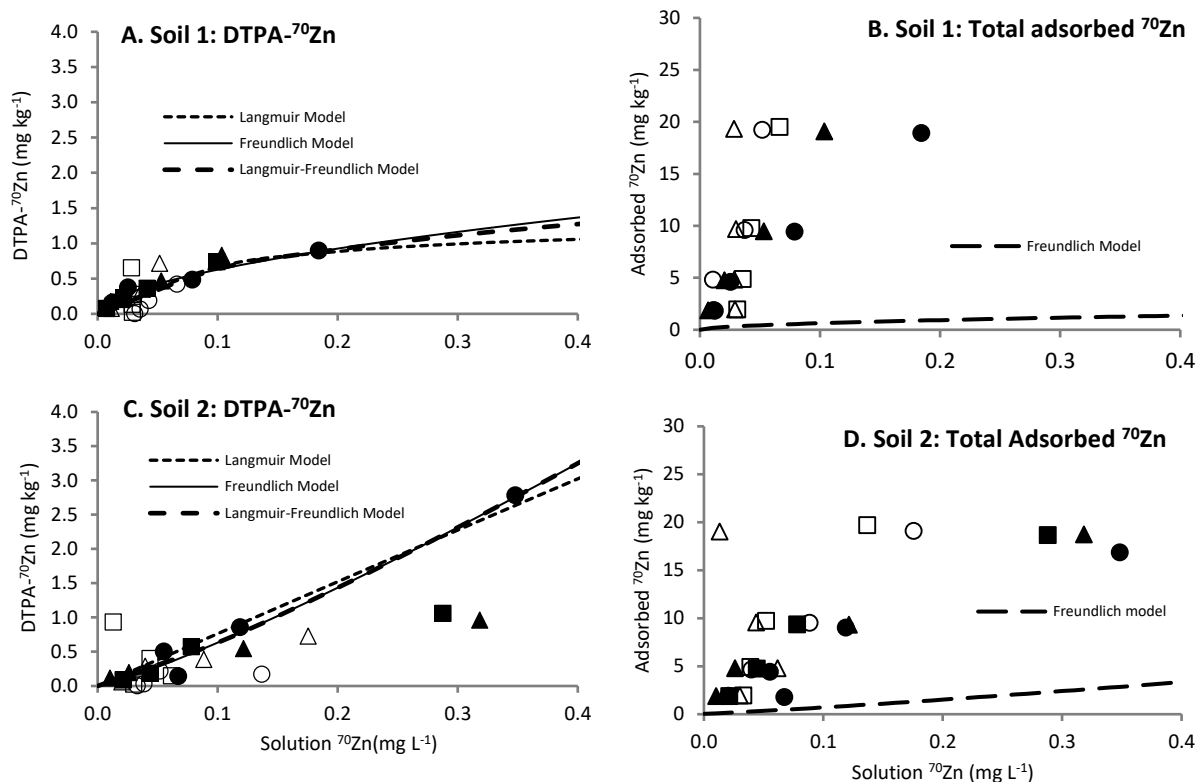


Fig. 4.7: Zinc adsorption isotherms for Soils 1 and 2, expressing solution ^{70}Zn concentration (X-axis) as a function of 'adsorbed DTPA- ^{70}Zn ' (A and C) and 'total adsorbed ^{70}Zn ' (B and D). The two calcareous soils from Kurdistan were incubated with ^{70}Zn for (●) 1 day, (▲) 7 days, (■) 14 days, (○) 28 days, (△) 56 days and (□) 84 days respectively. In graphs A and C three models are fitted to the entire 'reactive ^{70}Zn ' dataset across all incubation times. In graphs B and D the best-fit model line achieved for the DTPA- ^{70}Zn data is shown to illustrate the progressive adsorption (fixation) reaction against a consistent relationship between solution ^{70}Zn and reactive adsorbed ^{70}Zn .

4.3.3.5 Zn solubility

The concentration of soluble Zn measured in the soil suspension (0.01 M $\text{Ca}(\text{NO}_3)_2$) can be used to determine the Zn mineral solid phases which may control the solubility of Zn in the studied soils. A solubility diagram for Zn minerals was developed by plotting 'logarithm activity of Zn^{2+} ($\log \text{Zn}^{2+}$) against soil pH. The activity of Zn^{2+} , pH and partial pressure of CO_2 (PCO_2) used to develop this diagram, were determined using measured values of pH and Zn concentration in solution with speciation using the geochemical

model WHAM-VII. This approach can indicate the likelihood of Zn minerals being present in soils at different pH values (Lindsay, 1979, Nazif et al., 2015, Urgan and Rimmer, 2000).

Figure 4.8 presents the soil solution composition of Zn^{2+} in relation to common Zn minerals in the two studied soils. The Zn data was mainly undersaturated with respect to Smithsonite initially across all Zn applied rates but moved towards the Smithsonite solubility line with time (day 1 to 84) due mainly to a gradual increase in pH. Jalali and Zinli (2012) reported that in the calcareous soils, the Zn solubility is controlled by Smithsonite (ZnCO_3) due to the reaction of Zn with carbonate ions and then precipitation as ZnCO_3 . Martinez and Motto (2000) studied solubility of Zn added to minerals soil and found that in calcareous soils a significant fraction of Zn reacts with carbonate. The results of the current study suggests that Smithsonite may be the stable form of Zn mineral for applied Zn in the two studied soils.

Similar results between Zn solubility and soil pH were obtained by (Sachdev et al., 1992) who studied the effect of soil pH on Zn activity in alkaline soils. The values of pH increased with increasing incubation time in both soils and so the Zn^{2+} free ion activity fell within the stability zone of smithsonite. This trend may have arisen because the initial addition of Zn caused a drop in pH and the time-dependent dissolution of CaCO_3 then compensated and eventually caused precipitation of ZnCO_3 . This result contrasts with the study of Nazif et al. (2015) who suggested that in calcareous soils, Zn was adsorbed on CaCO_3 but undersaturated with Smithsonite. However, they worked with native Zn and did not initially add soluble Zn to the soil. The progressive fixation of Zn seen in the trends in Fig. 4.7 B and D should also be interpreted as being influenced by the gradual rise in pH seen and possibly as a consequence of ZnCO_3 precipitation rather than a continuing adsorption process. Inevitably, adding soluble Zn to the soils has an

initial acidifying effect which the soil's buffer capacity will counter as a time-dependent process.

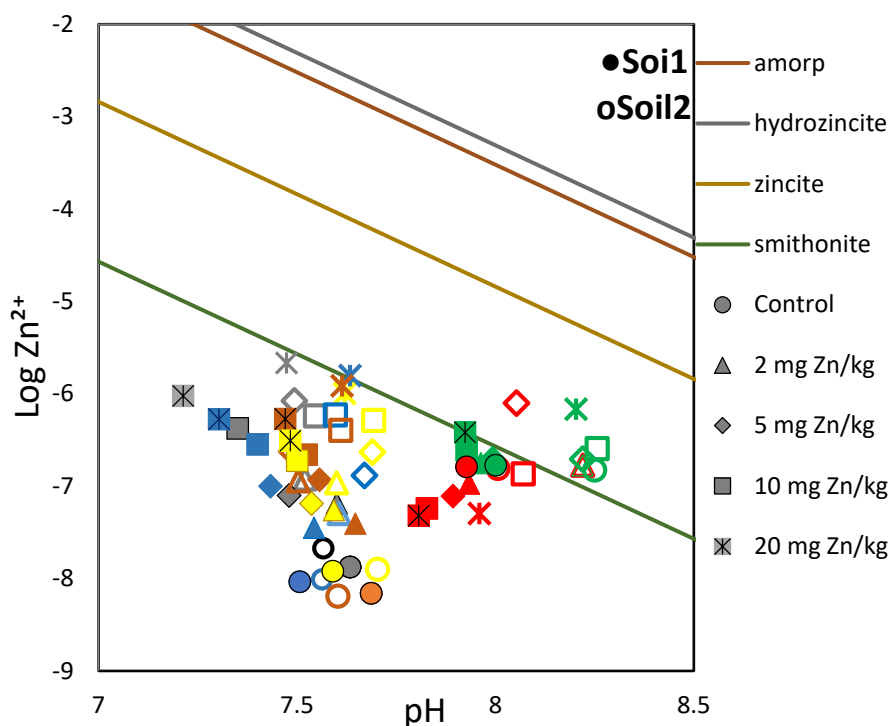


Fig. 4.8: Zinc solubility in studied soils in relation to crystalline Zn minerals; amorphous (ZnCO_3), hydrozincite, zincite, soil- zinc and smithsonite. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Nazif et al., 2015, Lindsay, 1979, Urgan and Rimmer, 2000). The two calcareous soils from Kurdistan were incubated with phosphate for (grey) 1 day, (blue) 7 days, (orange) 14 days, (yellow) 28 days, (red) 56 days and (green) 84 days respectively.

4.4 Conclusion

The current experiment has demonstrated that the calcareous soils of Kurdistan, which are used for wheat grain production, have a substantial capacity for P and Zn fertilizer adsorption and fixation into forms which are unavailable to plants. Phosphorus and Zn adsorption increased with applied rates and incubation time at rates dependent on key soil characteristics. The highest adsorption strength for P was found in Soil 1 due to its higher clay content, CEC value, Fe and Al concentrations. Although Zn adsorption was initially greater in Soil 2 due to its high CaCO_3 content and low organic matter adsorption was also greater in Soil 1 by the end of the experiment (84 days). Langmuir-Freundlich and Freundlich models described available P and Zn adsorption (P_{Olsen} and Zn_{DTPA}) in both soils. The relationships between available adsorbed P and Zn and concentrations in solution were remarkably consistent regardless of incubation time. This justifies the use of both the Olsen test and DTPA extraction to determine the available P and Zn pools which are in equilibrium with soil pore water P and Zn (the immediately available fractions). For both P and Zn there was progressive fixation with time which followed a very consistent pattern for both analytes and both soils. This was partly associated with a gradual increase in pH with time. The solubility of P and Zn increased with increasing application rates but, as expected, decreased with increasing time of incubation and pH value. The solubility of P appeared to be controlled by DCP and HA and Zn solubility gradually moved into the stability zone controlled by smithsonite with time. Both nutrients are therefore likely to exhibit plant uptake limitations - as expected in calcareous soils. The progressive changes in Zn solubility were confirmed by the progressive increase in apparent Zn_E values demonstrating the gradual mixing of the spiked Zn isotope with a larger soil Zn fraction.

5 PHOSPHORUS AND ZINC INTERACTION IN MAIZE, COWPEA AND TOMATO PLANTS GROWN IN THE CALCAREOUS SOIL OF KURDISTAN

5.1 Introduction

Phosphorus (P) and zinc (Zn) deficiencies are common nutritional limitations of crop production in several parts of the world, and P-Zn interaction has been extensively studied (Zhu et al., 2001a, Imran et al., 2016). The application of high amounts of P fertilizer can decrease the solution concentration of Zn (Lambert et al., 2007) due to the formation of insoluble Zn-P compounds ($\text{Zn}_3(\text{PO}_4)_2$) (Hafeez et al., 2013a), thereby decreasing plant Zn uptake. Wang et al., (2008) reported that TSP reduced Zn concentrations in shoots of cabbage (*Brassica chinensis* L.). In calcareous soils, Zn uptake and accumulation in crops generally decreases with increasing P application. Ma et al. (2016a) reported that increasing P causes a decrease in available Zn due to increased adsorption of Zn by Fe and Al oxides and precipitation of Zn-P compounds. However, Zhang et al. (2016) found that at low P application rates of 0-50 kg ha⁻¹, Zn accumulation at the flowering stage was increased in roots but then slightly decreased by increasing application rates to 100-400 kg ha⁻¹; this was thought to be related to root dry matter. In addition to directly influencing Zn solubility in the soil, P is also thought to have an antagonistic effect on Zn uptake by plant roots (Mousavi et al., 2013, Watts-Williams et al., 2013), but there is little information about specific mechanisms.

Zinc movement from plant roots to shoots decreases with an increase in the application rate of P fertilizer (Zhang et al., 2016). Bogdanovic et al. (1999) reported several possible reasons for this phenomenon including, (i) a simple dilution effect on Zn concentration in the plant shoots due to a P-related growth response, (ii) P-Zn

interactions in the soil, (iii) slower rates of translocation of Zn from the roots to the plant shoots, (iv) a metabolic change in plant cells related to changes in P and Zn concentrations, or (v) interference with the metabolic function of Zn at certain sites in the cell at extreme concentrations of P. It is also possible that root morphology could be altered due to P fertilization which may have an effect on Zn uptake by plants (Zhang et al., 2016). It has been recognised that the main interactions between Zn and P appear to be at the plant metabolic level (Bogdanovic et al., 1999).

In plants, phytic acid is an anti-nutrient factor because it contains six negatively charged phosphate groups (Lopez et al., 2002a), fixed to the inositol ring (Okazaki and Katayama, 2014) which forms extremely stable complexes with nutrient cations (Lopez et al., 2002a) and forms insoluble salts (Sakai et al., 2015, Gupta et al., 2015). Phytate is the cation-complexed form of phytic acid which causes a decrease in micronutrient cation bioavailability (Liu et al., 2006) especially in the case of Zn (Abebe et al., 2007). Phytic acid (PA) is thought to play the main role in inhibiting Zn bioavailability (Karunaratne et al., 2008, Gupta et al., 2015). To predict Zn absorption from edible foods containing PA, it has been usual to use their molar ratio in the food as an index of Zn bioaccessibility (Roohani et al., 2012).

Potential Zn bioavailability may be estimated from the PA/Zn molar ratio (Wang et al., 2015a, Gargari et al., 2007). Karunaratne et al. (2008) divided diets into three groups based on PA/Zn molar ratio including (i) < 5 high Zn availability, (ii) 5-15 moderate Zn availability, and (iii) >15 low Zn availability. Approximately, 55% of the food content of Zn is estimated to be absorbed if the PA/Zn molar ratio is less than 5, while it would be 35% if the ratio is between 5 to 15 and only 15% if it is higher than 15 (Vafaei and Sarraf, 2014). Wang et al. (2015b) list three major strategies for increasing mineral concentrations and bioavailability in agricultural products, including (i) plant genetic engineering, (ii) biofortification of Zn, and (iii) application of Zn fertilizer. They found

that the application of Zn fertilizer causes a decrease in the PA content of winter wheat and a decrease in the PA/Zn molar ratio to <15.

There are also several methods to reduce the inhibitory effect of PA and its concentration in foods. It is possible to hydrolyse PA by thermal processing, applying a phytase enzyme, soaking seeds and grains, and fermentation during the bread-making process. Saunders et al. (2012) reported that during the baking process heat destroys more than 50% of the PA in flour. Wu et al. (2010b) reported that the daily intake of PA is about 2 g for developing countries and 0.2-0.8 g for industrialized countries. The enzyme phytase decreases the negative effect of PA on Zn availability in foods; Tavajjoh et al. (2011) reported that about 60% of PA can be hydrolysed by phytase during bread making.

The objectives of the current study were to discover: (i) the effects of P and Zn co-fertilization on P and Zn concentration in maize and cowpea seeds and tomato fruit, (ii) the factors affecting PA/Zn molar ratio in those crops and the effects on estimated bioavailable Zn.

5.2 Materials and methods

5.2.1 Site description

The research site was located in a field of the Halabja Technical College of Applied Science, Halabja city, Sulaimanyah Polytechnic University, Kurdistan region, Iraq (35°12'46"N, 45°57'33"E). The site is at 730 m above sea level and located to the west of Halabja city. The soil in the studied area is a calcareous Mollisol as classified according to the USDA system of soil taxonomy (USDA, 1988).

5.2.2 Maize, tomato, and cowpea experiments

A field experiment to study the role of P on Zn uptake by maize, cowpea and tomato was carried out during the early cropping season (April–August) of 2016. A completely randomized block design experiment (CRBD) was undertaken with five-fold replication. The land was prepared by tillage and levelling and then divided into three areas for the three crops with 2 m space between each. Composite soil samples were collected from the plots before the experiment was initiated on 20th March 2016. Sixteen random soil samples (0–30 cm) across each area were taken using a stainless steel trowel and then sealed in a plastic bag for transport to the soil laboratory of the Agricultural Technical college of Halabja. Soil samples for each area were mixed together and 1 kg of each homogenized soil was dried at room temperature in an aluminium tray, disaggregated, sieved to obtain the <2 mm fraction and then stored in a plastic bag.

5.2.2.1 Maize Trial

Seeds of corn (*Zea mays* L) were purchased locally in Halabja city and were soaked in irrigation water for 24 h before planting on 7th April 2016. The seeds were planted by hand in rows 70 cm apart with 30 cm between plants at a depth of 3–5 cm. Each plot consisted of 3 rows of length 2 m, spaced 70 cm apart to give an area of 4 m². The space between seeds on the rows was 30 cm (18 seeds per plot), oriented in an east-west direction. The maize seed germinated 12 days after planting. Irrigation was carried

out twice every week during the growing period with well water (clean water) located in the field. Weed control was undertaken on two occasions; 4 and 6 weeks after planting. An insecticide, Delta 25, (a synthetic pyrethroid ester) was sprayed once to protect plants from diseases caused by insects. Nitrogen fertilizer (Urea 46% N) was applied as a basal dressing at the recommended rate of 120 kg N ha⁻¹ to plots. A first application (60%) of nitrogen (N) was applied to each plot and two weeks after the first application the remaining application (60%) of N fertilizer was applied after two and four weeks from the germination date.

5.2.2.2 Tomato Trial

Local genotype tomato seeds were purchased in Halabja city and planted in pots on 1st March 2016 to produce seedlings. When the seedlings were 10 cm high they were planted in lines and irrigated with water on 8th April 2016. Each plot consisted of 3 rows each of 1.5 m long spaced 50 cm apart. The space between plants along the rows was 30 cm (15 seedlings per plot), oriented in an east-west direction. Irrigation was carried out twice weekly during the growing period. Weed control and insecticide application were as for the maize plots. An application of 100 kg ha⁻¹ of N was made uniformly to all plots at sowing using urea, as recommended by the Agriculture Department of Halabja. The urea was broadcast and then incorporated into the soil. A first application (50%) of the nitrogen (N) was made to each plot two weeks after planting with the remaining 50% applied with irrigation water two and four weeks later.

5.2.2.3 Cowpea Trial

Local genotype cowpea seed (American) was purchased in Halabja city. Seeds were soaked in irrigation water for 24 h before planting on 27th April 2016. Seeds were planted at a depth of 3-5 cm, by hand, in rows 50 cm apart with 30 cm between plants. Each plot consisted of three rows of length 1.5 m, spaced 50 cm apart (2.25 m²), oriented in an east-west direction. Seeds germinated after one week. Irrigation was

carried out twice a week during the growing period. Weed control and insecticide application were as described in the maize trial. The recommended dose of urea was applied as a basal dressing at the rate of 20 kg N ha⁻¹ to plots in one dose with irrigation water after the cowpea plants had produced four leaves.

5.2.2.4 Treatments

Twelve treatments consisting of four P levels as Triple Super Phosphate (TSP 20% P) and three levels of Zn as Zn-chelate were applied. The P levels were 0, 50, 100 and 150 kg ha⁻¹ for maize plots and 0, 40, 80, 120 kg ha⁻¹ for tomato and cowpea plots applied to the soil by broadcasting in April 2016. Zinc levels were 0, 800, and 1200 g ha⁻¹ for maize plots and 0, 400, and 800 g ha⁻¹ for tomato and cowpea plots applied as a foliar spray four times during the growing season: four, six, eight and ten weeks after seed germination.

5.2.3 Managing the trials

5.2.3.1 Harvesting maize, cowpea and tomato plants

At harvest, three plants per treatment were harvested from the central row of each plot. Maize plant harvested at maturity stage but cowpea and tomato plants harvested after two weeks of last Zn foliar application (or after 12 weeks of germination) in single harvest. Tomato fruits, maize and cowpea seeds were collected by hand. The shoots of three plants in the central row were cut at the soil surface with a stainless steel knife and the roots excavated with a stainless steel trowel. All samples were sealed in a polyethene bags to transport to the soil laboratory of the Agriculture Technical college of Halabja. At the laboratory, the tomato fruit samples and roots were washed with distilled water and blotted dry with tissue paper and the fresh weight recorded. The tomato fruit samples were sliced with a stainless steel knife. The seeds of maize separated by hand and the cowpea seeds separated from their shells by hand. All tomato fruit and the tomato, maize and cowpea shoots and roots were oven dried in Al

trays at 45 °C in a forced air oven (model LDO, 060E) to a constant weight. All plant samples then finely ground in a grinder with a stainless steel chamber and blades (model IKA A11 basic, Minho Analytic) and sealed in plastic bags.

5.2.3.2 Soil sampling and preparation

After harvesting plants, soil samples were collected in the central area of each plot using a stainless steel trowel and then sealed in plastic bags for transport to the laboratory at Halabja Technical College of Applied Science. Approximately 1 kg of each soil was dried at room temperature in an aluminium tray, disaggregated, then sieved to obtain the <2 mm fraction. Approximately 75 g of each sample was then stored in a plastic bag, double labelled, and transported to the University of Nottingham, UK. A portion of each sample was finely ground using an agate ball mill (Retsch, Model PM400), and then stored in polyethene bags.

5.2.4 Soil analysis

5.2.4.1 Soil pH value

Soil pH was determined as described in Section 2.1.1 in Chapter 2.

5.2.4.2 Loss of Ignition (LOI %)

LOI % was determined as described in Section 2.1.2 in Chapter 2.

5.2.4.3 Soil digestion

Soil samples were digested as described in Section 2.1.3 in Chapter 2.

5.2.4.4 Determination of available phosphate (Olsen-P)

The Olsen-P determined as described in Section 2.1.4 in Chapter 2.

5.2.4.5 Extractable Zn by DTPA

The DTPA-Zn was determined as described in Section 2.1.5 in Chapter 2.

5.2.4.6 Determine soluble P and Zn

Approximately 2.0 g of air-dried soil (< 2 mm) were transferred into a 50 ml centrifuge tube with 20 ml of 0.0058 M calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), and shaken for 48 h followed by centrifugation (2500 rpm for 15 min), filtration (0.22 μm) and acidification of 5 ml with 0.2 ml of 50% HNO_3 for analysis of P, Zn, Ca, Na, K, Mg, and S. A separate aliquot was retained for pH determination and measurement of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and determination of the amount of phosphate as described by Murphy and Riley (1962a) in Section 2.2.6 chapter 2. Concentrations of soluble P in samples were determined by using Equation 5.1.

$$\text{Soluble P} = \frac{(\text{Abs} \times \text{Slope})}{V_{\text{Ext.}}} \dots\dots\dots \text{Eq. 5.1}$$

In Eq. 3.1 Soluble P is the concentration of phosphate in the soil solution ($\mu\text{g mL}^{-1}$), Abs is the absorbance measured with a spectrophotometer at 880 nm, Slope is the slope of standard P solution, and V_{Ext} is the volume of the supernatant used in the colour development.

5.2.4.7 Phosphate solubility

The solubility of phosphate determined as described in Section 2.1.7 in Chapter 2.

Zinc solubility

The solubility of Zn determined as described in Section 2.1.8 in Chapter 2.

5.2.4.8 WHAM speciation

WHAM speciation was determined as described in Section 2.1.10 in Chapter 2.

5.2.5 Plant analysis

5.2.5.1 Plant digestion

Plant samples were digested as described in Section 2.2.1 in Chapter 2.

5.2.5.2 Elemental analysis

Elemental analysis was undertaken as described in Section 2.2.2 in Chapter 2.

5.2.5.3 Phytic acid determination

The phytic acid concentration in seeds and fruit was determined as described in Section 2.2.3 in Chapter 2.

5.2.5.4 Phytic acid to zinc molar ratio (PA/Zn molar ratio)

Phytic acid to Zn molar ratio determined as described in Section 2.2.4 in Chapter 2.

5.2.5.5 Human Zn bioavailability/bioaccessibility in seeds and fruit

Human Zn bioavailability in seeds and fruit were determined as described in Section 2.2.5 in Chapter 2

5.2.5.6 Determination of solid=liquid partition coefficient for phosphorus as ($\log K_d P$)

Solid=liquid partition coefficient of P determined by dividing P_{Olsen} (mg kg^{-1}) on P_{Sol} (mg L^{-1}).

5.2.6 Determination of Zn contamination

To determine the percentage of Zn contamination which comes from soil, dust and chemical fertilizer. Equation 5.2 was used

$$\% \text{ Zn from soil dust} = \left(\frac{Zn_{\text{Soil}}}{V_{\text{Soil}}} \right) \times \left(\frac{V_{\text{Plant}}}{Zn_{\text{Plant}}} \right) \times 100 \quad \text{Eq. 5.2}$$

Where

% Zn is the percentage of Zn contamination in plant parts arising from soil dust, Zn_{Soil} and V_{Soil} are the concentration of Zn and vanadium (V) in soil and in Zn_{plant} and V_{plant} are concentrations of Zn and V in plants part. The same equation is used to determine the proportion of each element contamination from the soil dust.

5.2.7 Statistical Analysis

Analysis of variance (ANOVA) was based on a two-factorial randomised complete block design and treatments were analysed by a Least Significant Difference (LSD) test as $P \leq 0.05$. The various statistical computation was run using SPSS v. 24 for windows.

5.3 Results and discussion

5.3.1 Meteorological data and soil properties of the site

Table 5.1 presents meteorological data for the growing season (March-August 2016); data were obtained from the Agriculture Department of Halabja city. The data indicate that the area is semi-arid, has low humidity and precipitation and high temperatures. The college site is used by the Agricultural Technical College to produce orchard fruit and arable crops and for student training.

Table 5.1: Meteorological data for growing season (March-August, 2016)*.

Parameters		March	April	May	June	July	August	September
Temperature (°C)	Max	23.4	32.7	40.0	44.8	47.8	47.0	44.2
	Min	3.20	2.00	2.00	18.7	24.2	25.4	15.2
	Ave.	13.6	18.3	24.4	31.6	35.7	36.1	29.5
Humidity %	Max	84.0	78.0	71.0	29.0	26.0	25.0	33.0
	Min	28.5	19.0	17.0	14.0	15.0	14.0	17.0
	Ave.	45.9	41.2	31.4	21.7	20.3	19.5	22.7
Precipitation (mm)		125	72.7	8.40	0.00	0.00	0.00	0.00
Evaporation (mm)	Max	4.60	6.30	12.5	14.5	15.2	14.2	12.4
	Min	0.40	1.30	1.80	9.30	110	10.1	6.30
	Ave.	2.88	4.33	6.98	11.6	12.8	11.9	9.20
Soil temperature (°C) (30 cm)	Max	14.5	20.8	25.6	31.6	33.1	33.1	32.9
	Min	12.5	13.8	19.3	22.2	31.2	32.1	27.8
	Ave.	13.6	17.1	22.0	28.4	32.3	32.6	30.7

*Data were provided by the Halabja agriculture department.

The soil chemical properties of the experimental site are given in Table 5.2. The measured P_{Olsen} and Zn_{DTPA} at the sites were below critical values for optimum plant growth (<10 mg P_{Olsen} and <1.0 mg Zn_{DTPA}) (Imran et al., 2016, Ahmad et al., 2012). However, Zn extracted by DTPA was in the range for Iraqi soils. Alloway (2009) reported that values of Zn_{DTPA} (mg kg^{-1}) in Iraqi soils lie between 0.1 and 8.70 mg kg^{-1} with an average of 0.30 mg kg^{-1} . The soil at the experimental site represents the typical combination of high Ca concentration and pH prevalent in the region due to the calcareous nature of the soils.

Table 5.2: Average soil chemical properties of the experimental plots.

Soil properties	Maize plots	Cowpea plots	Tomato plots
pH	8.09	8.14	8.25
LOI %	9.88	9.84	10.0
Total Ca (g kg ⁻¹)	27.1	31.8	28.8
P _T (mg kg ⁻¹)	693	723	760
Zn _T (mg kg ⁻¹)	98.7	102	104
P _{Olsen} (mg kg ⁻¹)	5.48	4.18	5.77
Zn _{DTPA} (mg kg ⁻¹)	0.15	0.27	0.40

5.3.2 Soil pH at harvest

The results in Table 5.3 show the effect of applied P fertilizer rates on soil pH at harvest time. Before planting, the average pH values were 8.09, 8.14 and 8.25 for maize, cowpea and tomato soils respectively and the application of P appeared to cause a significant decrease in soil pH ($p < 0.05$), especially in the case of maize plots ($< \text{pH } 8.0$). This result is agreement with Fayaz et al. (2014) who found a significant negative relationship ($r^2 = 0.83$) between single super-phosphate rate and pH in calcareous soil. However, the decline in pH may also have been associated with the greater application of urea to maize and subsequent nitrification of ammonium. Maize received 120 kg ha⁻¹ of urea-N compared to 20 and 100 kg ha⁻¹ for the cowpea and tomato plots respectively. Li et al. (2006) and Lambert et al. (2007) also reported that the application of N fertilizer causes a decrease in pH value.

Table 5.3: The average pH values for maize, cowpea and tomato plots at harvest.

P added	Zn added	Maize plots	P added	Zn added	Cowpea plots	Tomato plots
(kg ha ⁻¹)		Average	(kg ha ⁻¹)		Average	Average
0	0	7.9	0	0	8.18	8.17
0	0.8	7.94	0	0.4	8.11	8.14
0	1.2	7.91	0	0.8	8.12	8.18
50	0	7.97	40	0	8.09	8.14
50	0.8	7.94	40	0.4	8.09	8.11
50	1.2	7.93	40	0.8	8.05	8.11
100	0	7.92	80	0	8.13	8.09
100	0.8	7.93	80	0.4	8.11	8.1
100	1.2	7.91	80	0.8	8.13	8.11
150	0	7.94	120	0	8.17	8.08
150	0.8	7.94	120	0.4	8.15	8.09
150	1.2	7.93	120	0.8	8.28	8.1

5.3.3 Available P (P_{Olsen} and P_{sol}) and solid-liquid partition coefficient

The phosphate fertilizer application rates (as TSP) increased P_{Olsen} and P_{sol} significantly ($p < 0.05$), although there was variation between replicates (Fig. 5.1). Garcia et al. (1997) and Al-Rohily et al. (2013) studied the effect of applied phosphate fertilizers, as TSP and KH_2PO_4 , on available P in calcareous soils; they found that available P increased with the rate of applied P fertilizers. Application of inorganic P fertilizer can increase the amount of P_{Olsen} in the soil immediately following application but available P may decrease with contact time due to fixation of P by soil components (Audette et al., 2016).

From the slopes of P_{Olsen} at harvest against P application (Fig. 5.1) it was estimated that about 83, 78 and 83% of applied P fertilizer was *fixed* by soil components in plots growing maize, cowpea and tomato respectively across all applied P levels. Abebe et al.

(2007) and Al-Rohily et al. (2013) studied the effect of incubation time of P availability in calcareous soils and found that about 50% of applied P as KH_2PO_4 was fixed by soil after just one week of incubation. The strong linearity of the trends in Fig. 5.1, and the solid-liquid partition coefficients ($\log K_dP$) in Table 5.4 suggest that the plot soils all had the same ability to adsorb P, a consequence of their common soil properties (Table 5.2). The results for P_{Olsen} and $\log K_dP$ suggest that the rate of fixation in the soil was independent of the P application rate. Thus, only soil properties (and time) appear to determine the fate of applied P fertilizer. It is reasonable to expect that fixation of P increases with a contact time between the applied P and soil; Mihoub and Boukhalfa-Deraoui (2014) showed that P availability decreased as incubation time increased for P fertilizer added to a calcareous sandy soil in Algeria. The high proportion of P fixation in plots probably reflects the calcareous nature of the soil (high Ca concentration and pH). Deraoui et al. (2015) studied the effect of incubation time and soil properties on P availability. They found that high concentrations of Ca and pH decreased available P. Applied P is thought to react preferentially with Ca^{2+} ions on the adsorption complexes and in the soil solution and then with the Ca^{2+} present on the surface of calcite (Akinremi and Cho, 1991). This series of reactions governs Ca-P minerals formation in calcareous soils (Tunesi et al., 1999). Aslam et al. (2000) studied P behaviour in various soil samples categorised by their Ca concentrations and showed that P fixation in the soil was associated with the level of free CaCO_3 .

Figure 5.2 shows that P_{Sol} was significantly correlated with P_{Olsen} in maize, cowpea and tomato plots: $r=0.92$, 0.71 and 0.67 , respectively ($p < 0.001$). Fayaz et al. (2014) also reported a high correlation between P_{Olsen} and P_{Sol} in calcareous soil.

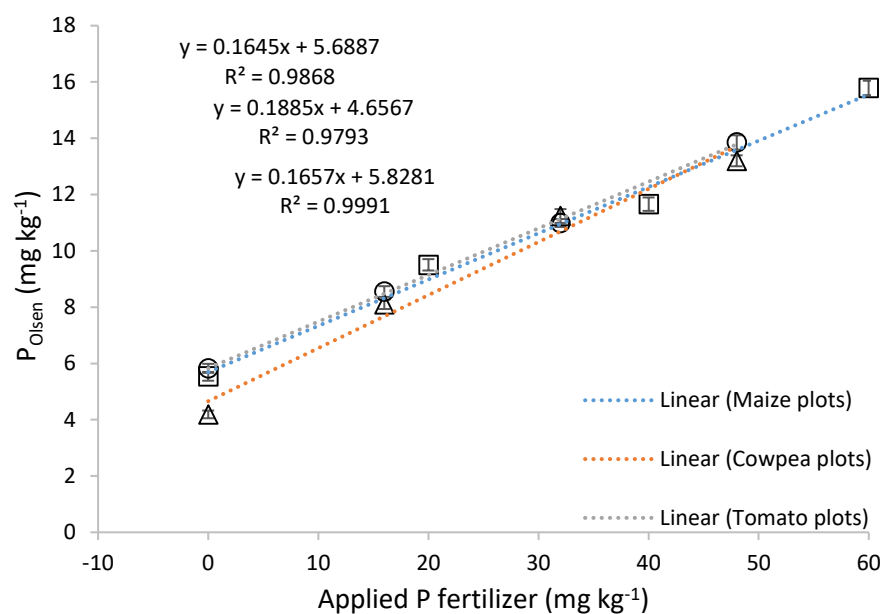


Fig. 5.1: The average values of P_{Olsen} for maize, cowpea and tomato plots at harvest as a function of fertilizer P application. For direct comparison, applied fertilizer values (kg ha⁻¹) were converted to units of mg kg⁻¹ assuming 2500 t ha⁻¹ of topsoil. (□) maize plots, (Δ) cowpea plots and (O) tomato plots.

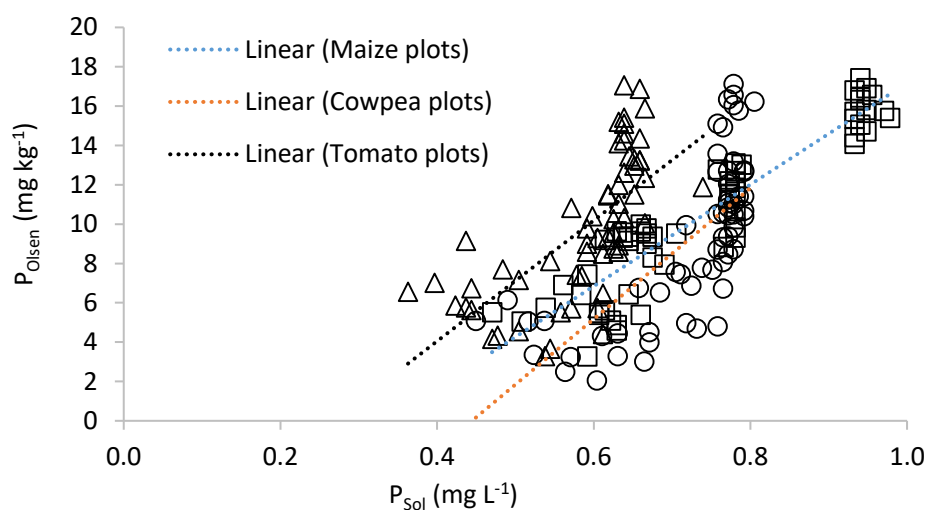


Fig. 5.2: Correlations between P_{Sol} and P_{Olsen} in (□) maize, (o) cowpea and (Δ) tomato plots.

Table 5.4: Effect of applied phosphate fertilizer on the solid-liquid partition coefficient of applied P (Log $K_d P$) for maize, cowpea and tomato plots. Values of log $K_d P$ were calculated as $\log_{10}(P_{\text{Olsen}}/P_{\text{Sol}})$.

P added (mg kg ⁻¹)	Maize Log $K_d P$	P added (mg kg ⁻¹)	Cowpea Log $K_d P$	Tomato Log $K_d P$
0	0.97	0	0.84	1.08
20	1.17	16	1.13	1.23
40	1.18	32	1.16	1.22
60	1.21	48	1.22	1.32

5.3.4 Soil exchangeable Zn (Zn_{DTPA})

Table 5.5 shows the average values of Zn_{DTPA} in maize, cowpea and tomato plots at harvest. The results indicated that the foliar application of Zn fertilizer increased Zn_{DTPA} in the soil of maize, cowpea and tomato plots. A significant increase ($p < 0.05$) was observed in maize plots, but not in cowpea and tomato plots when overall Zn rates were compared with control values. The Zn_{DTPA} values increased by 9%, 1% and 5% for maize, cowpea and tomato plots respectively. This suggests that some of the foliar-applied Zn fertilizer was transferred to the soil surface during application. Thomas and Omuetti (2012) reported that the application of Zn to soil causes an increase in Zn solubility. Again compared to control values, the application of P fertilizer also appeared to cause an increase in Zn_{DTPA} , especially at low P levels between 0-50 kg P ha⁻¹ for maize plots and 0-40 kg P ha⁻¹ for cowpea and tomato plots. This may have been related to the acidification of plots soil with increasing TSP application rate; the plot pH values decreased from 8.09, 8.14 and 8.25 to 7.93, 8.13 and 8.10 for maize, cowpea and tomato plots respectively. Wang et al. (2008) studied the application of phosphate fertiliser (single superphosphate, SSP) on availability and uptake of Zn by cabbage plant in contaminated soil. They also found that the application of SSP slightly increased Zn availability due to a decrease in soil pH from 5.23 to 4.50-4.80. However, the increase in the Zn_{DTPA} may be also related to N fertilizer application which, as discussed previously, caused a decrease in soil pH values. Hafeez et al. (2013) reported a positive correlation between Zn_{DTPA} and N fertilizer in soils.

Table 5.5: The average concentrations of Zn_{DTPA} in maize, cowpea and tomato plots.

P added	Zn added	Maize plots	P added	Zn added	Cowpea plots	Tomato plots
(kg ha ⁻¹)					(mg kg ⁻¹)	
0	0	0.15 ^b	0	0	0.33 ^a	0.25 ^a
0	0.8	0.34	0	0.4	0.32	0.26
0	1.2	0.28	0	0.8	0.35	0.26
50	0	0.19 ^a	40	0	0.33 ^a	0.26 ^a
50	0.8	0.36	40	0.4	0.35	0.30
50	1.2	0.31	40	0.8	0.36	0.32
100	0	0.30 ^a	80	0	0.30 ^a	0.30 ^a
100	0.8	0.27	80	0.4	0.43	0.29
100	1.2	0.31	80	0.8	0.42	0.32
150	0	0.27 ^a	120	0	0.33 ^a	0.28 ^a
150	0.8	0.30	120	0.4	0.36	0.33
150	1.2	0.32	120	0.8	0.29	0.30

Different letter indicate significant difference at (P≤0.05)

5.3.5 Determining controls over phosphate and zinc solubility in soils

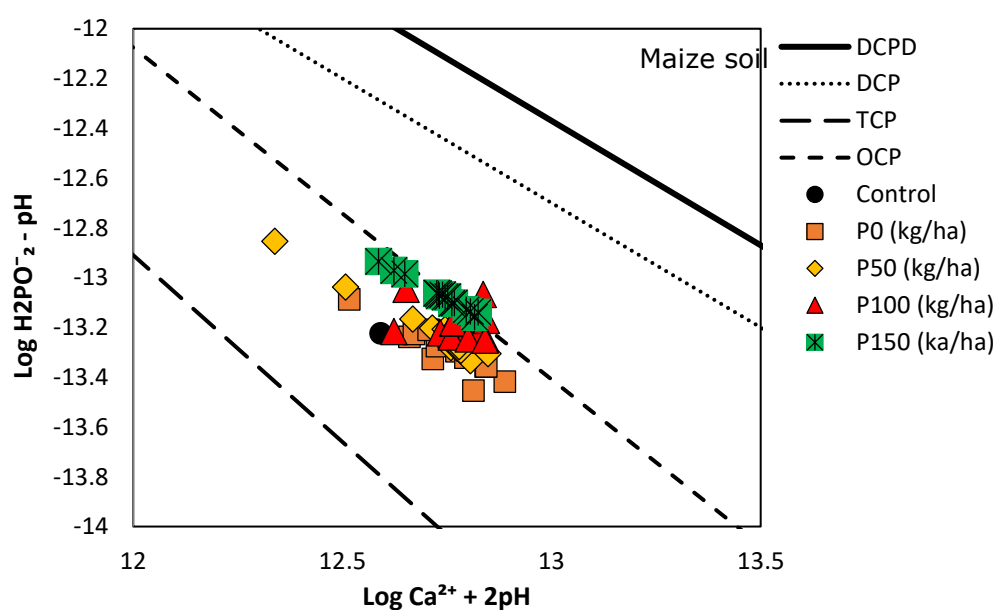
5.3.5.1 The solubility of calcium phosphate minerals

Possible solubility control by calcium phosphate minerals was investigated by plotting 'phosphate potential' ($\log \text{H}_2\text{PO}_4^- - \text{pH}$) against lime potential ($\log \text{Ca}^{2+} + 2\text{pH}$). The activity of Ca^{2+} , H_2PO_4^- and pH used to develop this diagram, were determined using measured values of pH and Ca and P concentration in solution with the determination of free ion activities using the WHAM (VII) geochemical speciation program. This approach can indicate the likelihood of specific phosphate minerals being present in soils with different pH values and different histories of P application (Zhang et al., 2014).

Figures (5.3a, b and c) present the soil solution composition (phosphate and lime potential) in relation to common Ca phosphate minerals for soils from the maize, cowpea and tomato plots. At harvest, the Ca and phosphate data fell between being slightly under-saturated with respect to Octa calcium phosphate (OCP) and super-

saturated with respect to β -Tricalcium phosphate (β -TCP) across all the P application rates. McDowella and Sharpley (2003) found that in a calcareous soil with a high Ca concentration and pH value, phosphate solubility was controlled by β -TCP. Zhang et al. (2014) studied the effect of different P application rates on phosphate mineral solubility and found that in native and heavily fertilized calcareous soils the solubility of P appeared to be controlled by a series of calcium phosphate minerals ranging from HA to β -TCP.

The results in (Fig. 5.3a, b and c) also suggest that the solubility of phosphate minerals in cowpea plots may be slightly higher than in the maize and tomato plots. This may have been due to a lower pH value in the cowpea plots and due to nitrification in the legume rhizosphere following N fixation. The application of higher rates of P fertilizer also appeared to cause a slight increase in the solubility of phosphate and at the highest rates of P application (120 or 150 kg ha⁻¹) the soil data fell very close to the OCP solubility line for all crops.



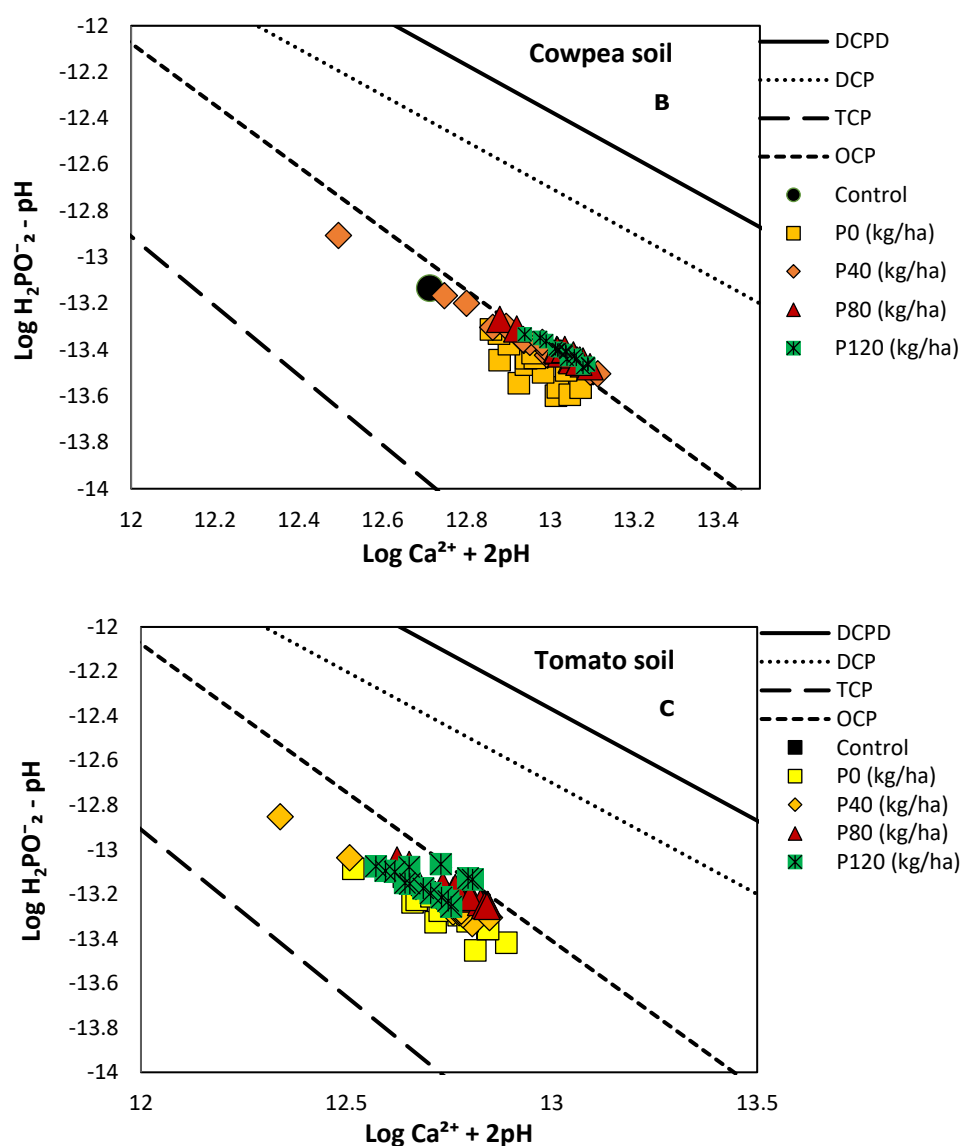


Fig. 5.3: Solubility diagram comparing soil phosphate with a range of crystalline Ca-P minerals: monocalcium phosphate, MCP; dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium phosphate, OCP; and β -tricalcium phosphate, β -TCP. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by Lindsay (1979). The soil was from plots used to grow (A) maize, (B) cowpea and (C) tomato; soil sampling was at harvest.

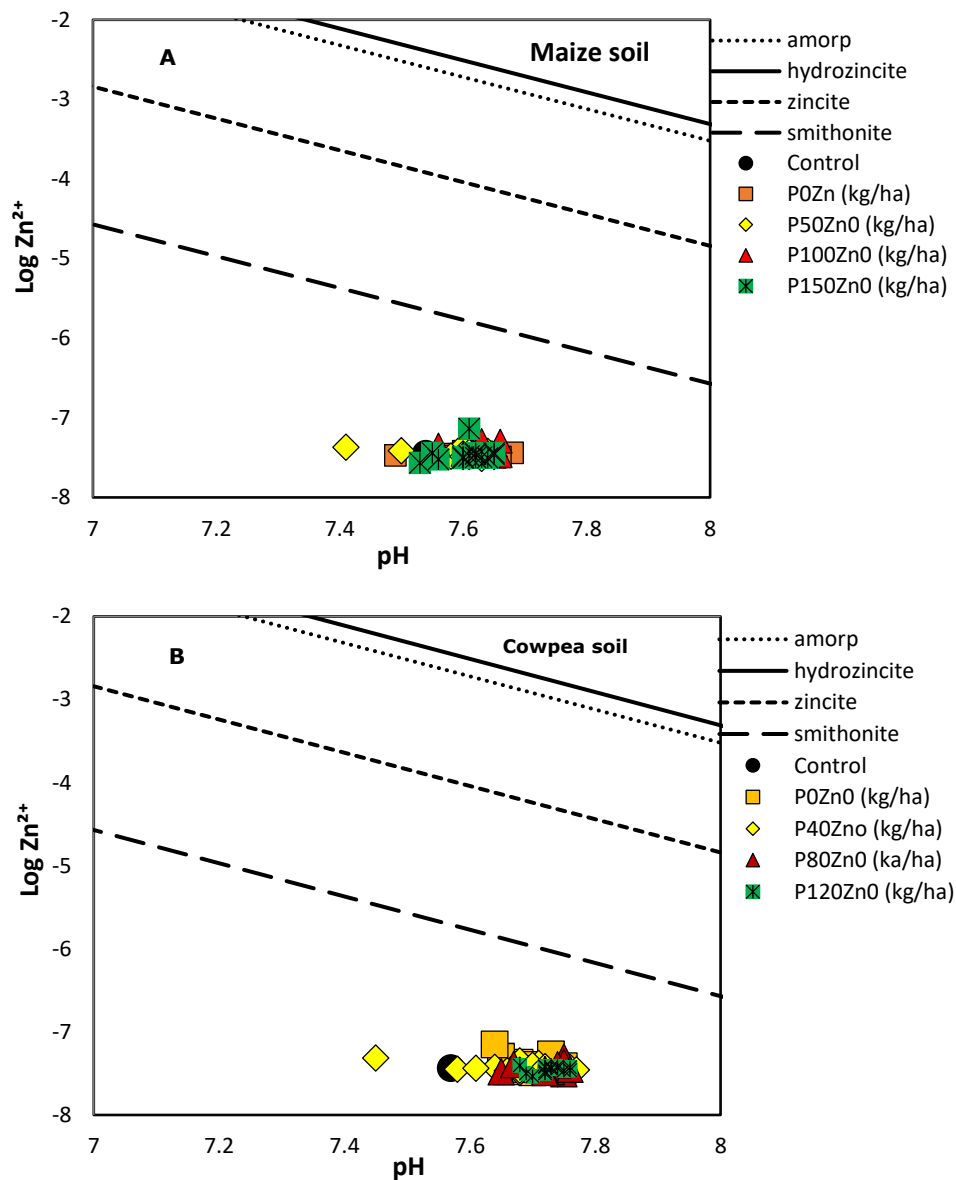
5.3.5.2 Zn solubility

A solubility diagram for Zn minerals was developed by plotting the free ion activity of Zn ($\log \text{Zn}^{2+}$) against soil pH. The activity of Zn^{2+} , pH and partial pressure (CO_2) used to develop this diagram, were determined using measured values of pH and Zn concentration in solution with chemical speciation resolved using WHAM-7. This approach can indicate the likelihood of Zn minerals being present in soils at different pH values (Lindsay, 1979, Nazif et al., 2015, Urgun and Rimmer, 2000).

Figures (5.4a, b and c) present the soil solution (Zn^{2+}) in soil suspensions from the maize, cowpea and tomato plots in comparison with common Zn minerals. The Zn data clearly indicate undersaturation with respect to Smithsonite (ZnCO_3) across all maize, cowpea and tomato plots. Sachdev et al. (1992) studied the measurement of Zn^{2+} activity in four soil samples, ranging in pH from 5.6 to 8.0, using soil suspension in EDTA solution. They reported that, in alkaline soils, the Zn was supersaturated with respect to the hypothetical 'Soil-Zn' compound as defined by Lindsay (1979), not shown in Fig. 5.4.

The results in (Fig. 5.4a, b and c) also suggest that the application of P fertilizer appeared to cause an increase in Zn solubility, notwithstanding the expected interaction between phosphate and Zn. However, the system remained undersaturated with respect to Smithsonite across all applied P rates and for maize, cowpea and tomato plots. Fayaz et al. (2014) undertook a laboratory incubation (28 days) to study the effect of P fertilizer (single super phosphate) on micronutrient availability in alkaline-calcareous soils from Pakistan. They showed that the application of single super phosphate decreased the pH of soil and thereby had a significant effect on the solubility of micronutrient metals.

Figure 5.4 suggests that Zn solubility in these soils is not controlled by the solubility product (K_{sp}) of a pure single compound; a similar result reported by Martinez and Motto (2000). In reality, the pattern observed is probably the net result of adsorption of Zn^{2+} on humus, Fe oxides and $CaCO_3$ as suggested by Nazif et al. (2015) and the assumption of solubility control by a pure compound is inappropriate.



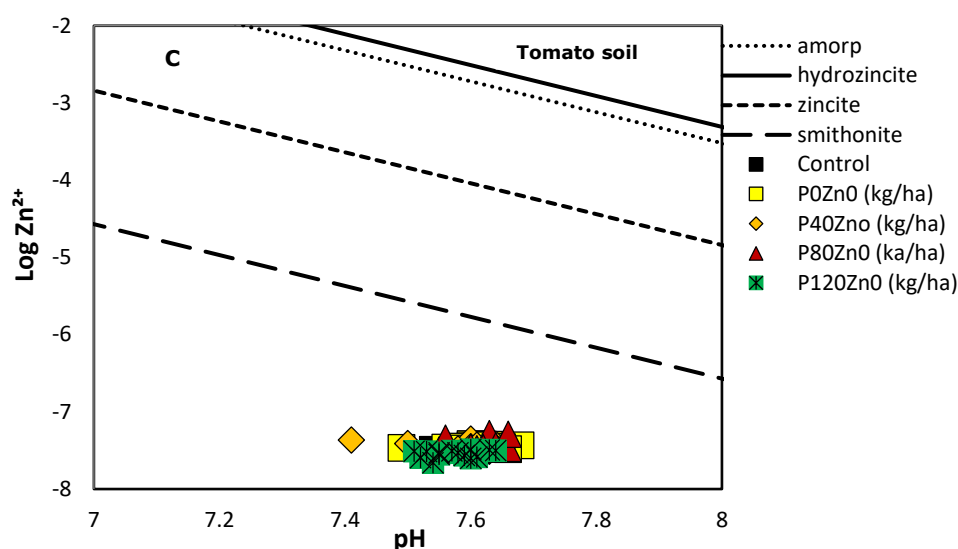


Fig. 5.4: Solubility diagram comparing test soils with a range of Zn minerals: amorphous, hydrozincite ($\text{Zn}_5(\text{CO}_3)_2(\text{OH})_5$), zincite (ZnO), and smithsonite (ZnCO_3). Equilibrium lines for mineral phases were calculated based on K_{sp} values reported by Nazif et al., (2015), Lindsay, (1979) and Urgun and Rimmer, (2000). For (A) maize, (B) cowpea and (C) tomato plots at harvesting.

5.3.6 Phosphorus fractionation in soil

Figure 5.5 shows the proportion of each P fraction in maize, cowpea and tomato plots (see Section 2.2.9); Table 5.5 shows the average concentrations (mg kg^{-1}) of each fraction. At harvest, the applied P fertilizer did not consistently increase the P concentration in individual fractions in any of the plots, at least partly due to the variability of measurements for individual fractions. Figure 5.5 suggests that there was a rapid assimilation of applied TSP phosphate into pre-existing soil fractions, in approximately the same ratio as the control soil. The comparative distribution of all P fractions in the control soils for maize, cowpea and tomato followed the order: $\text{Ca}_{10}\text{-P}$, organic-P, occluded-P, $\text{Ca}_2\text{-P}$, re-adsorption, Al-P and $\text{Ca}_8\text{-P}$. After application of P fertilizer rates the order was $\text{Ca}_{10}\text{-P} > \text{organic-P} > \text{Occluded-P} > \text{Ca}_2\text{-P} > \text{reabsorbed-P} > \text{Ca}_8\text{-P} > \text{Al-P}$ for maize plots and $\text{Ca}_{10}\text{-P} > \text{organic-P} > \text{Occluded-P} > \text{Ca}_2\text{-P} > \text{reabsorbed-P} > \text{Al-P} > \text{Ca}_8\text{-P}$ for cowpea plots and tomato plots. Only $\text{Ca}_2\text{-P}$ and the organic-P fractions in maize plots, $\text{Ca}_2\text{-P}$, re-adsorbed-P and Al-P fractions in cowpea plots and Ca_2 , $\text{Ca}_8\text{-P}$, re-adsorbed-P and Al-P fractions in tomato plots were increased compared with the

control. Yang et al. (2012) studied inorganic P fractionation and translocation in low P soils and found that application of ^{32}P - P_i increased $\text{Ca}_2\text{-P}$ while the transformation of P into $\text{Ca}_8\text{-P}$, $\text{Ca}_{10}\text{-P}$, re-adsorbed-P Al-P and Fe-P fractions decreased significantly when compared with soil to which no P fertilizer was applied. They suggested there was a transformation of water-soluble P to more slowly available and unavailable P fractions in deficient soils – more so than in 'sufficient' soils. The results of P fractionation indicated a negligible Fe-P fraction; values were below the detection limits of 0.05 mg kg^{-1} . Adhami et al. (2006) studied the inorganic P fraction in calcareous soils and reported that Fe-P only accounted for 5% of the sum of P fractions but was strongly correlated with the form of Fe oxide present in the calcareous soils of Iran.

Abolfazli et al. (2012) and Jun et al. (2010) reported that Ca-P form was the dominant form of P in calcareous soils and accounted for about 69-71% of the total inorganic P, including DCP ($\text{Ca}_2\text{-P}$), OCP ($\text{Ca}_8\text{-P}$) and HA ($\text{Ca}_{10}\text{-P}$). The results in Fig. 5.5 support the above conclusion. In the current study, the Ca-P content accounted for the greatest proportion of total P (65-78%) for control plots and (67-72%) for fertilized plots, suggesting a non-significant decrease in the P fractional distribution. The proportions of plant-unavailable P fractions (Occluded-P and $\text{Ca}_{10}\text{-P}$) were approximately 84, 76 and 71% in control soils and 76, 72 and 71% in fertilized maize, cowpea and tomato plots respectively. These results suggest that the proportion of 'plant-unavailable' P decreased slightly after application of P fertilizer especially in the case of maize and cowpea plots.

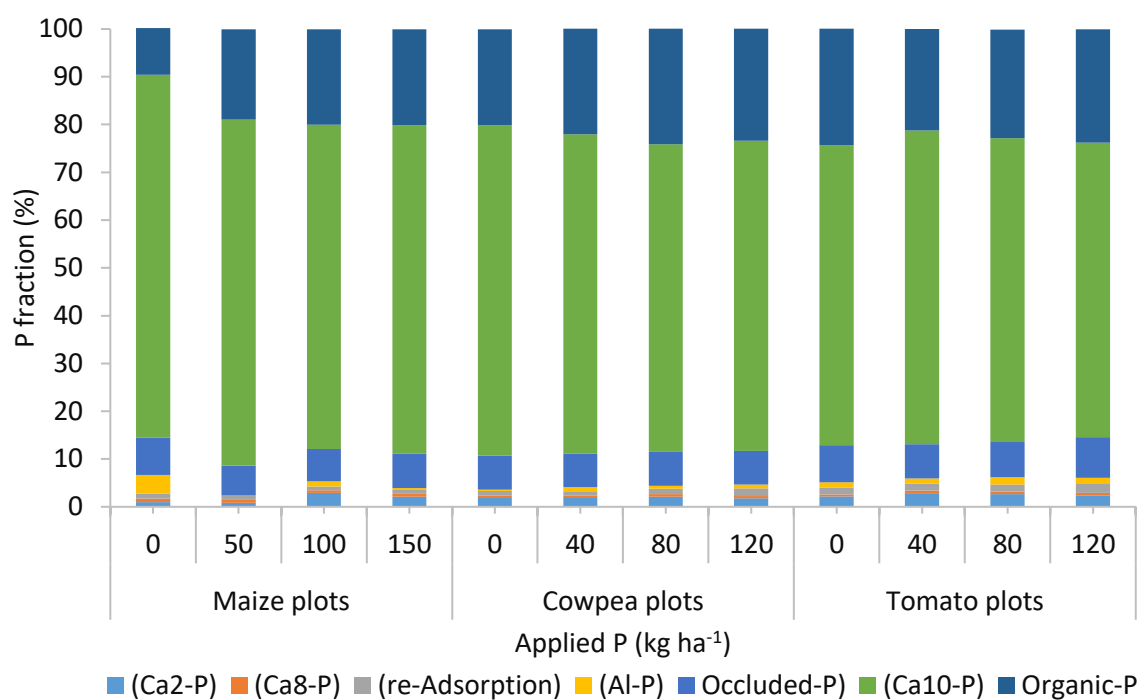


Fig. 5.5: Fractionation (%) of P in soils: $\text{Ca}_2\text{-P}$ = Dicalcium phosphate; $\text{Ca}_8\text{-P}$ = P associated with OCP; Re-adsorbed-P; Al-P = associated Al oxides; Occluded P; $\text{Ca}_{10}\text{-P}$ = HP; Organic-P.

Table 5.6: Phosphorus fractions in maize, cowpea and tomato plots.

Plants	P added (kg ha^{-1})	($\text{Ca}_2\text{-P}$)	($\text{Ca}_8\text{-P}$)	(re- Adsorption)	(Al-P)	(Fe-P)	Occluded- P)	($\text{Ca}_{10}\text{-P}$)	Organic-P	Total P
		(mg kg^{-1})								
Maize	0	2.32	1.68	2.59	9.26	0	18.5	180	23.1	237
	50	2.19	1.97	2.01	0.00	0	16.3	189	49.4	261
	100	8.35	1.55	2.32	2.99	0	19.4	194	57.1	286
	150	6.10	1.66	2.67	0.70	0	20.4	196	57.2	285
Cow pea	0	5.26	1.55	2.54	0.71	0	20.1	195	56.6	282
	40	5.44	1.69	2.57	2.40	0	20.8	197	65.3	295
	80	6.78	1.74	3.7	1.79	0	22.7	206	77.4	320
	120	5.43	1.53	5.03	2.29	0	21.8	200	72.1	308
Tomato	0	6.81	1.33	4.35	3.59	0	24.5	197	76.6	314
	40	8.67	1.67	4.37	3.22	0	21.8	199	64.2	303
	80	8.33	1.73	4.78	4.71	0	23.3	201	71.8	316
	120	7.71	2.16	6.61	3.88	0	28.7	207	79.8	336

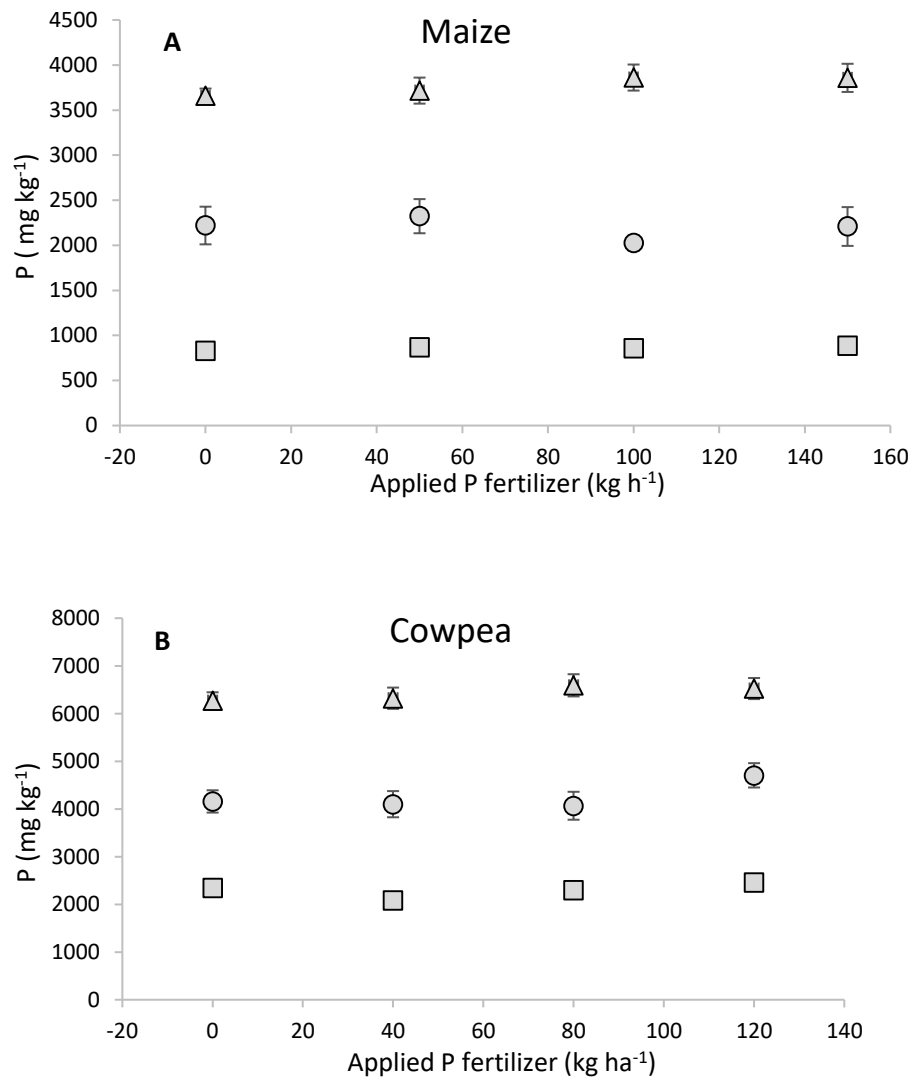
5.3.7 The concentration of P and Zn in crops

5.3.7.1 *The concentration of P in roots, shoots, grain, seeds and fruit*

The results in (Fig. 5.6 a, b and c) show that the P application rates to soil increased P concentration in all crop parts significantly ($p < 0.05$), except for maize shoots. As P application rate increased from 0 to 150 kg ha⁻¹, the percentage of P increased by 6, 4 and 5% in maize roots, shoots and grain respectively. Similarly, as P fertilizer application rate increased from 0 to 120 kg ha⁻¹, the percentage of P increased by 5, 26 and 11% and 5, 15 and 12% in cowpea and tomato roots, shoots, seed and fruit respectively. Apart from the influence of plant controls on the uptake, the comparatively small increase in P concentration in the crop may have been partly due to the fact that more than 80% of applied P was fixed by soils due to their calcareous nature (Fig. 5.5. and preceding discussion). According to Imran et al. (2016) application of 60 kg P ha⁻¹ as DAP combined with 16 kg Zn ha⁻¹ as ZnSO₄·7H₂O, increased P concentration by 5.8% in the maize grain of a Neelam genotype and by 7.8% by sole P application (60 kg ha⁻¹) grown in the calcareous soil of Pakistan. In the current study, the maize response to applied P rates was much lower – possibly due to differences in genotype and P fertilizer type. Again, the low response of maize, cowpea and tomato plants to applied P rates might simply be due to the high rate of applied P transformation to unavailable P forms.

The results in Fig. 5.6 indicate that tomato plants had the highest response to applied P rates and maize plants had the lowest. Holloway et al. (2001) and Zhang et al. (2017) reported that plants differ in their response to P applied depending on moisture, cultivar and growth dilution. It is clear that the field experiment is not under the same level of control as a greenhouse experiment and is more affected by environmental factors. The results of the current study indicated that the optimum P application rate was (100, 80 and 40) kg ha⁻¹ providing the maximum increase in the percentage of P (5, 5 and 15%) for maize grain, cowpea seed and tomato fruit respectively.

The evidence provided from the P fractionation scheme is supported by single estimates of plant-available P (P_{Olsen} and P_{Sol}). At harvest about 17, 22 and 17% of the applied P fertilizer remained available, as estimated from P_{Olsen} . Mohasses (2015) reported that the application of TSP alone to calcareous soils not useful due to the abundance of calcium ions; phosphate ions simply react with active calcium in the soil resulting in relatively insoluble apatite formation. Zhu et al. (2018) reported that a short time after application, about 80% of the applied P is rendered unavailable for plant uptake due to adsorption and precipitation of applied P by Ca^{2+} ions in calcareous soils.



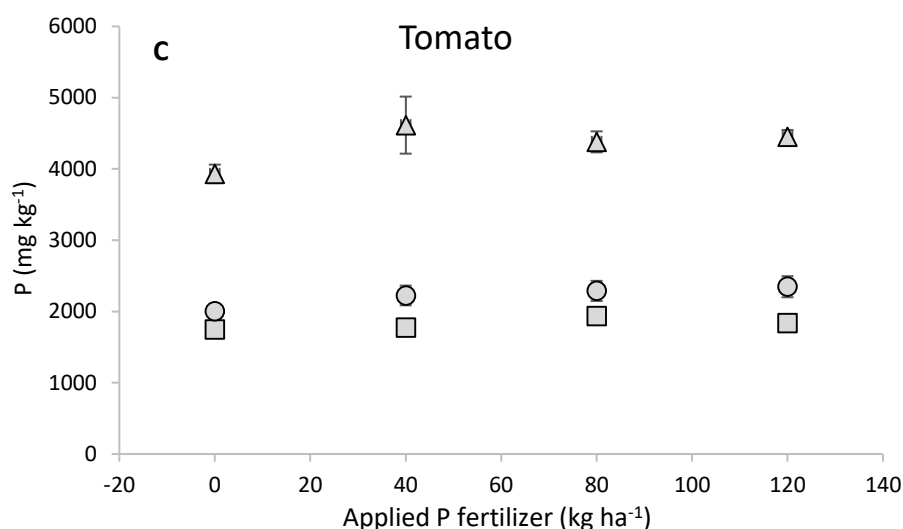


Fig. 5.6: The concentration of phosphorus (mg kg^{-1} dry matter) in (□), root, (○) shoot and (Δ) grain, seed or fruit of (A) maize, (B) cowpea and (C) tomato plants at harvest.

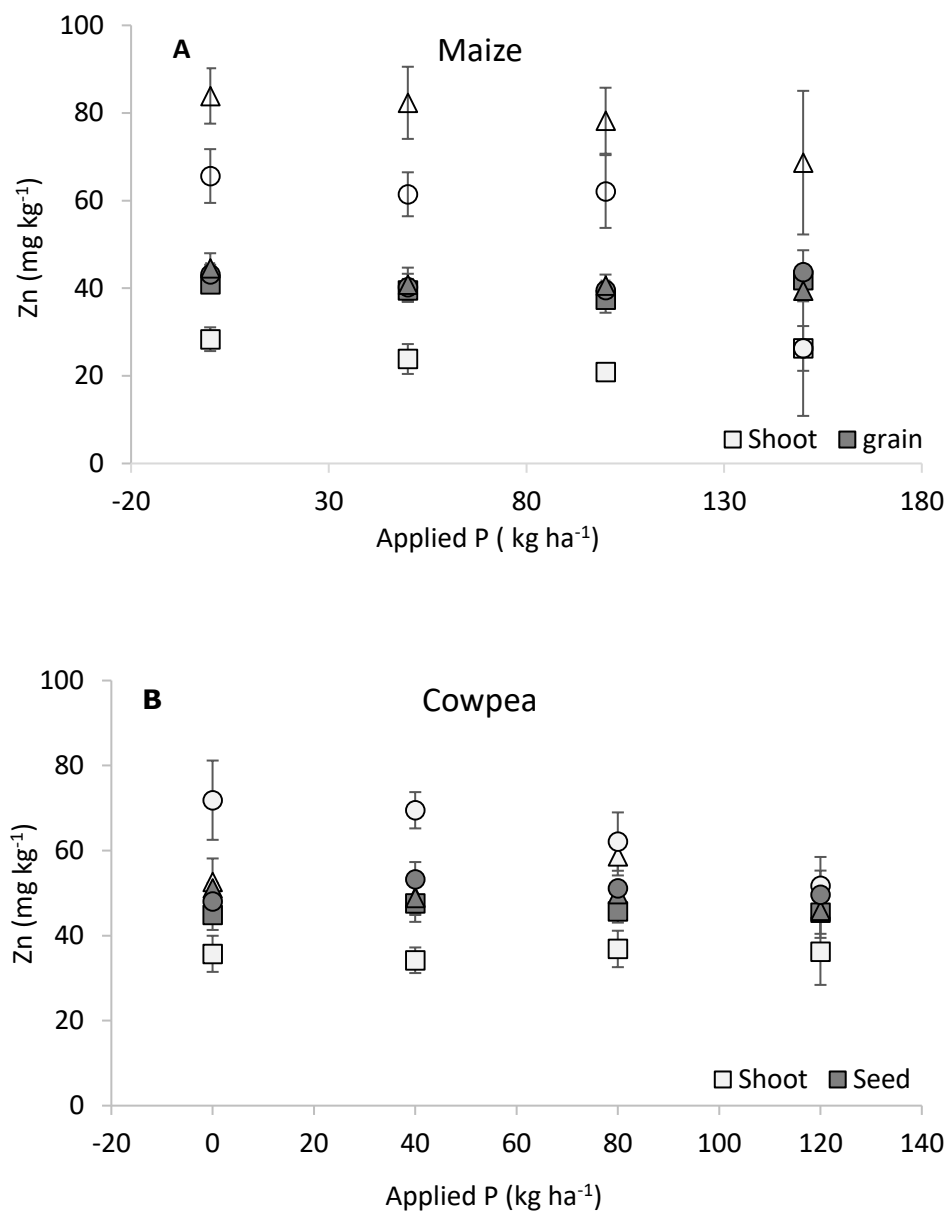
5.3.7.2 The concentration of Zn in plant shoots, grain, seed and fruit

At harvest, the foliar application of Zn fertilizer increased Zn concentration in plant shoots significantly ($p < 0.05$) as shown in Fig. 5.7a, b and c. The proportion of Zn concentration differed between plants and at all Zn rates. The concentration of Zn in increased by 69%, 45% and 54% for maize, cowpea and tomato shoots at 1.2 and 0.8 kg Zn ha^{-1} rates respectively when compared with control plants. However, Zn concentrations increased by only 4% at 0.8 and 1.2 kg Zn ha^{-1} rates in maize grain, by 6 and 9% for cowpea seed and by 3 and 4% for tomato fruit at 0.4 and 0.8 kg Zn ha^{-1} rates. The increase in shoot Zn concentration must have arisen due to the direct foliar application of Zn fertilizer but clearly, there was a considerable inhibition to subsequent transfer to edible crop compartments. In contrast, Wang et al. (2012) studied the foliar application of Zn to maize and wheat plants, they found that foliar application of Zn fertilizer (4 kg Zn ha^{-1} as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) increased Zn concentrations in maize and wheat grain by 27% and 28% respectively. This may be because they used a larger application amount of Zn fertilizer, and without P fertilizer application.

The results in (Fig. 5.7) indicate that Zn concentration in maize grain, cowpea seed and tomato fruit was not improved by the foliar Zn application rates used. This may also have been due to an inhibitory effect from the application of P fertilizer – especially as much of the foliar Zn application ended up in the soil. The concentration of Zn decreased by 8, 4 and 3% in maize grain, cowpea seed and tomato fruit respectively as P application rate was increased from 0 to 150 kg P ha⁻¹ for maize plots and 120 kg ha⁻¹ for cowpea and tomato plots. Yadav et al. (1985) found that Zn concentration in different cowpea parts decreased as the P application rate was increased in a glasshouse pot experiment. Singh et al. (1988) studied the antagonistic reaction mechanism between P and Zn in beans; they found that Zn concentration in plant tops decreased significantly with the application of P fertilizer and they recorded the greatest reduction with 40 kg P ha⁻¹ rate. Reduction in Zn concentrations in wheat grain and maize grain was reported (Zhang et al., 2012a, Imran et al., 2016). Zhang et al. (2017) studied the overuse of P fertilizer on Zn bioavailability in winter wheat and found that Zn concentration decreased by 58% when P application was increased from 0 to 400 kg ha⁻¹. Thus, the minor effect of P on Zn in maize, cowpea and tomato edible parts in the present study may be related to an inhibitory effect of applied P fertilizer within the plants, because Zn fertilizer was applied as a foliar dressing, or it may have arisen from interaction in the soil as the applied Zn was detected in the soil at harvest.

Thus, results indicate that, although the Zn foliar application increased Zn concentration in crop leaves, even the maximum application of 1.2 kg Zn ha⁻¹ for maize and 0.8 kg Zn ha⁻¹ for cowpea and tomato plants did not improve Zn concentration in grain, seed and fruit of crops. Future work should examine the effect of increasing the amount of foliar-applied Zn; it may yet prove to be a satisfactory method for increasing the concentration of Zn in edible crop parts. Because the soils of Kurdistan region are calcareous, there must be only limited soil Zn uptake by crops (see Fig. 5.7 control soils) due to strong Zn absorption on CaCO₃ producing low solubility (see Fig. 5.4) and

this may be aggravated by the relatively high levels of P fertilizer used in the area. Abdoli et al. (2016) reported that in calcareous soils, application of Zn fertilizer to the soil is a non-viable method because of rapid adsorption of Zn.



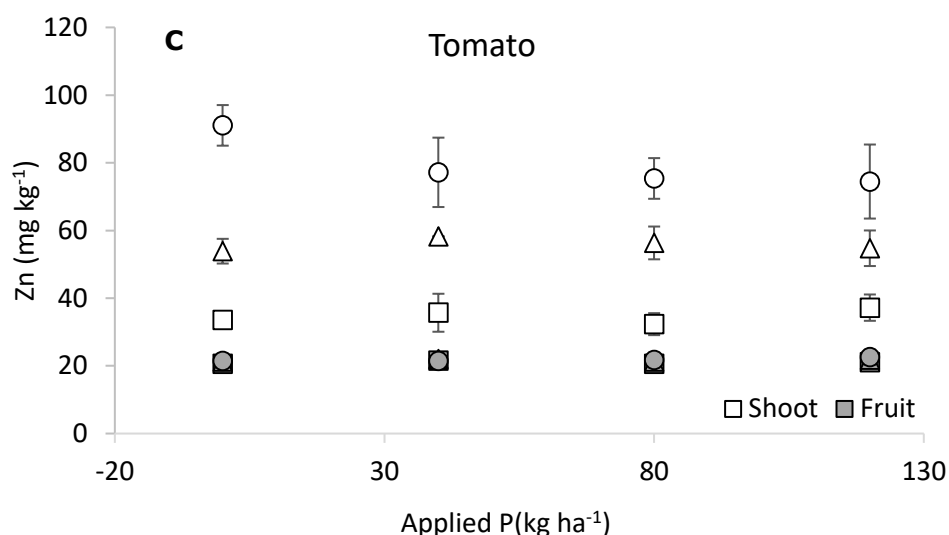


Fig. 5.7: Concentration of Zn (mg kg^{-1} dry matter) in the shoot, grain, seed and fruit of maize, cowpea and tomato plants at harvest and at different P and Zn application rates; (0, 50, 100 and 150) kg P ha^{-1} and (($\square$) 0, (Δ) 0.8 and (O) 1.2) kg Zn ha^{-1} for maize and (0, 40, 80 and 120) kg P ha^{-1} and (($\square$) 0, (Δ) 0.4 and (O) 0.8) kg Zn ha^{-1} for cowpea and tomato.

5.3.8 The interaction between P and Zn in shoots, seeds, and fruit

Table 5.7 shows that the highest P concentration occurred in the treatment P150_Zn1.2 and P120_Zn0.8 in maize and cowpea shoots while for tomato shoots the highest P concentration was in the P120_Zn0 treatment. In maize grain, cowpea seed and tomato fruit the highest P concentration were in treatments P100_Zn0, P80_Zn0.8 and P40_Zn0.4.

Cowpea and tomato shoots had the highest Zn concentration in the P0_Zn0.8 treatment but in maize shoots, this was in the P150_Zn1.2 plots. In maize grain, cowpea seed and tomato fruit had the highest Zn concentration at P0_Zn1.2, P40_Zn80 and P120_Zn0.8 treatments respectively.

In cowpea and tomato shoots, treatments without P application had the highest Zn concentration while P application alone caused a decrease in the Zn concentration. These results are similar to Imran et al. (2016) and Zhang et al. (2017) for maize grain

and wheat grain, respectively, grown in calcareous soils. A decrease in Zn concentration due to the application of P fertilizer may result from increased plants shoot growth, which causes dilution of the plant Zn concentration (Gianquinto et al., 2000). Phosphorus also restricts the translocation of Zn from shoot to the top (Singh et al., 1988) at a high level of P Zn becomes chelated to the cell wall in the plant leaves (Yang et al., 2011). Further study is required to investigate the individual processes that control Zn uptake and translocation in crops and how these are affected by high levels of P application.

Table 5.7: The concentration of P and Zn in (A) maize, (B) cowpea and (C) tomato roots, shoots, seeds and fruit.

A	Maize	Root		Shoot		Seed	
Added	Added	P	Zn	P	Zn	P	Zn
P	Zn						
(kg ha ⁻¹)					(mg kg ⁻¹)		
0	0	881	15.8	2690	28.4	3540	40.9
0	0.8	857	14.1	2510	65.6	3870	43.2
0	1.2	889	14.3	2200	70.8	3810	44.6
50	0	882	18.8	2750	23.8	3830	39.5
50	0.8	847	14.1	2220	61.4	3720	40.2
50	1.2	877	18.1	2000	82.3	3600	40.8
100	0	815	19.4	1920	20.9	4120	37.4
100	0.8	910	18.4	2010	62.1	3440	39.6
100	1.2	846	14.3	2270	83.1	3470	40.6
150	0	905	17.2	1850	26.3	3830	41.9
150	0.8	977	16.5	2070	71.8	3930	43.7
150	1.2	890	16.5	2700	84.6	3410	39.4

B Added P	Cowpea Added Zn	Root P	Zn	Shoot P	Zn	Seed P	Zn
(kg ha ⁻¹)		(mg kg ⁻¹)					
0	0	2520	21.9	4670	35.7	6060	44.9
0	0.4	1930	23.6	3340	52.6	6710	51.1
0	0.8	2580	20.1	4330	71.9	6030	48
40	0	1610	15.9	3580	34.2	5930	47.6
40	0.4	2090	19.3	4590	49.1	6210	48.9
40	0.8	2370	24.1	4130	69.5	6930	53.2
80	0	2350	24.1	4200	36.9	6090	45.7
80	0.4	2340	24.6	4220	58.6	6640	49.8
80	0.8	2240	19.6	3790	62.1	6960	51.1
120	0	2590	23.9	3370	36.2	6710	45.4
120	0.4	2490	26.4	4060	46.3	6120	45.8
120	0.8	2290	21.1	4500	57	6750	49.6

C Added P	Tomato Added Zn	Root P	Zn	Shoot P	Zn	Fruit P	Zn
(kg ha ⁻¹)		(mg/kg)					
0	0	1800	29.9	2210	33.6	4250	20.6
0	0.4	1670	27.8	1870	53.9	4110	21.3
0	0.8	1770	27	1940	91.1	4220	21.5
40	0	1800	37.4	2400	35.7	4750	21.5
40	0.4	1690	28.3	2120	58.2	5260	22.1
40	0.8	1730	31.8	2160	60.9	3830	21.5
80	0	1940	28.4	2060	32.3	4820	20.6
80	0.4	1930	31.4	2580	56.3	4270	21.3
80	0.8	1940	32.7	2230	75.4	4060	21.8
120	0	2010	34.2	2540	37.2	4150	21.1
120	0.4	1480	31.5	2430	54.8	4250	21.8
120	0.8	1580	31.9	2080	83.4	4510	22.6

5.3.9 Proportion (%) of plant element concentration arising from soil dust

The objective of this calculation was to distinguish between systemic uptake of elements by roots from the soil and the fraction that arises from extraneous soil dust. Dust includes small (micrometre and nanometre diameter) solid particles transferred by wind and water (rain and irrigation splash) from the soil to plant leaves and stems (Farmer, 1993). Considering the high ratio of soil-to-plant concentrations of trace metals even a small level of plant contamination with soil may substantially increase the apparent concentration of elements in plants.

To test for possible contamination of plants by soil dust Fig. 5.8 shows a correlation between vanadium (V) and iron (Fe) which may be indicative of soil dust. High positive correlations (r^2) between Fe and V were found for roots (0.98, 0.99 and 0.98) and shoots (0.98, 0.96 and 0.97) of maize, cowpea and tomato respectively (Fig. 5.9a and b). This indicates possible contamination of plant parts by soil dust, which may substantially affect the concentrations of macro and micronutrients reported for the crops. Equation 5.2 (Section 5.2.5, Chapter 2) was used to determine the contamination proportion of each element. Table 5.3.7 presents the percentage of metal concentration arising from soil dust in maize, cowpea and tomato roots, shoots and edible parts.

The crop Zn concentrations (mg kg^{-1}) were between 14.1-19.4, 15.9-26.4 and 27.0-37.4 for plants roots, 20.9-84.6, 34.2-71.9 and 32.3-91.1 for shoots and 37.4-44.6, 45.4-53.2 and 20.6-22.6 mg kg^{-1} for edible parts of maize, cowpea and tomato respectively. The results in Table 5.7 showed that about 23.5, 5.35 and 16.4% of Zn in roots and 1.22, 2.52 and 2.54% of Zn in shoots arises from soil dust. For tomato fruit, maize grain and cowpea seed, the contributions of soil dust to Zn concentration were negligible.

The contributions from soil dust to each element was calculated (Table 5.7). As for Zn, lowest contamination was found in the edible parts of the plants and the greatest was in their roots. The greatest contributions from soil dust were for elements which form major structural components in soils (Al, Mn, Al) but which undergo only limited uptake by plant roots and elements generally associated with metal oxides (e.g. As, Cr).

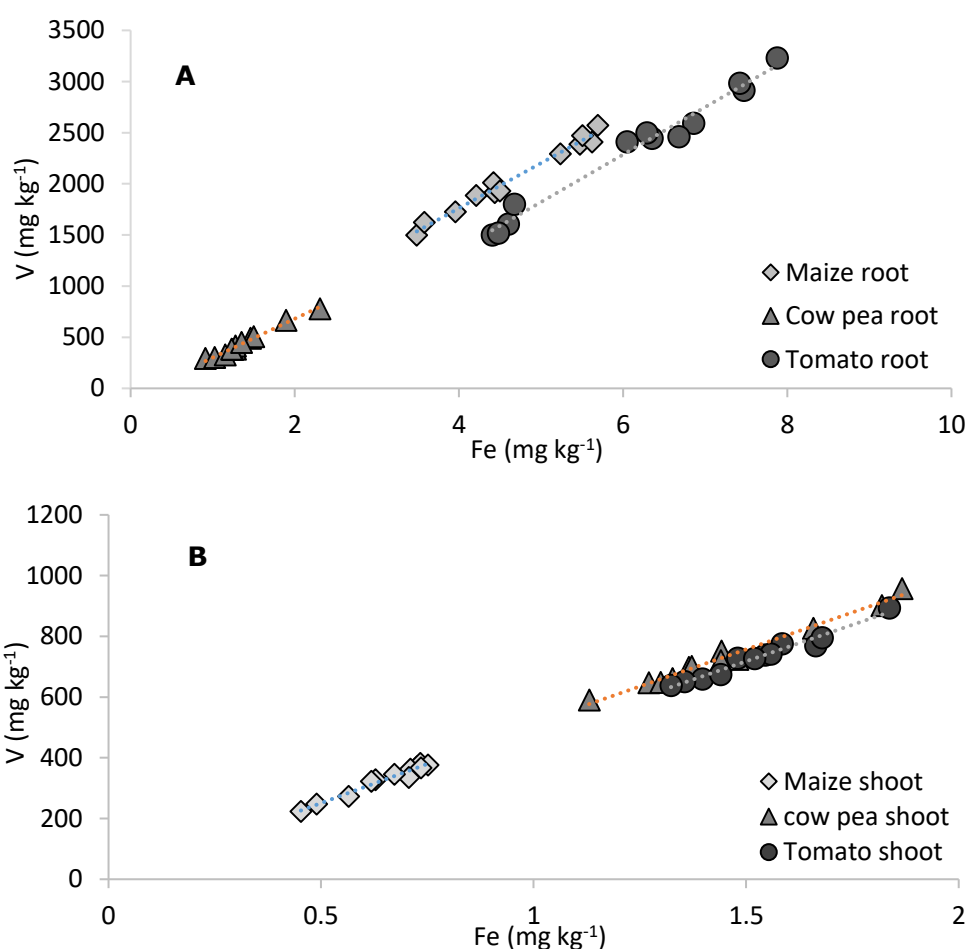


Fig. 5.8: Correlations between V and Fe elements in maize, cowpea and tomato (A) shoots and (B) roots.

Table 5.8: The estimated proportion (%) of macro and micro-element concentration in maize, cowpea and tomato roots, shoots, grain, seed and fruit arising from soil dust.

Element	Maize			Cowpea			Tomato		
	Root	Shoot	Grain	Root	Shoot	Seed	Root	Shoot	Fruit
Na	35.1	29.8	0.00	1.45	31.2	0.13	43.6	11.8	0.21
Mg	33.8	3.09	0.00	5.23	3.43	0.01	20.7	3.31	0.16
P	3.17	0.18	0.00	0.37	0.22	0.00	2.06	0.42	0.00
K	2.19	0.34	0.00	1.12	0.56	0.00	2.67	0.53	0.01
Ca	25.9	1.61	0.00	7.80	1.11	0.03	5.45	0.83	0.32
Al	97.9	107	45.1	137	104	0.00	120	113	125
Cr	79.7	49.2	6.28	100	70.3	457	89.7	62.6	19.8
Mn	79.6	4.49	0.00	47.5	3.31	0.04	68.2	6.48	1.18
Fe	77.3	67.5	0.00	107	67.3	1.16	90.2	71.1	22.8
Co	34.5	55.6	0.00	53.6	36.4	0.14	65.9	43.2	2.42
Ni	52.0	51.0	0.00	51.0	40.8	0.03	71.1	54.6	7.36
Cu	19.0	1.92	0.00	8.49	3.06	0.00	12.6	4.31	0.08
Zn	23.5	1.22	0.00	5.35	2.52	0.00	16.4	2.54	0.09
As	88.7	51.7	36.2	114	64.3	35.6	79.6	71.1	29.3
Se	13.2	2.67	0.00	5.69	6.30	0.01	13.4	2.71	0.10
Mo	10.6	1.08	0.00	2.45	0.36	0.00	6.87	1.92	0.06
Cd	16.8	1.80	0.00	6.17	10.9	0.05	7.07	1.15	0.04
Ba	90.3	22.4	0.40	46.1	15.4	0.00	56.9	9.57	1.35

5.3.10 Effect of applied P and Zn fertilizers on PA and PA/Zn molar ratio

The bioavailability to humans of Zn in grains and cereal crops is negatively correlated with the concentration of phytic acid (PA). This is due to the antinutritive property of PA, which inhibits the absorption of Zn and decreases its bioavailability (Gupta et al., 2015). It is well documented that dietary Zn absorption is affected by the quantities of PA and Zn in the diet (Miller et al., 2015) because PA chelates Zn ions forming insoluble complexes in the gastrointestinal tract that cannot be absorbed (Gibson et al., 2010).

Miller et al. (2015) studied the relation between Zn absorption and dietary PA intake in infants and young children based on modelling combined data from various studies. They found that when Zn intake was controlled, the absorption of Zn decreased by 0.04

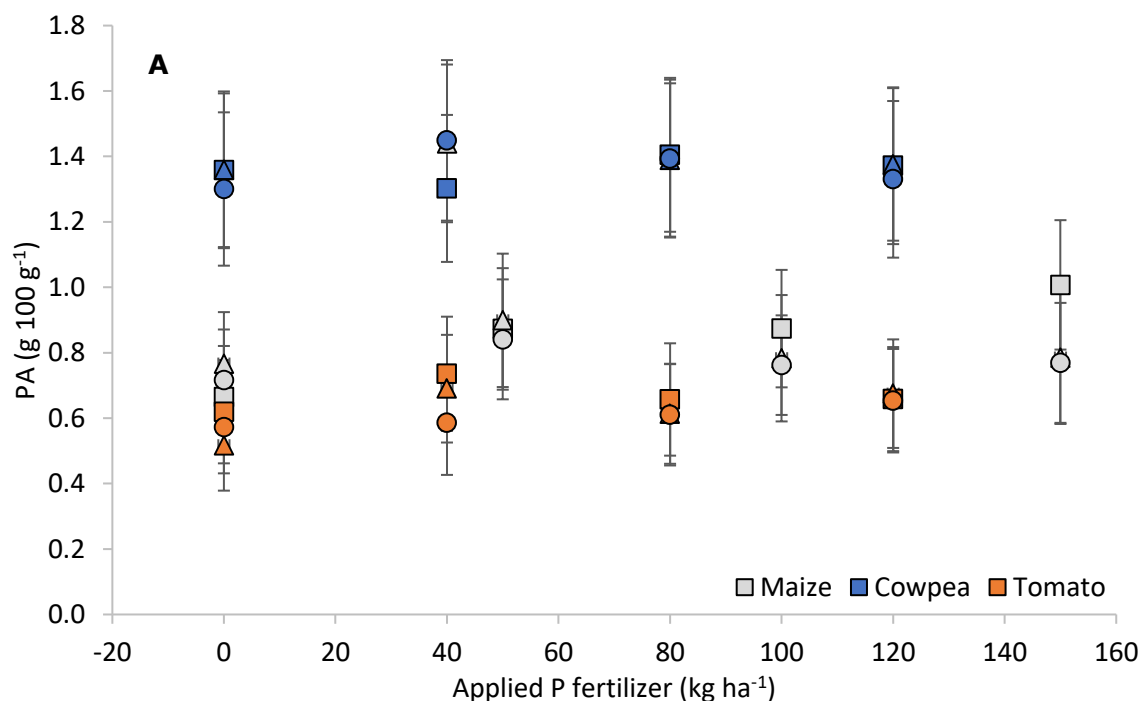
mg d⁻¹ when the concentration of PA intake increased by 500 mg d⁻¹ but this varied with height, age and weight.

Thus PA and the PA/Zn molar ratio are thought to be a reliable guide to Zn bioavailability (Li et al., 2017). Figure (5.9a and b) shows the effect of different rates of P and Zn fertilizers on PA concentration and PA/Zn molar ratio in maize grain, cowpea seed and tomato fruit. Phytic acid content (g 100g⁻¹) ranged between 0.66-1.01, 1.30-1.49, and 0.52-0.67 and the PA/Zn molar ratio ranged between 16.2-22.9, 26.9-31.0 and 24.9-32.3 for maize grain, cowpea seed and tomato fruit respectively.

Phytic acid content and the PA/Zn molar ratio decreased by 5 and 7% at an application rate of 1.2 kg Zn ha⁻¹ in maize grain, by 1 and 7% in cowpea seed and by 9 and 8.5% at 0.8 kg Zn ha⁻¹ in tomato fruit respectively when overall Zn and PA levels were compared with control (Fig. 5.9a). These apparently decreased values were significant (p<0.05) only in maize grain and tomato fruit but not in cowpea seed. On average, tomato fruit showed the greatest decrease in PA and PA/Zn ratio; cowpea seed had the lowest. Abdoli et al. (2016) studied the effect of soil and foliar Zn application on bread and durum wheat in calcareous soils and found that PA and PA/Zn molar ratio decreased as Zn rate increased. It is possible that Zn application does not only increase Zn concentration in crops but also reduces P and PA concentration in crops. Decreasing PA and PA/Zn molar ratio may also arise from growth dilution in crops. Various studies have shown that PA and PA/Zn molar may be significantly decreased with Zn fertilization (Imran et al., 2016, Bharti et al., 2013, Erdal et al., 2002). Erdal et al. (2014) and Yang et al. (2011) found that PA concentration and PA/Zn ratio decreased by 29 and 50% in lentil seed and 13 and 29% in wheat grain in hydroponic culture by application of 14 kg Zn ha⁻¹ and 3.0 µM L⁻¹ as ZnSO₄.7H₂O to soil and nutrient solution respectively, without P application. The lower decrease in % PA and PA/Zn molar ratio in the current study

may be due to lower Zn applications to plants which were intended to reflect local farming practice.

The results in Fig. 5.9 also indicate that fertilizing the crops with P increased PA contents and PA/Zn molar ratio significantly ($p < 0.05$). Phytic acid content and PA/Zn molar ratio in maize grain, cowpea seed and tomato fruit increased by 15, 21 and 19% and 23, 6 and 8% respectively comparing the average overall P application levels with controls but there was a fluctuation between the P application rates. About 60% to 80% of total P in crops is in the form of PA and, in particular, most of the P in grain is present as PA (Zhang et al., 2012b). The results of this study are in agreement with the finding of (Imran et al., 2016). They studied the effect of different rates of P fertilizer, applied to calcareous soils, on PA and PA/Zn molar ratio in maize grain and found that PA and PA/Zn molar increased by 22 and 34% following application of 60 kg P ha⁻¹ as DAP in DK-6142 maize genotype grains grown in calcareous soils in Pakistan.



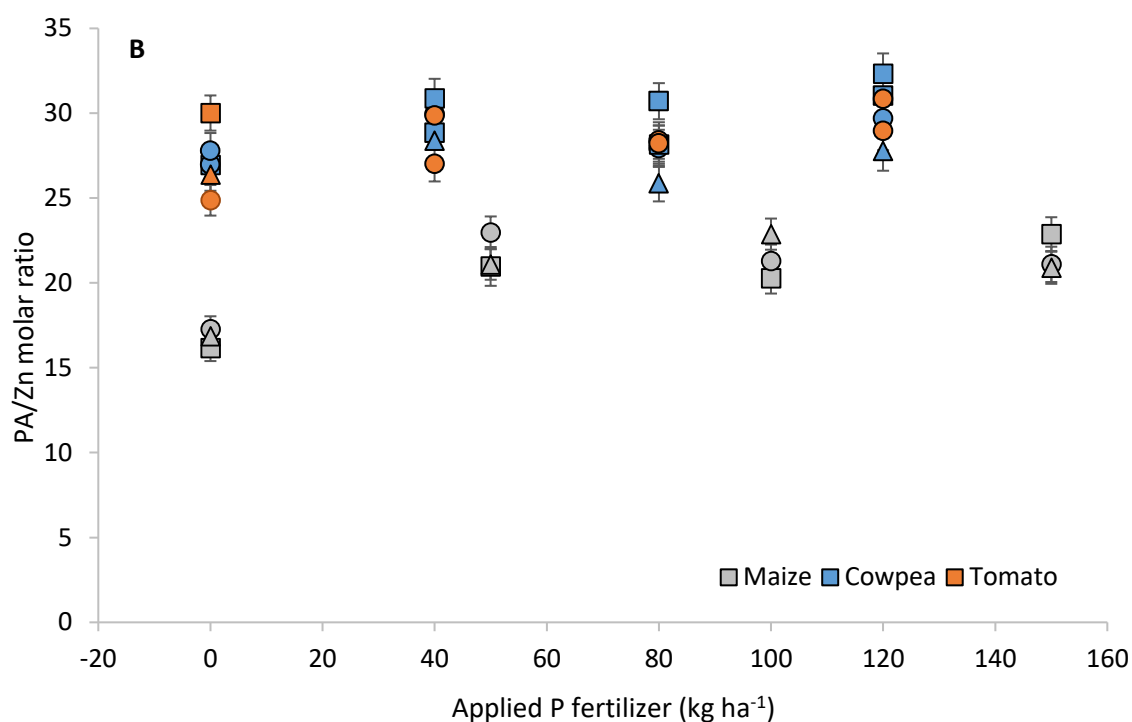


Fig. 5.9: Effect of P and Zn fertilizer application on (A) PA and (B) PA to Zn molar ratio in maize grain, cowpea seed, and tomato fruit; (0, 50, 100 and 150) kg P ha⁻¹ and ($\square$) 0, (O) 0.8, and (Δ) 1.2 kg Zn ha⁻¹ for maize grain and (0, 40, 80 and 120) kg P ha⁻¹ ($\square$) control, (O) 0.4, and (Δ) 0.8) Zn ha⁻¹ for cowpea grain and tomato fruit.

5.3.11 Estimated Zn bioavailability to humans

Zinc absorption in the human intestine is a complex process. 'Trivariate models' have been developed to estimate total Zn absorption depending on the relative concentrations of Zn and PA in the diet (Hussain et al., 2012b, Miller et al., 2007). These models may account for about 80% of the variation in the amount of Zn absorbed (Abebe et al., 2007, Liu et al., 2017).

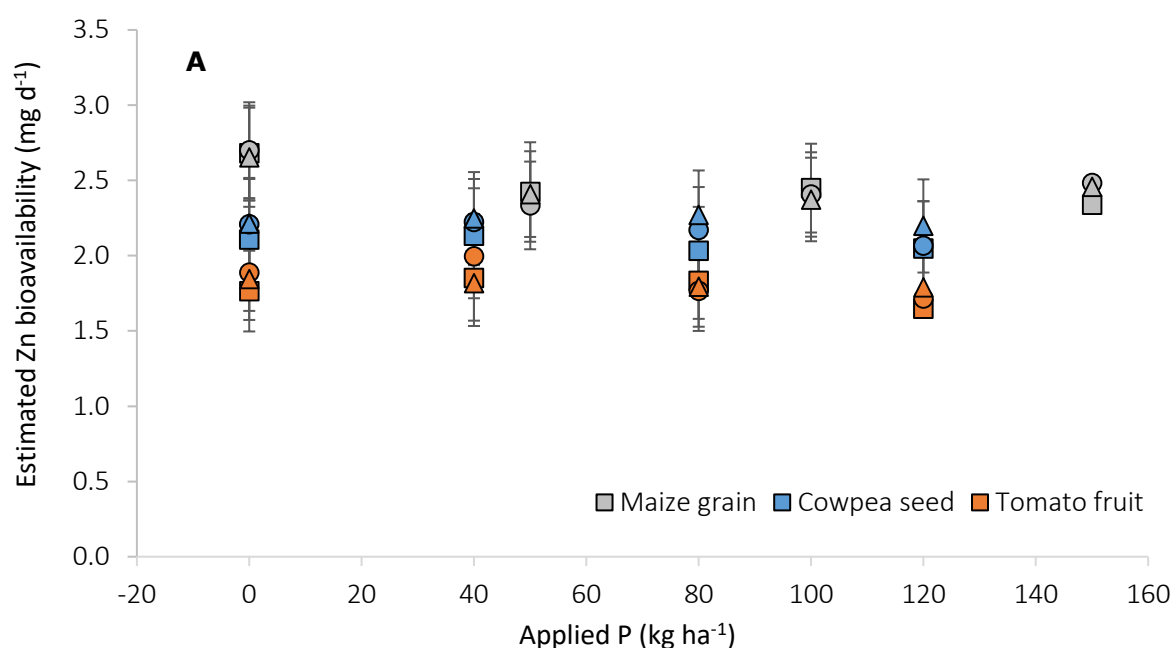
A standard per-capita level of consumption by adults was set at 300 g d⁻¹ (FAO Database, 2011) and the estimated bioavailable Zn was calculated for maize grain, cowpea seed and tomato fruit as a single daily diet. Three different model parameter values for A_{MAX} , K_R and K_P (see Section 2.5.4; Eq. 2.8) have been reported by Imran et al. (2016), Miller et al. (2007) and Brown et al. (2010) and these alternative values were used to calculate estimated bioavailable Zn.

Using the parameter values from Imran et al. (2016), Miller et al., (2007) and Brown et al., (2010), respectively, estimated bioavailable Zn in (mg d^{-1} for 300 g consumption) ranged between 2.33-2.70, 2.18-2.51 and 1.45-2.34 for maize grain, 2.11-2.27, 1.89-2.15 and 1.76-2.05 for cowpea seed and 1.72-2.00, 1.44-1.82 and 1.34-1.68 for tomato fruit (Fig. 5.10a, b and c).

Foliar Zn fertilization increased estimated bioavailable Zn by 11, 4 and 6% in maize grain, cowpea seed and tomato fruit respectively when all Zn rates were compared with controls. A significant increase ($p < 0.05$) was observed for maize grain and tomato fruit while in cowpea seed the level of improved Zn bioavailability was not significant. On average, the highest increase in the estimated bioavailable Zn was observed in maize grain and this followed by tomato fruit and cowpea seed. This may be because the maize plants were fertilized with higher Zn rates than cowpea and tomato crops. Zhao et al. (2014) studied the effect of foliar Zn application on wheat grain Zn content in Zn-deficient calcareous soil and found that grain Zn content and Zn bioavailability increased by 61 and 36% with foliar applications of $1.5 \text{ kg ZnSO}_4 \cdot 7\text{H}_2\text{O ha}^{-1}$ in a laboratory experiment. Imran et al. (2016) found that estimated bioavailable Zn increased by 34 and 24% in Neelam and DK-6142 maize grain genotypes by soil application of 16 kg Zn ha^{-1} as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in calcareous soils in Pakistan. Compared to these studies, lower increases in the estimated bioavailable Zn were achieved in the present study. Again, the results suggest that to increase the estimated bioavailable Zn in crops grown in the calcareous soils of Kurdistan, foliar application of Zn fertilizer is an acceptable method but application rates should be increased over current local norms.

Fertilizing the crop plants with P decreased the estimated bioavailable Zn in maize grain and tomato fruit significantly. The estimated bioavailable Zn decreased by 12, 2 and 5% in maize, cowpea and tomato fruit when overall applied P levels were compared

with controls but there was variation between application rates and crops. Vafaei and Sarraf (2014) studied the effect of P and Zn application on increasing the quality of winter wheat and found that application of 200 kg P ha⁻¹ as DAP caused a significant decrease in estimated bioavailable Zn in the winter wheat. Ryan et al. (2008) also found that Zn bioavailability decreased due to the increase in crop PA content through PA/Zn molar ratio, consistent with previous results (Zhang et al., 2017, Imran et al., 2016). Thus the results from the current study indicate that the application of P fertilizer had a small effect on estimated bioavailable Zn in all crops grown in the calcareous Kurdistan soils, possibly due to rapid fractionation of P fertilizer into relatively unavailable P fractions in the soil. Overall, the result suggests that increasing estimated bioavailable Zn should focus on foliar Zn fertilizer rates.



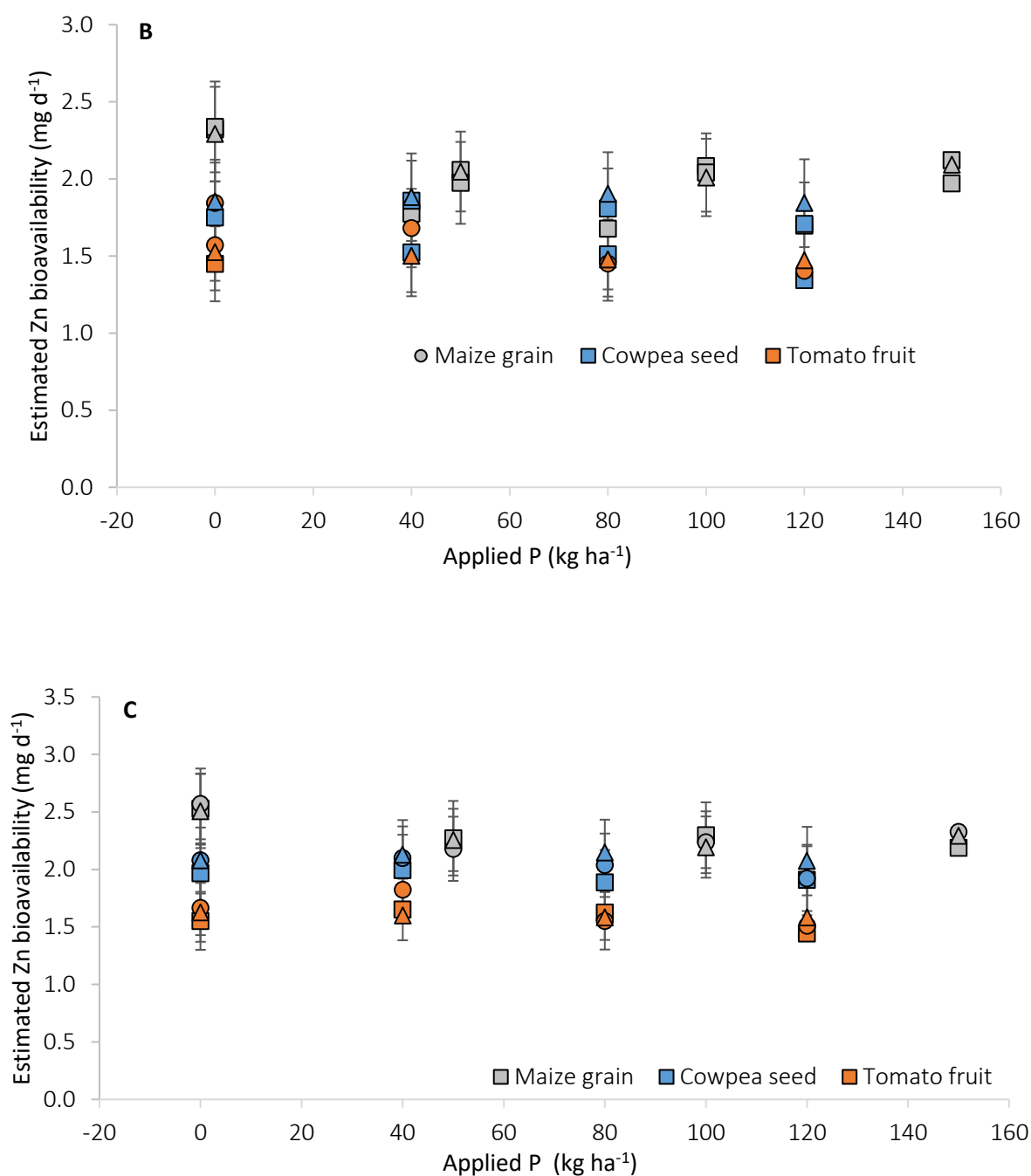
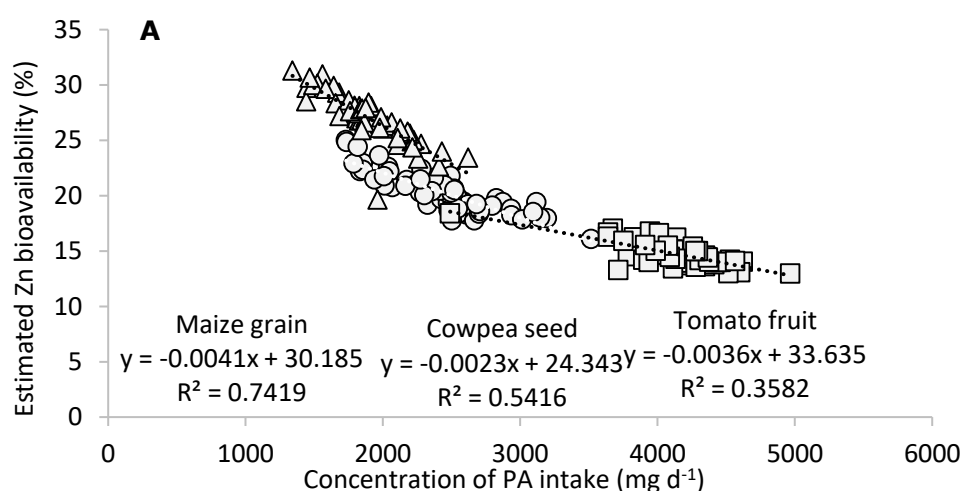


Fig. 5.10: Effect of P and Zn fertilizers application rates on estimated bioavailable Zn in maize grain, cowpea seed, and tomato fruit; (0, 50, 100 and 150) kg P ha^{-1} ; ($\square$) 0, ($\circ$) 0.8, and (Δ) 1.2 kg Zn ha^{-1} for maize grain and (0, 40, 80 and 120) kg P ha^{-1} and ($\square$) control, ($\circ$) 0.4, and (Δ) 0.8 Zn ha^{-1} for cowpea grain and tomato fruit. Using three different sets of parameter values for K_R and K_P from (A) Imran et al. (2016), (B) Brown et al. (2010) and (C) Miller et al. (2007).

5.3.12 Effect of PA on the proportion of bioavailable Zn

The results in (Fig. 5.11a, b and c) show the extent to which the proportion (%) of estimated Zn bioavailability in crops decreased with increasing concentration of PA intake for all the available crop data.

The percentage of estimated bioavailable Zn in maize grain decreased from 25, 23 and 21% to 16, 15 and 12% when the concentration of PA (mg d^{-1}) increased from 1730 to 3520. In cowpea seed, the decrease was from 18, 18 and 16% to 13, 12 and 11% when the concentration of PA intake (mg d^{-1}) increased from 2490 to 4970. In tomato fruit the decrease was from 31, 28 and 26% to 20, 19 and 18% when the concentration of PA intake (mg d^{-1}) increased from 1340 to 2620 when the Imran et al. (2016), Miller et al. (2007) and Brown et al. (2010) parameters were used respectively. The results also indicate that highest bioavailable Zn was observed in tomato fruit followed by maize grain and cowpea seed due to differences in their PA concentrations.



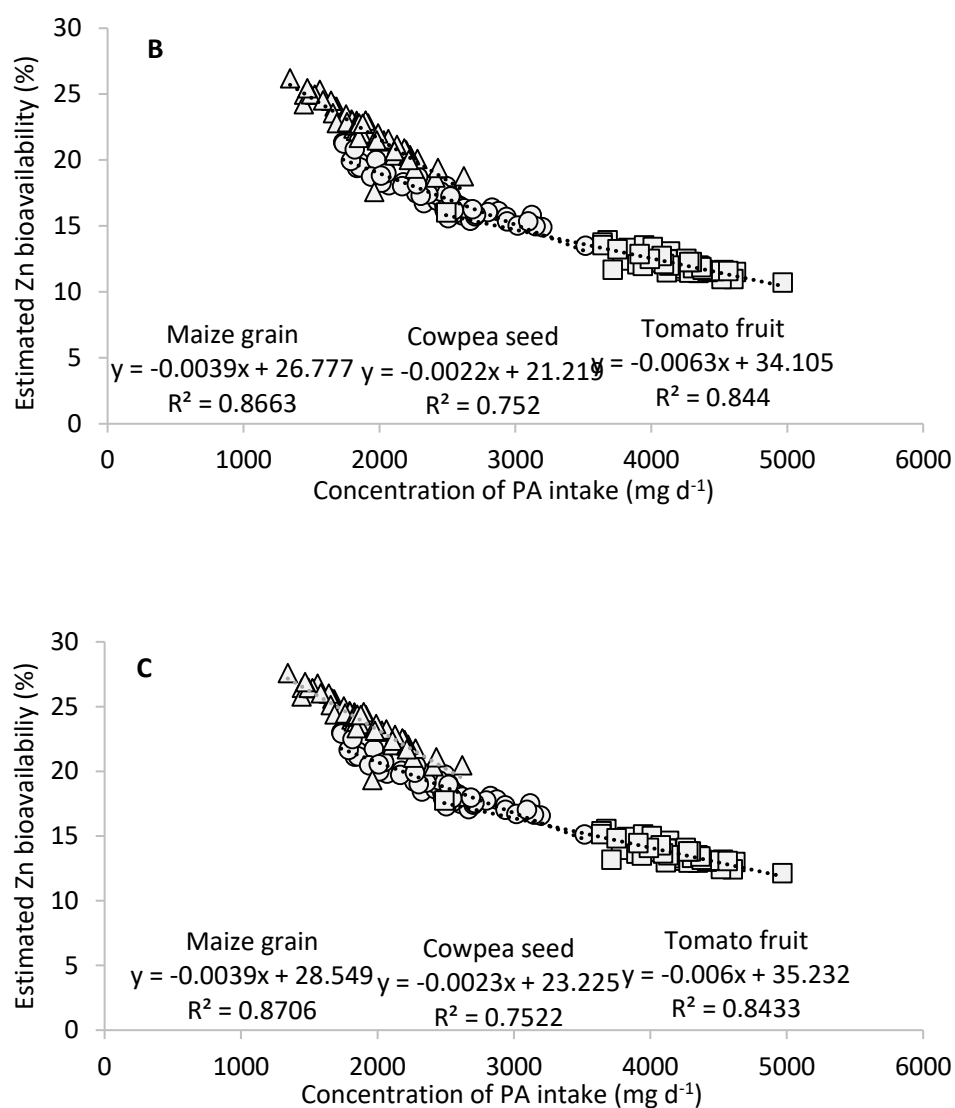


Fig. 5.11: Effect of the concentration of PA intake (mg d⁻¹) on the percentage of estimated Zn bioavailability in 300 g of (O) maize grain, (□) cowpea seed and (Δ) tomato fruit; using three different parameters of K_R and K_P from (A) Imran et al. (2016), (B) Brown et al. (2010) and (C) Miller et al. (2007).

5.4 Conclusions

Chapter 5 presents the results of a field trial examining the effects of soil P and foliar Zn applications to cowpea, tomato and maize grown in calcareous soils in Kurdistan. An overall impression from the data is that Zn is deficient, as expected, but agronomic practices have offset P deficiency by heavy applications of phosphate fertilizer. This has had potential consequences for human nutrition by increasing the phytic acid: Zn ratio in the edible parts of crops.

At harvest, calcium phosphate solubility data suggested that soils were slightly under-saturated with respect to Octa calcium phosphate (OCP) and super-saturated with respect to β -Tricalcium phosphate (β -TCP) but data fell close to the OCP line. Zinc solubility showed clear undersaturation with respect to Smithsonite (ZnCO_3) by approximately two orders of magnitude in (Zn^{2+}) free ion activity across all P applied rates in maize, cowpea and tomato plots.

The application of P increased P_{Olsen} in all plots, but a large proportion (average = 81%) of applied P was fixed by the calcareous soils. An attempt to fractionate P showed that applied P fertilizer had been rapidly redistributed within pre-existing soil P fractions in approximately the same proportion of each fraction. An isotopic study would be required to verify this in future work.

Foliar application of Zn successfully increased Zn concentration in all three crops; Zn content in the edible portions of the crops was systemic in origin, rather than soil dust contamination. The Zn concentration increased by 66%, 45% and 54% for maize, cowpea and tomato shoots and 4% in maize grain, 9% for cowpea seed 4% for tomato fruit. Applied P had no significant effect on P concentration in crop parts, either due to physiological control or the relatively high P status of the soils as a consequence of

prolonged P fertilizer application. The percentage of P increased by 6, 4 and 5% in maize roots, shoots and grain respectively. Similarly, the percentage of P increased by 5, 26 and 11% and 5, 15 and 12% in cowpea and tomato roots, shoots, seed and fruit respectively. Combined application of P and Zn showed no interactive effects.

Phosphate application had a depressing effect on Zn concentration in crop parts. Foliar application of Zn fertilizer had a very small depressing effect on PA content but a much greater effect on PA/Zn molar ratio in maize grain, cowpea seed and tomato fruit. Phytic acid content and the PA/Zn molar ratio decreased by 5 and 7% in maize grain, by 1 and 7% in cowpea seed and by 9 and 8.5% in tomato fruit.

By contrast, PA and PA/Zn increased with P application rates. Phytic acid content and PA/Zn molar ratio in maize grain, cowpea seed and tomato fruit increased by 19, 4 and 19% and 23, 3 and 12% respectively. As a consequence, foliar application of Zn fertilizer increased the estimated bioavailable Zn by 11, 4 and 6% in maize grain, cowpea seed and tomato fruit respectively when all Zn rates were compared with controls. Zn in maize grain, cowpea seed and tomato fruit decreased with P application rates by 11, 4 and 6% in maize, cowpea and tomato fruit when overall applied P levels were compared with controls there was variation between application rates and crops.

Foliar application of Zn in calcareous soils seems beneficial in the long run to decrease grain PA/Zn molar ratio and thereby increase the quality of maize grain, cowpea seed and tomato fruit. A more focussed investigation of P requirement is also required as currently the calcareous soils may be maintained at an unnecessarily high phosphate status with consequences for trace nutrient metal availability to crops and human populations.

6 PHOSPHORUS AND ZINC BIOAVAILABILITY IN WHEAT GROWN IN THE CALCAREOUS SOILS OF THE KURDISTAN REGION

6.1 Introduction

In calcareous soils, the bioavailability of Zn limits crop production due to the presence of CaCO_3 as an adsorbent for Zn^{2+} ions, high pH values which encourage Zn^{2+} adsorption on Fe oxides and humus, and the application of P fertilizer which may co-precipitate with Zn^{2+} (Duffner et al., 2012, Wang et al., 2017, Mousavi and Galavi, 2012, Wu et al., 2017). It is not clear whether Zn-P interactions occur both in the soil and in the plant (Fageria, 2001, Mousavi and Galavi, 2012).

To explain the interactions between P and Zn, (Fageria, 2001, Mousavi and Galavi, 2012) reported three mechanistic hypotheses including: (i) interference of P at the level of plant metabolism including effects on uptake, utilization and translocation of Zn, (ii) difference of P/Zn ratio due to changes in dry matter production with P application and (iii) soil P and Zn interactions. Furthermore, these interactions with P cause a decrease in the bioavailability of Zn within wheat grains, both through decreasing the total concentration of Zn and increasing the PA/Zn molar ratio (Magallanes-Lopez et al., 2017, Khoshgoftarmanesh et al., 2017).

Wheat is the main cereal crop produced in Kurdistan (Ziadi et al., 2013). Wang et al. (2017) reported that in many developing countries it provides about 50% of daily calorie intake. To prevent Zn deficiency in humans, the concentration of Zn in wheat grain should reach a level of around 40 mg kg^{-1} (Chen et al., 2017b, Bouis et al., 2011). However, the total Zn contents of the wheat grain, flour, and bread are not the major factor controlling dietary assimilation of Zn. The presence of 'anti-nutrients' compounds

such as phytic acid (PA), is one of the main problems affecting the nutritional quality of crops (Poblaciones and Rengel, 2016) and the proportion of trace metals, such as Zn, that is readily absorbed (Magallanes-Lopez et al., 2017). To determine Zn bioavailability in foods, it is necessary to measure the molar ratio of PA to Zn. A decrease in this molar ratio increases Zn bioavailability.

Wheat variety, growing conditions, and location are all factors that may cause differences in the bioavailability of Zn (Gabaza et al., 2018). The wheat growing area in the Kurdistan Region amounts to about 570,000 ha; approximately 500,000 t is harvested every year and wheat production is mostly rain-fed (Mazid, 2015). Therefore, the aims of this survey were:

- (i) To determine the bioavailability of P and Zn in wheat-producing soils, wheat grain and local types of bread;
- (ii) To evaluate PA concentration, the PA/Zn molar ratio and the estimated human Zn bioavailability in wheat varieties, flours and bread consumed in Sulaimanyah province;
- (iii) To evaluate the nutrient status of wheat grain grown in Kurdistan calcareous soils.

6.2 Methods and materials

6.2.1 Soil and wheat survey in Sulaimanyah province

A total of 120 wheat grown field, soil, and wheat grain samples were collected from main wheat production areas in Sulaimanyah province/ Kurdistan region, Iraq in May-June 2017 in ten locations include Halabja, Sirwan, Khormal, Sharazoor, Said Sadiq, Barznja, Piramagrun, Chamchamal, Darbandikhan and Kalar (Fig 6.1). The number of samples was dependent on the size of a location area (i.e. more samples were collected in a large size area). Areas selected for soil and wheat grain sampling were chosen on the basis of agriculture activity for wheat production, soil types, wheat variety and geographical variety. Adana and Aras are the two main wheat varieties grown in the Kurdistan region with other varies such as Rashidi, Barcelona, and Wafia grown in a few locations (Table 6.1).

Table 6.1: Wheat varieties collected at ten different locations in Sulaimanyah province.

Location	Code	No. of samples	Adana	Aras	Rashidi	Barcelona	Wafia
Halabja	HAL	13	8	5	-	-	-
Sirwan	SIR	8	6	2	-	-	-
Khormal	KHO	7	3	2	-	1	1
Sharazoor	SHA	15	6	6	-	1	2
Said Sadiq	SSQ	22	7	13	-	2	-
Barznja	BAR	8	1	7	-	-	-
Piramagrun	PIR	12	5	5	-	1	1
Chamchamal	CHA	10	4	4	2	-	-
Darbandikhan	DAR	10	3	6	-	1	-
Kalar	KAL	15	11	1	2	-	-

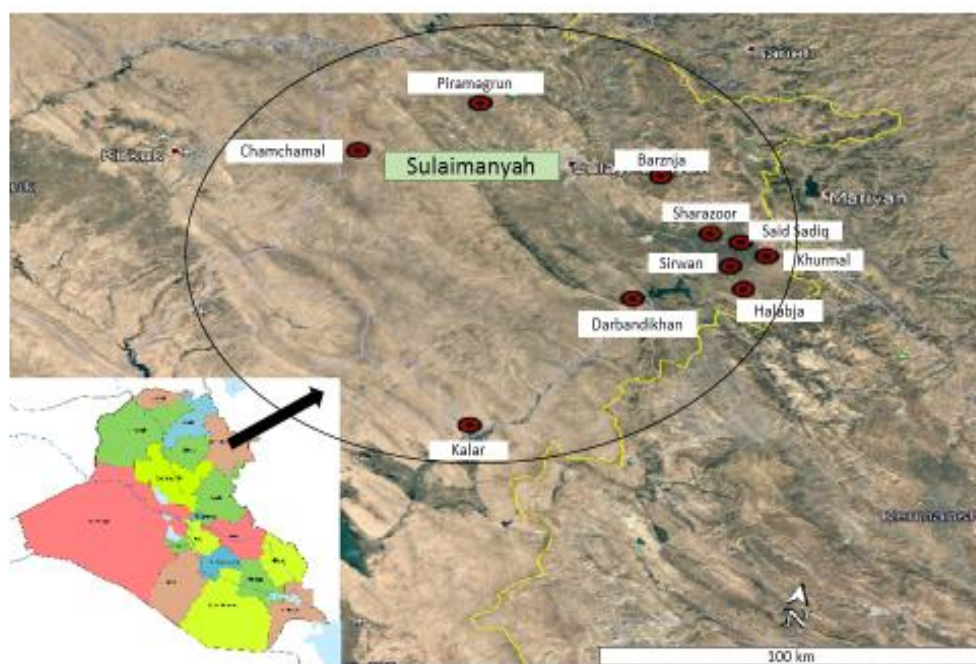


Fig. 6.1: Surveyed wheat and corresponding soil samples locations in Sulaimanyah province, Kurdistan region, Iraq.

For each site, wheat grain samples were collected from five different sampling points in the field after selecting about 100 m² in each wheat farm (shown in Fig. 6.2). The samples from five points were mixed to produce a composite sample, sealed in plastic bags for transport to a laboratory at the Halabja Technical College of Applied Science. 120 samples in W-transect with each sample covering 15-20 heads of wheat. Grain was separated from straw, mixed, and a representative sample taken. Approximately 10 g of each sample was then stored in a plastic bag labelled and transported to the Nottingham University, UK. A portion of each sample was finely ground using a centrifugal mill (Retsch, Model ZM200), and then stored in a polyethene bag for further analysis.

6.2.1.1 Soil sampling and preparation

Soil samples were collected from same areas as wheat grain sampling as shown in Fig. 6.2 of at a depth of 0-15 cm, using a stainless steel trowel and then sealed in plastic bags for transport to a laboratory at the Halabja Technical College of Applied Science. About 1 kg of each soil was dried at room temperature in an aluminium tray, disaggregated, then sieved to obtain the <2 mm fraction. Approximately 50 g of each sample was then stored in plastic bags, labelled and transported to Nottingham University, UK. A portion of each sample was finely ground using a gate ball mill (Retsch, Model PM400), and then stored in a polyethene bag for further analysis.

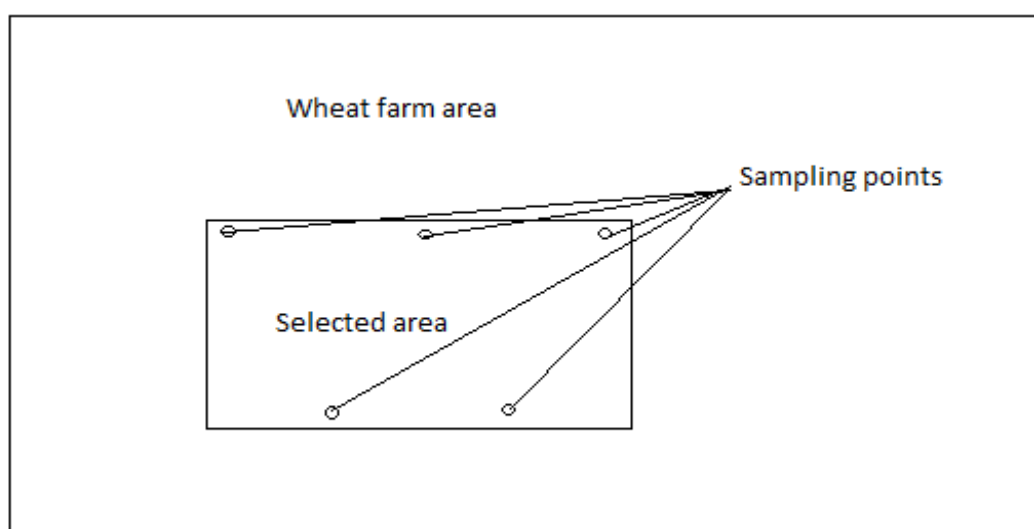


Fig. 6.2: Wheat and corresponding soils sampling design.

6.2.1.2 Flour and bread sampling

Using simple systematic random sampling, 50 samples of various types of bread, including Lawasha, Mashini, Samoon, Hawrami and Tiry, were collected from different bakeries in Sulaimanyah, Halabja, Khormal, Said Sadiq, Chamchamal and Kalar as shown in Table 6.2. For each bread, sample corresponding flour samples were also collected. All bread samples were completely dried at room temperature for 3 days and then powdered using a stainless steel electric mixer. Approximately 5 g of flour and

bread samples were stored in separate plastic bags, labelled and transported to the University of Nottingham, UK for further analysis.

Table 6.2: Bread type with corresponding flours collected at seven different locations in Sulaimanyah province.

Location	Code	No. of samples	Lawasha	Mashini	Samoon	Hawrami	Tiry
Sulaimanyah	SUL	7	2	2	2	-	1
Halabja	HAL	10	2	2	2	2	2
Khormal	KHO	5	3	-	2	-	-
Said Sadiq	SSQ	8	2	2	2	1	1
Piramagrun	PIR	6	2	2	2	-	-
Chamchamal	CHA	5	2	1	2	-	-
Kalar	KAL	6	4	-	2	-	-
Home made	HMD	3	1	-	-	1	1

6.2.2 Soil analysis

6.2.2.1 *Soil pH*

The pH of soils was determined according to Section 2.1.1 in Chapter 2.

6.2.2.2 *Loss of Ignition (LOI %)*

The LOI% for soils were determined according to Section 2.1.2 in Chapter 2.

6.2.2.3 *Soil digestion*

Soil samples were digested according to Section 2.1.3 in Chapter 2.

6.2.2.4 *Olsen-P*

The Olsen-P was determined for soils according to Section 2.1.4 in Chapter 2.

6.2.2.5 *DTPA-Zn*

The DTPA-Zn for soils were determined according to Section 2.1.5 in Chapter 2.

6.2.2.6 *Soluble P and Zn*

The soluble P and Zn were determined according to Section 2.1.6 in Chapter 2.

6.2.2.7 *WHAM speciation*

The WHAM speciation was determined according to Section 2.1.10 in Chapter 2.

6.2.3 Wheat grain, flour, and bread analysis

The elements in soil, wheat grain, flour and bread samples were determined according to Section 2.2.2 in Chapter 2.

6.2.3.1 *Wheat grain, flour, and bread digestion*

The wheat grain, flour, and bread samples were digested according to Section 2.2.1 in Chapter 2.

6.2.3.2 *Elemental analysis*

Elements were analyzed according to Section 2.2.2 in Chapter 2.

6.2.3.3 *Phytic acid*

The PA in wheat grain, flour, and bread samples were determined according to Section 2.2.3 in Chapter 2.

6.2.3.4 *Phytic acid to zinc molar ratio*

The PA to Zn molar ratio for wheat grain, flour, and bread samples was determined according to Section 2.2.4 in Chapter 2.

6.2.3.5 *Estimated human Zn bioavailability*

The estimated human Zn bioavailability for wheat grain, flour, and bread samples was determined according to Section 2.2.5 in Chapter 2.

6.2.4 Statistical analysis

The data were analyzed using SPSS 24.0 for Windows. A one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) ($p < 0.05$) was performed to test the significance of treatments. The relationships between variables were determined via Pearson's correlation analysis ($p \leq 0.05$).

6.3 Results and discussion

6.3.1 Hierarchical clustering analysis for surveyed soils

A dendrogram of soil properties (Fig. 6.3) showed that seven coherent groups could be identified with similarity levels greater than 65%. From left to right in Fig. 6.3, Group I (pH and Ca concentration), reflects the calcareous nature of soils (pH around 8) while Group II indicates Na^+ and K^+ in exchangeable forms or in primary minerals. Group III (LOI and Cu) might arise from Cu adsorption on humus particles although this would suggest that the total Cu concentration in the soil depends on the soil humus content retaining the Cu which seems unlikely. Group IV represents the presence of an oxide mineralogy (Al, Fe, As and Mn). Group V, includes Cd, U and Pb, possibly indicating a common source of soil contamination such as phosphate fertilizers although Cd and U often reflect a calcareous mineralogy too. Jia et al. (2010) reported that heavy metals may be added to soils through agricultural fertilizers, especially phosphate, and this is the most likely source of the Cd and U. Ahmed et al. (2014b) studied factors affecting U fractionation and distribution in the soil profile of arable and woodland soils; they reported that U was strongly associated with soil Ca ($r=0.94$) and P ($r=0.86$) contents and that U probably arose from the long-term application of phosphate fertilizer. A correlation between Cd and P would not necessarily be expected given the offtake of the P and retention of the Cd that would be expected over decades of fertilizer application. Nevertheless, Group VI (P_{Olsen} and total P) does suggest a relationship arising from fertilizer application. The final identifiable group (VII) (Zn and Mo) is difficult to explain and may reflect primary mineralogy in the parent materials of the region.

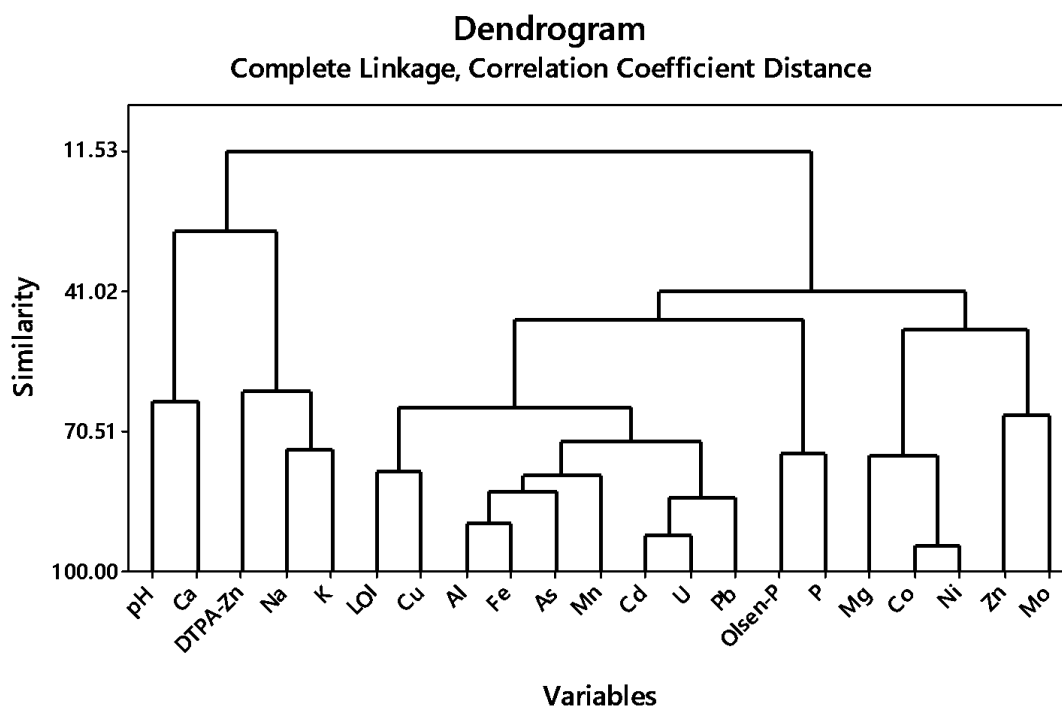


Fig. 6.3: Dendrogram for hierarchical cluster analysis of soil characteristics, macro and microelement contents in surveyed soils (n=120), collected at ten locations in Sulaimanyah province, Kurdistan region, Iraq.

6.3.2 Soil characteristics

Table 6.3 shows the chemical characteristics of the surveyed soils in the surface layer of 0-15 cm, a significant difference was found between individual sample locations ($p < 0.05$). The soil pH values ranged from 7.46 to 8.67. Calcium concentrations ranged from 6.75 to 155 g kg⁻¹ (a 23-fold variation). The LOI ranged from 3.15% to 20.9%, (a 7-fold variation).

Table 6.3: Maximum, median and minimum values of P_T , Zn_T and Ca, P_{Olsen} , Zn_{DTPA} , P_{Sol} , Zn_{Sol} , soil pH and LOI for all the studied soils (n=120): Data are arranged in order of decreasing average Ca concentration for each location

Soil variable		Location									
		SHA	KHO	BAR	HAL	SSQ	SIR	DAR	PIR	KAL	CHA
No. of samples		15	7	8	13	22	8	10	12	15	10
P_T (mg kg ⁻¹)	Maximum	1220	2350	2930	3740	2550	1130	800	1100	740	680
	median	754	746	779	667	803	817	625	638	554	580
	Minimum	403	653	698	416	645	690	482	411	426	492
Zn_T (mg kg ⁻¹)	Maximum	106	150	135	81.9	143	91.3	78.1	196	92.2	105
	median	85.1	84.5	96.2	71.5	86.1	74.8	63.9	67.3	60.8	66.4
	Minimum	71.0	73.1	73.6	42.6	69.9	70.0	53.3	59.6	43.1	54.4
Ca (g kg ⁻¹)	Maximum	101	53.7	10.2	142	119	90.7	109	155	136	134
	median	21.3	30.7	19.1	42.4	59.0	78.6	81.4	88.3	98.9	100
	Minimum	6.75	10.7	97.1	17.8	23.8	36.4	31.1	27.1	26.0	54.3
P_{Olsen} (mg kg ⁻¹)	Maximum	8.22	11.1	8.97	24.6	21.2	28.1	11.5	9.60	10.8	6.65
	median	5.60	5.83	4.96	8.98	10.0	10.8	6.46	6.16	3.61	4.89
	Minimum	3.03	4.66	2.24	5.28	6.03	6.46	4.94	4.29	2.13	2.25
Zn_{DTPA} (mg kg ⁻¹)	Maximum	0.75	0.48	5.23	0.77	0.37	0.70	3.75	2.80	0.63	0.81
	median	0.28	0.24	0.40	0.39	0.00	0.31	1.95	0.33	0.31	0.46
	Minimum	0.14	0.20	0.22	0.24	0.69	0.25	0.70	0.18	0.14	0.37
P_{Sol} (mg L ⁻¹)	Maximum	9.83	0.83	9.91	3.14	3.98	1.21	2.08	2.41	2.80	0.93
	median	1.41	0.10	0.19	2.28	0.11	0.38	1.09	0.11	0.35	0.36
	Minimum	0.07	0.07	0.08	0.28	0.08	0.07	0.86	0.08	0.17	0.14
Zn_{Sol} (μg L ⁻¹)	Maximum	2.75	3.70	2.11	1.73	4.80	2.29	7.80	9.46	5.30	2.94
	median	1.08	1.16	0.55	1.36	1.21	1.03	1.62	1.08	1.42	1.22
	Minimum	0.65	0.55	0.21	0.57	0.34	0.49	0.80	0.40	0.57	0.65
pH	Maximum	8.19	8.18	8.04	8.09	8.10	8.21	8.67	8.14	8.43	8.33
	median	8.10	8.03	7.95	7.94	7.98	8.04	8.15	8.01	8.18	8.06
	Minimum	7.60	7.93	7.58	7.83	7.75	7.88	7.46	7.83	7.83	7.77
LOI%	Maximum	13.8	11.9	14.1	10.9	10.9	12.0	8.6	20.9	6.66	8.55
	median	11.0	10.2	10.4	9.54	9.22	9.93	5.10	9.59	4.75	5.10
	Minimum	7.28	9.13	6.72	6.12	7.76	9.06	3.79	6.58	3.15	3.79

SD= standard deviation

At harvest, P_T concentration (mg kg^{-1}) ranged from 1240 ± 845 to 562 ± 82.5 for BAR and KAL locations respectively (Fig. 6.4). Zhu et al. (2018) reported that the concentrations of P and Zn in all soil types in the world ranged between 50-3000 mg kg^{-1} . Thus, the concentration of P_T in surveyed soils were in the global range. In the majority of surveyed soils, P_{Olsen} was insufficient for optimum wheat growth. As shown in Table 6.1, in about 87% of surveyed soils, P_{Olsen} was lower than the accepted critical level of 10 mg kg^{-1} (Ahmed, 2013). The variation of P_T and P_{Olsen} concentrations between locations probably arise from several factors but principally from differences in parent material and P fertilizer application. It was apparent that farmers in the region routinely apply different amounts of P fertilizer when interviewed at the time of the survey; the ranges of P_T and P_{Olsen} determined were 400-3740 and 2.13-28.1 mg kg^{-1} respectively. Chen et al. (2017a) also reported that, in calcareous soils, parent material and applied phosphate fertilizers are main sources for total P content. Al Harbi et al. (2013) reported that the application of 450 kg ha^{-1} of P fertilizer as diammonium phosphate increased available P from 5.85 to 18.3 mg kg^{-1} . Wang et al. (2015a) studied the effect of long-term application of phosphate fertilization on P sorption and accumulation in calcareous soils of 15-year old orchards in China. They found that P_T increased from 540 to 904 mg kg^{-1} and P_{Olsen} increased from 3.40 to 30.7 mg kg^{-1} ; similarly, P_{Sol} (CaCl_2 -P extraction) increased from less than 0.1 to 0.66 mg L^{-1} . At the same time, about 47% of the surveyed soils contained values of P_{Sol} , which were higher than the required amount for optimum plant growth of 0.5 mg L^{-1} in the soil solution (Imran et al., 2016; Table 6.1). This is also probably a result of extensive applications of phosphate fertilizers by farmers, typically 100-200 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ in every growing season. Ali et al. (2014a) reported that P_{Sol} increased linearly with the application of SSP from 0 to 150 kg ha^{-1} in alkaline-calcareous soils. The *reservoirs* of available P (P_{Olsen}) constituted a very small proportion of P_T , varying from 0.22 to 3.78%. Furthermore, P_{Sol} was about 3.20 to 35% of P_{Olsen} , indicating that a relatively high proportion of available P was immediately available to plants.

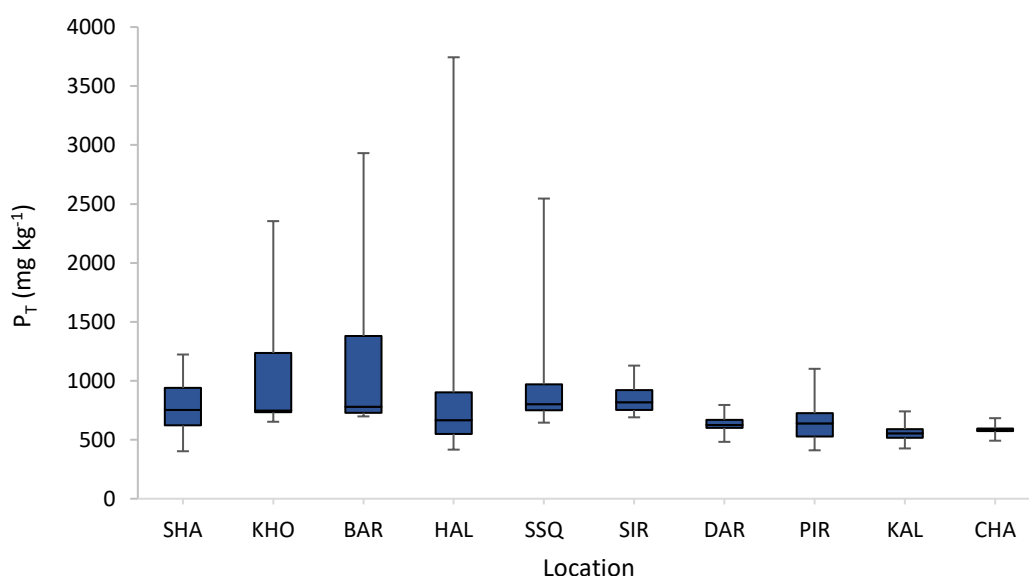


Fig. 6.4: The distribution of P_T in the studied soils ($n=120$) collected from wheat farms at ten locations in Sulaimanyah province, Kurdistan region, Iraq.

The concentration of Zn_T ($mg\ kg^{-1}$) ranged from 60.4 ± 11.9 to 102 ± 19.3 for BAR and KAL locations respectively (Fig. 6.5), a significant difference was found between locations ($p < 0.05$). Alloway (2008b) reported that the total concentration of Zn in all soil types in the world ranged between 10-300 $mg\ kg^{-1}$. Compared to this, the concentration of Zn_T in surveyed soils were in the global range. In the majority of surveyed soils, Zn_{DTPA} was insufficient for optimum plant growth; with about 78% of soils Zn having lower a concentration than the accepted critical level of 0.5 $mg\ kg^{-1}$ (Poblaciones and Rengel, 2016). The apparent Zn deficiencies in the surveyed fields can be attributed to the high soil Ca concentrations and high pH value. Maqsood et al. (2014) found that about 75% of Punjab calcareous soils are Zn deficient due to strong adsorption on $CaCO_3$ at high pH. Reyhanitabar and Gilkes (2010) studied the kinetics of Zn_{DTPA} extraction in calcareous soils and found that Zn *desorption* was negatively ($r = -0.67$) correlated with soil pH. The variation in Zn_{DTPA} between locations may be due to geochemical differences between locations. The low availability of Zn in soils may also be related to high levels of applied P fertilizer applied in every growing season. Ali et al. (2014a) studied the effect of applied P fertilizer on micronutrient availability in

alkaline-calcareous soils and found that the concentration of Zn regularly decreased with increasing application of SSP fertilizer. However, in the soils surveyed in the current study, indices of available Zn (Zn_{DTPA} and Zn_{Sol}) were not negatively correlated to available P (P_{Olsen} and P_{Sol}). In about 98% of the surveyed samples, Zn_{Sol} was under the critical value for optimum plant growth of $4-270 \mu g L^{-1}$ in soil solution (Alloway, 2009), indicating high Zn retention due to high $CaCO_3$ content, high pH and the absence of Zn fertilizer application. Hussain et al. (2011) studied Zn release characteristics in calcareous soils and found a negative correlation between plant-available Zn and $CaCO_3$ content. The *reservoirs* of available Zn (Zn_{DTPA}) constituted a very small proportion of Zn_T , varying from 0.18 to 5.58 %. Furthermore, whereas the Zn_{Sol} was only 0.14 to 0.18% of Zn_{DTPA} indicating that a relatively small proportion of the available Zn was immediately soluble.

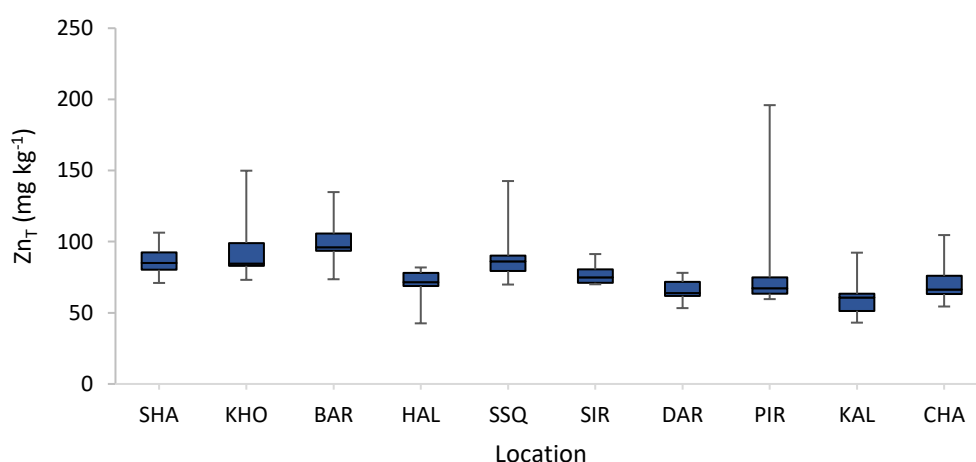
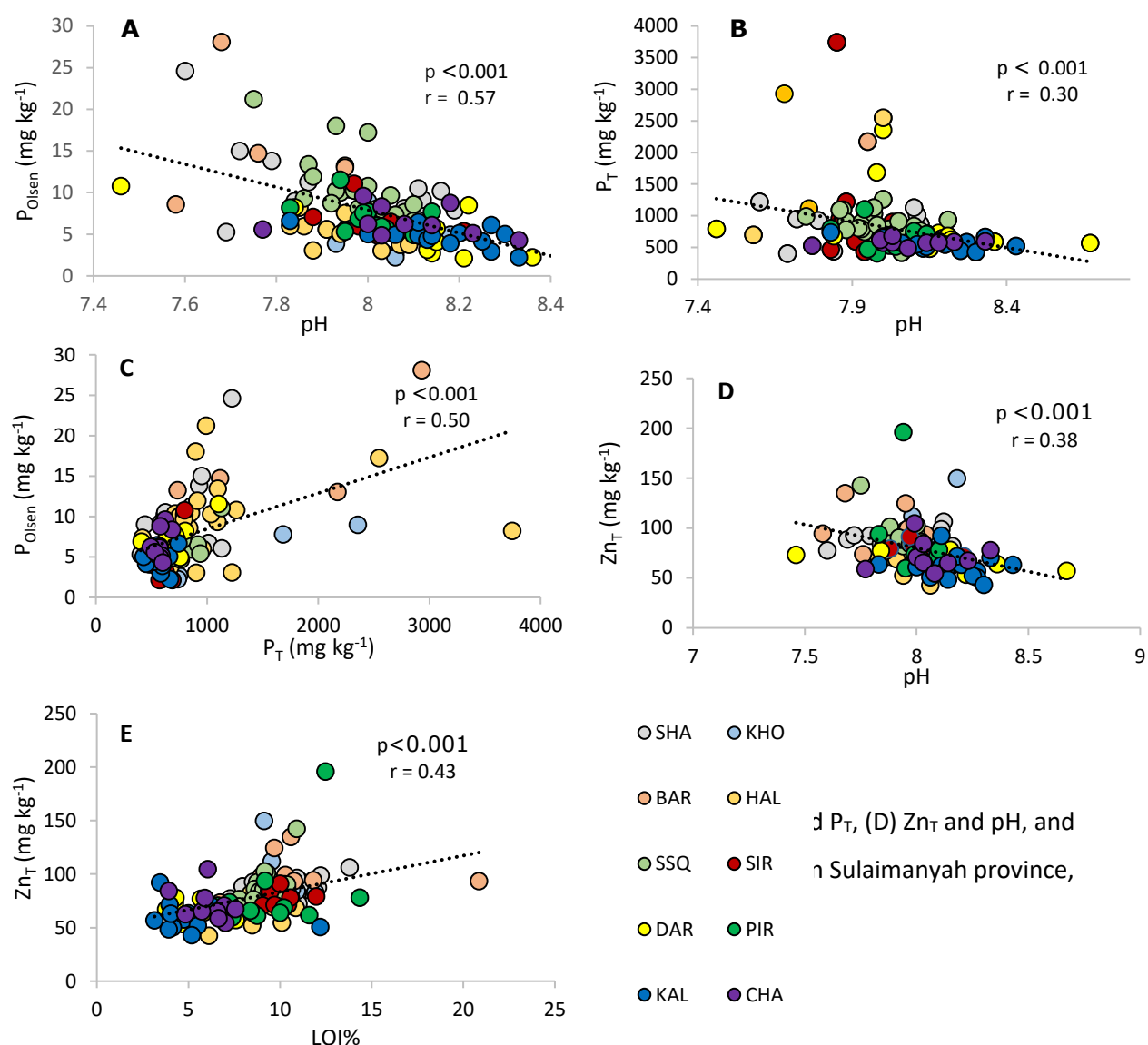


Fig. 6.5: The distribution of Zn_T in the studied soils ($n=120$) collected from wheat farms at ten locations in Sulaimanyah province, Kurdistan region, Iraq.

A correlation showed that P_T , P_{Olsen} and Zn_T were negatively ($r= -0.57$, -0.30 and -0.38 , $p<0.001$) correlated with pH (Fig. 6.6a, b and d) while P_{Olsen} and Zn_T were positively correlated to P_T ($r=0.50$, $p<0.001$) and LOI% ($r=0.43$, $p<0.001$) (Fig. 6.6c and e) respectively. Among other soil characteristics, no significant correlations were found.



6.3.3 Solid $\rightleftharpoons$ liquid partition coefficient (Log K_d)

In this survey, K_d values were calculated ($C_{\text{ads}}/C_{\text{soln}}$), where C_{ads} (mg kg^{-1}) and C_{soln} (mg L^{-1}) refer to the concentrations of solid and solution phase P and Zn, which together define the partitioning of P and Zn between solid and solution phases. The results in Table 6.4 indicate that all locations have very similar values of Log k_d P when P_T was considered but values differed when P_{Olsen} was considered. This is consideration of the

significant difference in P_{Olsen} values between locations. The result of $\log K_d P$ also indicates low adsorption of P in the surveyed soils. This may be due to greater solubility of P due to the application of P fertilizer manually.

$\log K_d Zn$ is different between locations when Zn_T and Zn_{DTPA} are considered Table 6.4. Jalali and Moradi (2013) studied sorption of heavy metal in polluted and unpolluted calcareous soils and found high sorption capacity for Zn in the soils.

The high $\log K_d Zn$ values may be due to the presence of low amounts of Zn_{Sol} in the soil solution. Shaheen et al. (2013) reported that values of K_d increased with decreasing initial concentration of metal in the soil solution. In the surveyed soils, low concentrations of Zn were found due to the absence of Zn fertilizer application and the calcareous nature of soils. This reflects strong adsorption on $CaCO_3$ which has a high specificity for Zn. Calcium carbonate may also cause precipitation of $ZnCO_3$. Papadopoulos and Rowell (1989) and Clemente et al. (2006) studied the reaction of Zn in calcareous soils and found that the $CaCO_3$ surface increased Zn adsorption and led to precipitation of $ZnCO_3$ respectively.

Soil pH and organic matter content are two of several soil properties that control metal and sorption distribution of soil solid and solution phases (Bradl, 2004). Correlation suggested that values of $\log K_d$ for P and Zn were not affected by soil pH and Ca concentration, possibly due to the narrow range of pH values (7.46-8.67) and relatively high Ca concentrations in all the surveyed soils. However, $\log K_d (Zn_T/Zn_{\text{Sol}})$ was significantly and positively related to soil LOI content ($r = 0.41$, $p < 0.001$) (Fig. 6.7) although the relationship was weak. Shaheen et al. (2013) reported that increasing soil organic matter and ion exchange cause increase the adsorption of soluble Zn.

Table 6.4: The logarithm of the solid-liquid partition coefficient (Log K_d) for P and Zn adsorption in surveyed soils (n=120) cultivated with wheat grain at ten locations in Sulaimanyah province, Kurdistan region, Iraq.

Location	Log k_d P				Log K_d Zn			
	P_T/P_{Sol}		P_{Olsen}/P_{Sol}		Zn_T/Zn_{Sol}		Zn_{DTPA}/Zn_{Sol}	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
SHA	3.02	0.78	1.11	0.69	4.87	0.23	2.43	0.25
KHO	3.74	0.47	1.44	0.38	4.84	0.31	2.33	0.30
BAR	3.51	0.62	1.53	0.57	5.21	0.30	2.94	0.40
HAL	2.56	0.29	0.40	0.28	4.76	0.21	2.50	0.19
SSQ	3.60	0.55	1.65	0.47	4.88	0.29	2.47	0.26
SIR	3.49	0.51	1.36	0.44	4.89	0.30	2.52	0.28
DAR	2.71	0.11	0.53	0.16	4.57	0.24	3.00	0.30
PIR	3.59	0.34	1.62	0.35	4.80	0.31	2.57	0.34
KAL	3.05	0.35	0.97	0.29	4.59	0.27	2.32	0.29
CHA	3.25	0.26	1.28	0.26	4.74	0.26	2.60	0.24

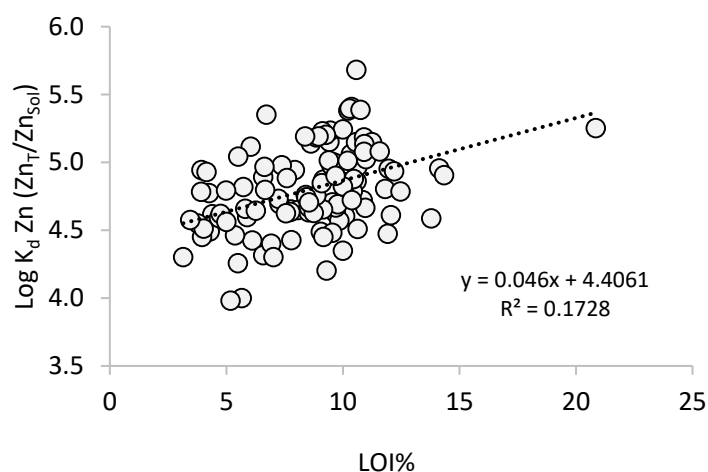


Fig. 6.7: Correlation between solid-liquid partition coefficient (Zn_T/Zn_{Sol}) and loss on ignition (LOI) in surveyed soils.

6.3.4 Phosphate and zinc solubility in surveyed soils

6.3.4.1 Phosphate solubility

A solubility diagram for calcium phosphate equilibria was developed by plotting 'phosphate potential' ($\log H_2PO_4^- - pH$) against lime potential ($\log Ca^{2+} + 2pH$). The

activities of Ca^{2+} , H_2PO_4^- and pH values used were determined using measured values of pH and Ca and P concentration in solution with speciation using the WHAM-7 program. This approach can indicate the likelihood of specific phosphate minerals being present in soils at different pH values (Zhang et al., 2014).

Figure 6.8 presents the soil solution composition (phosphate potential and lime potential) in relation to common Ca phosphate minerals in the studied soils. The Ca and phosphate data fell between saturated with respect to dicalcium phosphate dihydrate (DCPD) and undersaturated with respect to β -Tricalcium phosphate (β -TCP) or supersaturated with respect to hydroxyapatite (HA) across all the surveyed soils.

Zhang et al. (2014) studied phosphate mineral solubility in native and cultivated calcareous soils and found that the range phosphate minerals including DCPD, DCP, TCP, OCP and HP apparently regulated the solubility of phosphate in the fertilized and native calcareous soils.

The results in Fig. 6.8 indicate that soils in several locations appear to have a common phosphate mineralogy. Thus soils from PIR are intermediate between the HA and TCP solubility lines, CHA soils appear to contain TCP, soil from KAL are intermediate between TCP and OCP, DAR soils appear to have OCP present and HAL and SHA soils are intermediate between OCP and DCP. Furthermore, soils that have more available phosphate (P_{Olsen}) appear to have DCPD and DCP present whereas soils that have less available P are undersaturated with respect to OCP, TCP and HA indicating that P_{Olsen} at least qualitatively reflects the type of Ca phosphate mineral present. Thus soils in SHA (OCP/DCP) had an average P_{Olsen} of 10.1 mg kg^{-1} while in PIR (HA/TCP) average P_{Olsen} was 6.62 mg kg^{-1} .

The high phosphate solubility in the surveyed soils may be due to heavy and continuous applications of phosphate fertilizers by farmers in every growing season. Al-Tameemi

et al. (2015) studied the solubility of Ca-phosphate minerals in some calcareous soils of south Iraq influenced by phosphate fertilizer application. They found that increasing P rates increased P solubility and Ca-phosphates minerals present were mono-calcium phosphates, octa-calcium phosphate and hydroxyapatite according to their saturation index values. The results of the current study indicate that DCP, OCP and β -TCP minerals are stable forms of P mineral in all studied soils. The results also suggest that the solubility of phosphate minerals appeared to decrease with increasing soil pH value and Ca concentration. Soils in the SHA and HAL locations, supersaturated with respect to OCP (OCP/DCP), contained 26.6 and 60.6 g kg⁻¹ Ca and pH values of 9.97 and 9.95 respectively whereas in PIR, KAL and CHA locations (HA/TCP/OCP) contained 87.5, 92.9 and 96.8 g kg⁻¹ Ca and pH values of 8.01, 8.17 and 8.10 respectively.

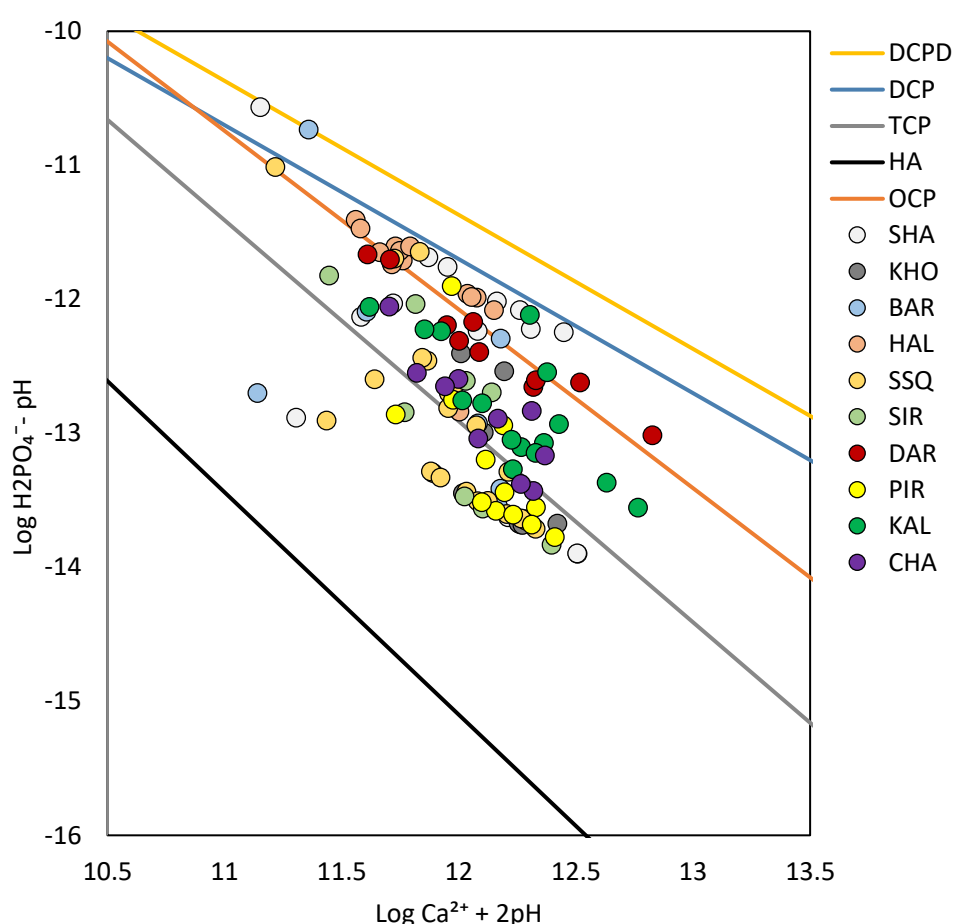


Fig. 6.8: Solubility diagram for phosphate minerals in the surveyed soils in relation to crystalline Ca–P minerals: dicalcium phosphate dihydrate, DCPD; dicalcium phosphate, DCP; Octacalcium

phosphate, OCP; β -tricalcium phosphate, β -TCP; and hydroxyapatite; HA. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Lindsay, 1979).

6.3.4.2 Zinc solubility

A solubility diagram for Zn minerals was developed by plotting the activity of Zn^{2+} ions ($\log Zn^{2+}$) against soil pH. The activity of Zn^{2+} , pH and partial pressure (CO_2) used to develop this diagram, were determined using measured values of pH and Zn concentration in solution with speciation estimated using WHAM-7. This approach can indicate the likelihood of Zn minerals being present in soils at different pH values (Lindsay, 1979, Nazif et al., 2015, Urgan and Rimmer, 2000).

Figure 6.9 presents the soil solution Zn^{2+} activities in relation to common Zn minerals in the studied soils. The solubility of Zn decreased with increasing soil pH as expected. The Zn data were undersaturated with respect to Smithsonite and Hydrozincite across all the surveyed soils. However, the data were remarkably close to the empirical 'Soil-Zn' solubility relation as suggested by Lindsay (1979). Martinez and Motto (2000) studied the solubility of Zn added to mineral soils and found that, in calcareous soils, Zn solubility was not determined by the formation of a single Zn metal compound. In reality, notwithstanding the similarity to the 'Soil-Zn' solubility relation of Lindsay, the data probably indicates *adsorption* of Zn^{2+} on the surfaces of $CaCO_3$, Fe oxides and on humus as suggested by Nazif et al. (2015).

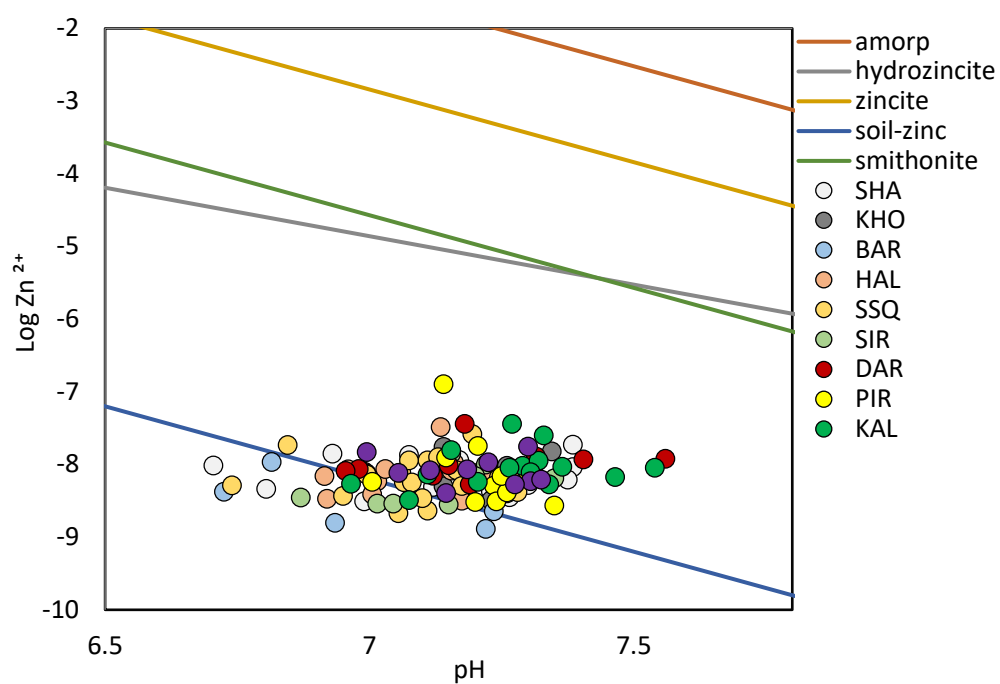


Fig. 6.9: The solubility of Zn in the studied soils in relation to possible Zn minerals: amorphous (amorphous, ZnCO_3), hydrozincite, zincite, soil-Zn, and Smithsonite. Equilibrium lines for mineral phases were calculated based on the K_{sp} values reported by (Nazif et al. 2015).

6.3.5 Phosphorus and Zn contents in wheat grain, flour, and bread

Figure 6.10 shows movement of P and Zn from wheat grain to flour and then to bread in (mg 300g⁻¹). A significant reduction of P and Zn concentrations recorded between wheat grain and flour due to milling process but slightly they are increased from flour to bread may be due to applying yeast and emission of CO₂ during baking process. Overall, the concentration of P and Zn in final product (bread) for 300 g daily intake are below recommended level of 500 mg P d⁻¹ and 4mg Zn d⁻¹ in 300 g bread.

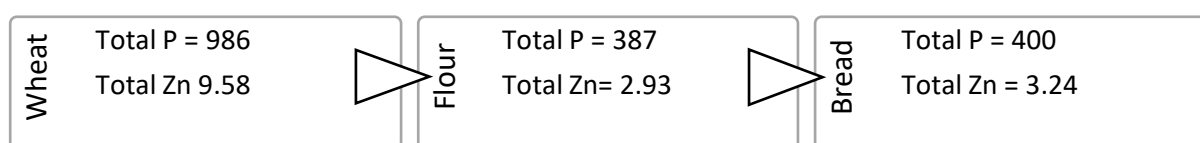


Fig. 6.10: The concentration of P_T and Zn_T in (mg 300g⁻¹) in wheat, flour and bread in the studied samples (120 wheat, 50 flour and bread samples).

6.3.5.1 Phosphorus and Zn distribution in wheat grain

At harvest, the average values of P and Zn concentrations in wheat grain showed significant variation ($p < 0.05$) between locations. The average concentrations of P (mg kg⁻¹) ranged from 2890±694 to 3630±490 for PIR and BAR locations as shown in (Fig. 6.11) respectively. About 40% of wheat grain samples contained higher amounts than the recommended value of 3570 mg kg⁻¹ of P (Databases, 2018), reflecting the high solubility of P in some of the soils probably due to the application of P fertilizer in each growing season by farmers. Alam and Azam Shah (2002) conducted a greenhouse experiment to study the influence phosphate fertilization on P concentration in wheat; they found that application of 40 kg kg⁻¹ of P as SSP increased P concentration in wheat grain from 1978 to 2942 mg kg⁻¹.

Regression analysis indicated that the P concentration in wheat grain was negatively related to soil pH ($r = -0.37$, $p < 0.001$) while, positively related ($r = 0.45$, $p < 0.002$) to

P_{Olsen} in soils as shown in (Fig. 6.12a and b) respectively. Boukhalfa-Deraoui et al. (2015) found a significant correlation ($R^2= 0.787$) between P fertilizer application as MAP and P uptake by durum wheat in the arid conditions in an alkaline sandy soil of southern Algeria. Thus, the high concentration of P in wheat grain samples may reflect the extensive application of P fertilizer to the fields surveyed in the current study.

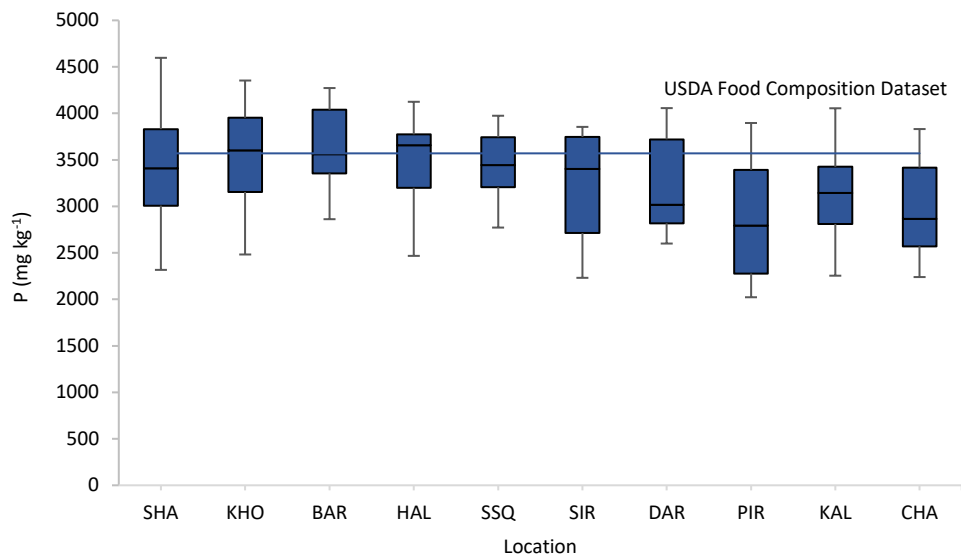
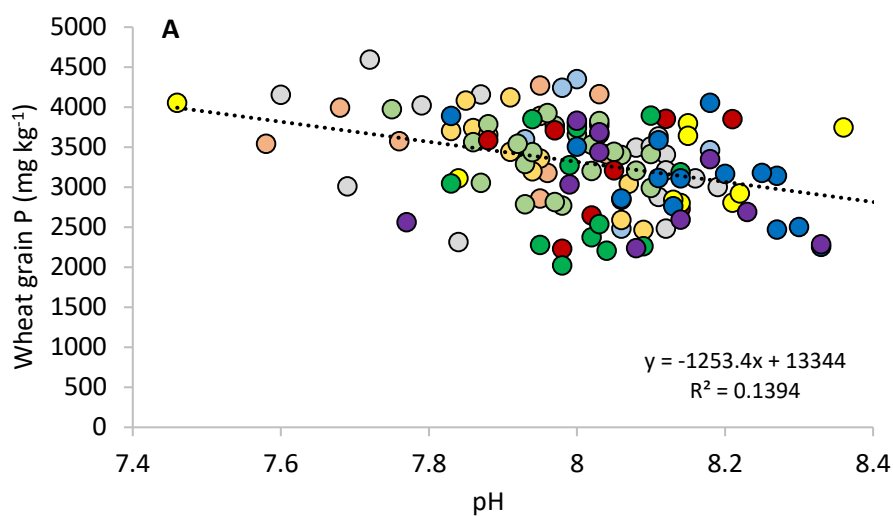


Fig. 6.10: The concentrations of phosphorus in wheat grain samples (n=120) collected at ten different locations in Sulaimanyah province, Iraq.



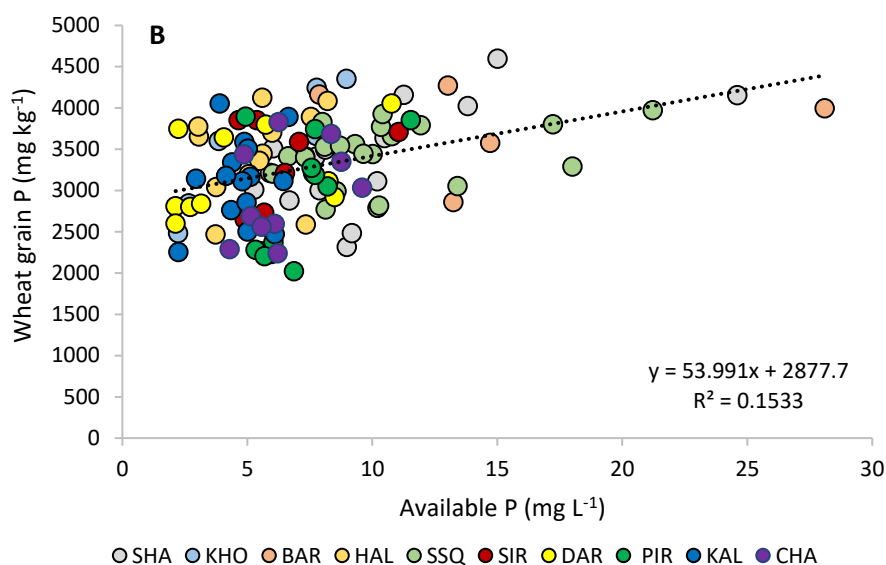


Fig. 6.11: Correlations between P concentration in wheat grain samples with (A) soil pH and (B) available P in soils across all the surveyed wheat grain samples (n=120) collected at different locations in Sulaimanyah province, Iraq.

The average Zn concentration in wheat grain (mg kg^{-1}) ranged from 26.3 ± 7.37 to 35.0 ± 6.11 for CHA and HAL locations respectively as shown (Fig. 6.13). Zinc concentrations in bread wheat ranged from 20 to 40 mg kg^{-1} in a study of 243 wheat genotypes cultivated in normal agricultural soils (Nikolic et al., 2016). Cakmak (2009) reported lower Zn levels of $< 12 \text{ mg kg}^{-1}$ for wheat grain grown in the highly deficient calcareous soils of Turkey. Zhang et al. (2010) reported 29 mg Zn kg^{-1} for three main wheat cultivars, grown in the calcareous soils of China which did not exhibit Zn deficiency. Liu et al. (2014) analysed 655 wheat grain samples grown in the calcareous soils of China from 2009 to 2011, they found that average Zn concentrations were 30.4 and 30.3 mg kg^{-1} for winter and spring wheat respectively. Guttieri et al. (2015) analysed 286 bread wheat cultivars in the USA and observed Zn concentrations ranging from 25 to 33 mg kg^{-1} . Compared to these extensive reports, the average value of 24.3

mg kg⁻¹, suggest Zn in wheat grain grown in Kurdistan is in the lower range of Zn concentrations reported globally.

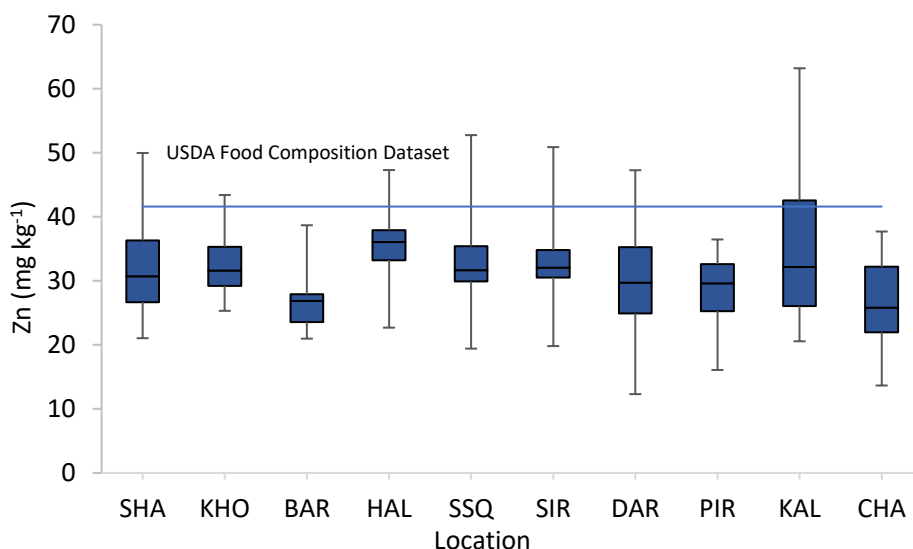
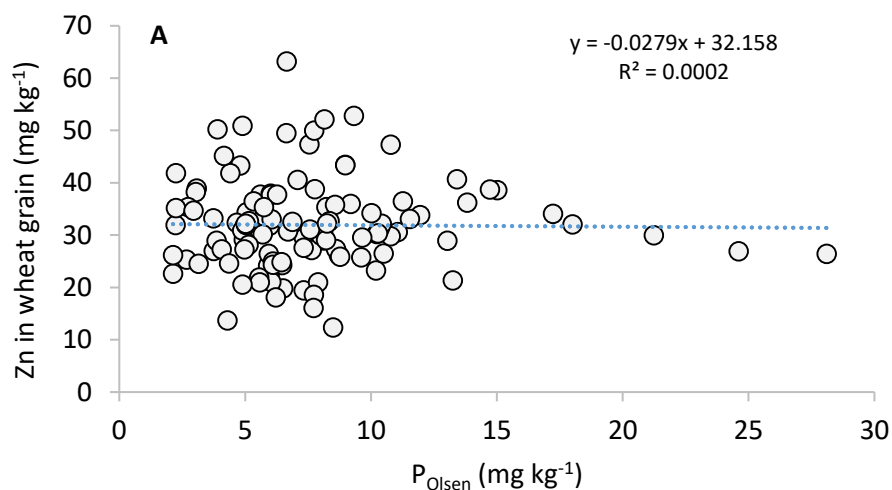


Fig. 6.12: The concentrations of zinc in wheat grain samples (n=120) collected at ten different locations in Sulaimanyah province, Iraq.

Approximately 88% of the surveyed wheat grain samples contained Zn concentrations below the value recommended for human nutrition of 41.6 mg kg⁻¹ by the USDA (Databases, 2018). According to Cakmak (2008) for populations with a predominantly cereal-based diet, the Zn concentration in wheat grain should be as high as 40-60 mg kg⁻¹ to provide an adequate level in the diet. Again, it is clear that the wheat grain grown in the calcareous soils of Kurdistan falls in the lower range of values recommended for adequate human nutrition. This situation arises because of the low availability of Zn in the Kurdistan soils and because farmers do not normally apply micronutrients to wheat farms in the Kurdistan region. The latter observation is presented based on farmer interviews undertaken during the soil and wheat grain surveys. Nikolic et al. (2016) studied the soil availability and wheat grain status of Zn in Serbia and found that wheat grain Zn concentration was negatively correlated with the application of phosphate fertilizer in calcareous soils. Li et al. (2010) studied the

effect of long-term fertilization on soil and wheat grain micronutrients in an Aquic Inceptisol and found that P application significantly decreased wheat grain Zn concentration. However, in the surveyed wheat grain samples, the concentration of Zn was not negatively correlated with P_{Olsen} or with the concentration of P in wheat grains (Fig. 6.14a and b). This may be because farmers apply N (as Urea) along with P fertilizers (as TSP and DAP) each growing season which could decrease the effect of P on Zn concentration in wheat grains due to the synergistic effect of applied N on Zn uptake by wheat plants. Akram et al. (2017) reported that nitrogenous protein has a major role in Zn uptake from soil to root, mobilization within the plant and accumulation. Svecnjaka et al. (2013) studied the effect of N fertilizer application on trace element uptake by wheat grain in a field experiment; they found that application of 194 kg N ha^{-1} as (Urea 46% N) increased Zn concentration from 34.9 to 38.9 mg kg^{-1} in wheat grain. Thus, the application of N fertilizer alongside P fertilizer to wheat grown in the calcareous soils of the Kurdistan region may reduce the antagonistic effect of P on Zn uptake.



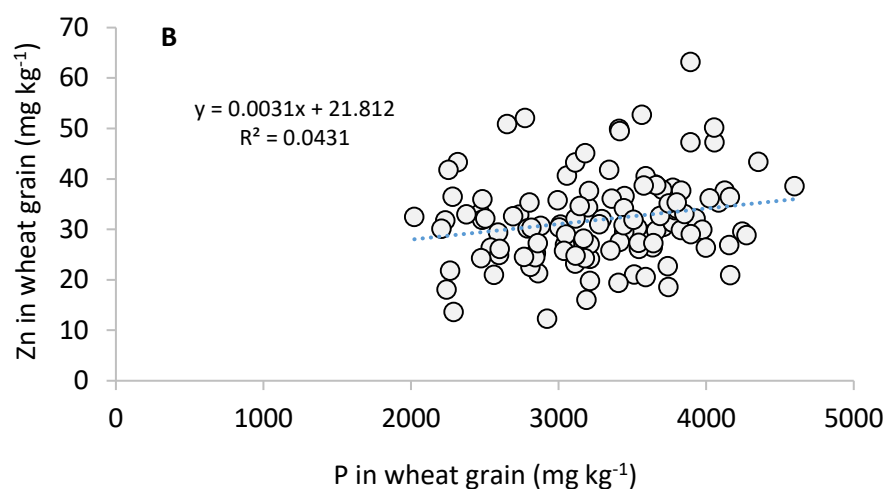


Fig. 6.13: The correlation between (A) P_{Olsen} and (B) P in wheat grain with Zn concentrations in the wheat grains and (n=120).

The principal reason for low concentrations of Zn in wheat grain is probably the presence of high amounts of calcium carbonate in the soils and high pH values. These are the conditions under which Zn fixation occurs, rendering it unavailable for plant uptake (Cakmak and Kutman, 2017, Li et al., 2010). It is well documented that wheat cultivated in calcareous soils contains relatively low concentrations of Zn due to low Zn availability. Thus the application of a Zn biofortification plan could make a substantial contribution to raising the Zn content wheat in Kurdistan.

A critical Zn level of 20 to 24 mg kg⁻¹ has been suggested (Nikolic et al., 2016) for rain-fed wheat grain grown on alkaline calcareous soils in Pakistan as the lowest Zn concentration in grain needed to deliver 95% of the highest grain yield (Karami et al., 2009). In the present survey, only 12% of surveyed samples had Zn concentrations below 24 mg kg⁻¹ dry matter, suggesting that Zn deficiency to the crop may actually be a minor consideration for wheat production in the Kurdistan region, compared to human dietary considerations. Correlations between concentrations of Zn in wheat grain samples with individual soil parameters were not significant.

6.3.5.2 Phosphorus and zinc concentrations in flour and bread

Table 6.5 shows the average P and Zn concentrations, and standard deviations, for five types of bread consumed in the Kurdistan region and their corresponding flours. The flours and bread were divided into five types: Hawrami, Tiry, Lawasha, Mashini and Samoon. All flour samples and most bread were factory-produced but three of the bread samples were homemade products. Hawrami, Tiry, Lawasha and Mashini bread all are thin flatbreads, whereas Samoon is a thick loaf. Samoon is usually used in fast food outlets in the Kurdistan region, while other types of bread are usually used domestically for lunch and dinner.

The data includes average values for the bread. Significant differences ($p < 0.05$) were found between locations and between bread types. The concentrations of P exceed the value of P concentration in wheat flour for bread of 970 mg kg^{-1} reported by the USDA (Databases, 2018). This again reflects the high solubility of P in Kurdistan arable soils. The difference in P concentration between bread types may also be due to the type of flour used for making individual bread types.

The average Zn concentration across all flours, bread types and locations in (mg kg^{-1}) was 9.76 for flours and 10.8 for bread. Results of the current survey also showed that the Zn content in about 76% of flours was lower than the recommended value of 14.7 mg kg^{-1} suggested by the USDA (Databases) to meet human nutritional requirements. Gargari et al. (2007) and Khaniki (2005) reported 14.0 ± 0.32 to 14.3 ± 3.56 and $12.2 \pm 1.91 \text{ mg Zn kg}^{-1}$ for different bread types consumed in Iran which are higher than many of the bread types analysed in the current Kurdistan survey. In fact, the Zn concentration in many of the Kurdistan samples is lower than the maximum limit allowed for baked products and bread in Iran (Khaniki, 2005). Tiry bread had the highest concentration of Zn in the five groups of bread, followed by Hawrami, Lawasha, Samoon and Mashini.

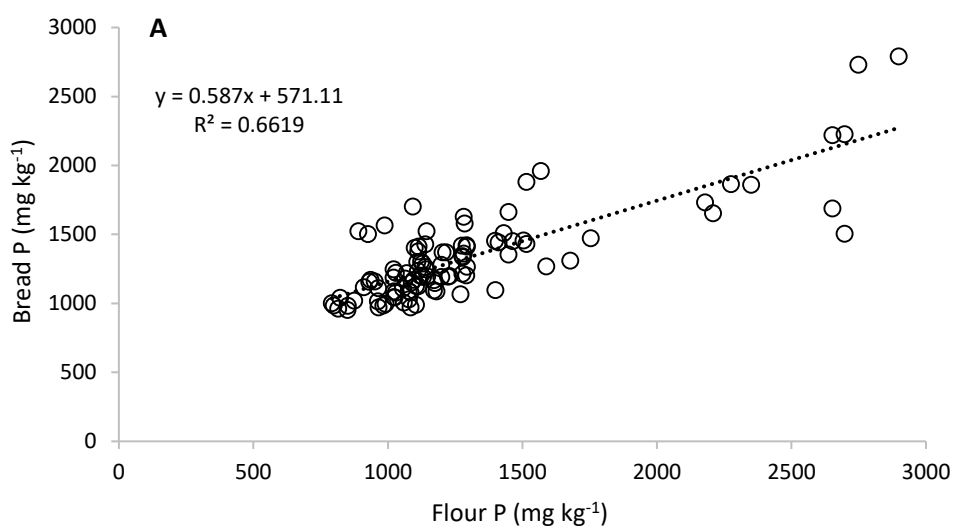
The results showed a slight increase in the concentration of Zn in bread samples during the bread making process. This may be related to loss of water and CO₂ during the bread making process. Flour contains 7% water as standard and loss of CO₂ occurs during the fermentation process from the dough. Furthermore, some Zn may be added in yeast during the fermentation process.

Regression analysis showed strong correlations ($r=0.81$, $p\leq0.001$, and $r=0.75$, $p\leq0.001$) between P and Zn concentrations in flours and bread as shown in (Fig. 6.15a and b) respectively. Phosphorus and Zn concentrations in bread increased with increasing concentrations in the corresponding flour, which helps to validate the study.

Table 6.5: The concentration of P and Zn in flour and corresponding bread samples collected at seven different locations in Sulaimanyah province. Data are arranged in descending order of the average P concentration in flours.

P (mg kg ⁻¹)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	2020	-	1290	-	-	-	-	2310	1870	530
	Bread	1790	-	1240	-	-	-	-	1860	1630	340
Tiry	Flour	2020	-	1290	1450	-	-	-	1540	1570	320
	Bread	1620	-	1380	1500	-	-	-	1920	1610	230
Lawasha	Flour	1050	1510	1150	1090	1240	1100	1190	2820	1400	600
	Bread	1080	1550	1130	1070	1180	1190	1240	2760	1400	570
Mashini	Flour	1300	-	998	1300	1090	1130	-	-	1160	130
	Bread	1210	-	1010	1360	1360	1430	-	-	1270	170
Samoon	Flour	1160	1120	1070	1280	984	807	933	-	1050	160
	Bread	1260	1330	1260	1370	1080	998	1070	-	1200	140

Zn (mg kg ⁻¹)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	16.6	-	9.63	-	-	-	-	25.4	17.2	7.92
	Bread	14.7	-	11.9	-	-	-	-	14.7	13.8	1.63
Tiry	Flour	16.6	-	9.63	12	-	-	-	8.34	11.6	3.64
	Bread	14.9	-	12.8	13.9	-	-	-	18	14.9	2.25
Lawasha	Flour	7.34	12.4	8.31	7.72	9.27	8.43	8.85	30	11.5	7.63
	Bread	7.45	13.2	9.37	8.43	8.82	10.4	10.4	30.5	12.3	7.55
Mashini	Flour	9.48	-	5.34	9.73	8.27	7.77	-	-	8.12	1.75
	Bread	8.5	-	6.54	10.6	10.3	8.5	-	-	8.9	1.65
Samoon	Flour	7.73	7.2	7.07	9.44	6.9	5.45	5.48	-	7.04	1.37
	Bread	8.94	10.5	10.2	9.96	9.09	7.9	7.96	-	9.23	1.05



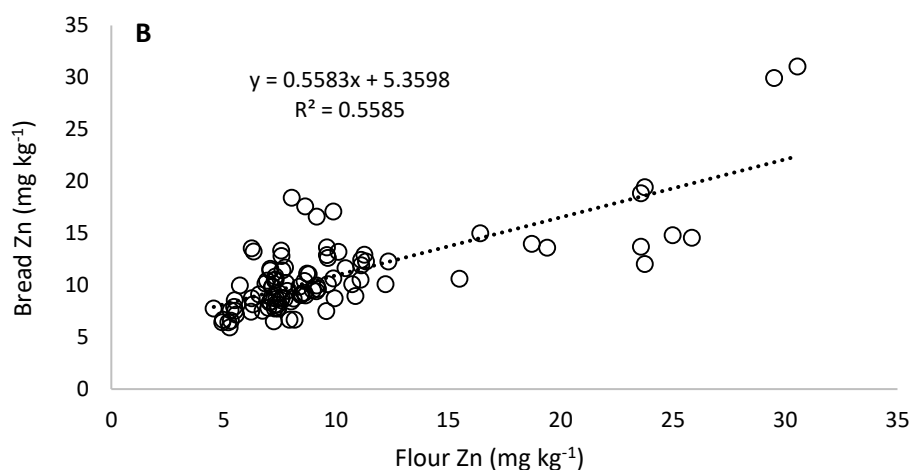


Fig. 6.14: Correlations between P (A) and Zn (B) concentration in flour and corresponding bread samples collected at locations in Sulaimanyah province.

6.3.6 Hierarchical clustering analysis in wheat grains

In order to investigate correlations between wheat grain elements and some selected soil parameters, hierarchical clustering analysis was used and presented as a dendrogram for 120 wheat grain samples (Fig. 6.16). There were three main groups with high similarity (about 75%). Group, I included S, Cu and Zn which may simply reflect metabolic processes in the wheat plants, rather than a geochemical association. Nan et al. (2002) found a strong positive correlation between Zn and Cu in spring wheat grown on contaminated soils which were ascribed to the metabolic processes of the wheat grain. Group II, included Ca, As, Al, Fe, Co, Pb, Ni, U and Mn which probably reflects contamination of wheat grains by soils. A high correlation was found between Fe with V and Fe with Al in the wheat grains, which strongly supports the contamination of wheat grains by soils (Fig 6.17). The pH value had a weak effect on heavy metals accumulation in wheat grains, while as being highly correlated with Ca be due to the co-occurrence with Ca in calcareous soils. Jia et al. (2010) found that most of the Cr, Ni, Cu, Zn, As, Cd and Pb in wheat grains reflected the composition of the soil in which it was grown. Group III, including the major nutrients Mg, P and K, probably indicates the effect of NPK fertilizer application on the composition of wheat grain. Cadmium was

also closely related to this group, possibly indicating contamination from P fertilizer application, as has been well documented.

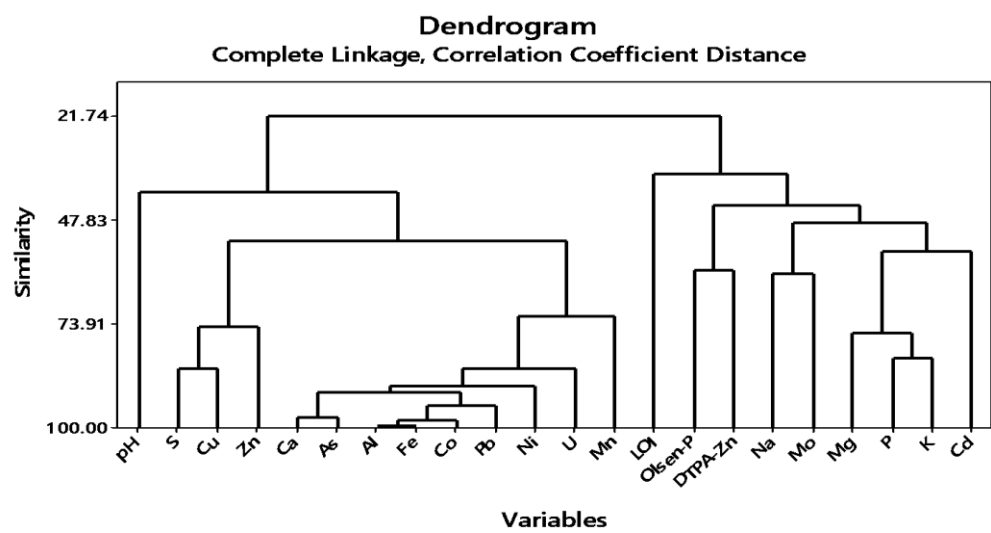
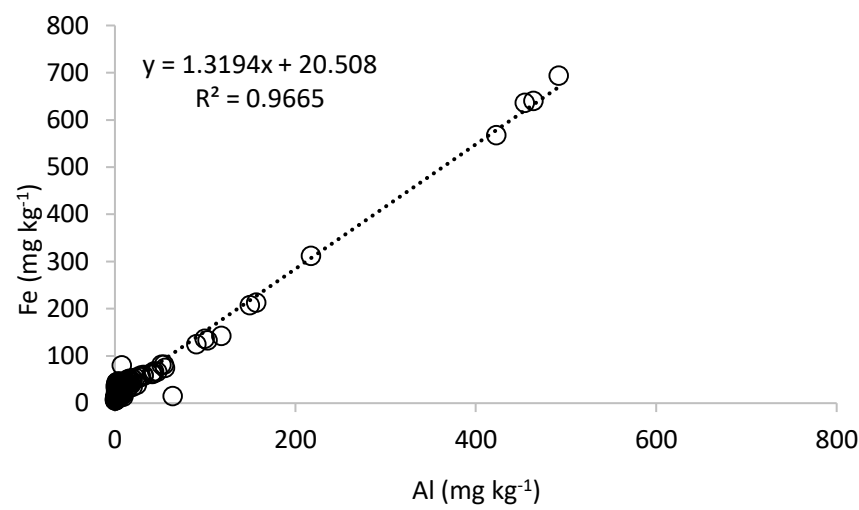


Fig. 6.15: Dendrogram analysis of the hierarchical cluster analysis of soil characteristics with the elemental composition of wheat grain samples (n=120).



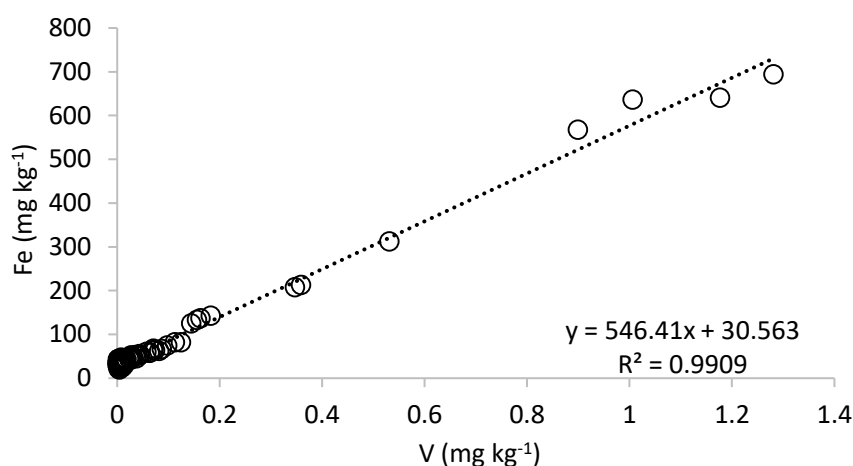


Fig. 6.16: Correlation between Fe and Al, and Fe and V, across all studied wheat grain samples.

6.3.7 PA concentration and PA/Zn molar ratio

6.3.7.1 PA concentration and PA/ Zn molar ratios in wheat grain

Figure 6.18 presents PA content in wheat grain samples; significant differences ($p < 0.05$) were found between locations. Average values of PA contents in wheat grains, in units of $\text{g } 100 \text{ g}^{-1}$, showed a large variation between locations (2-fold variation). In the present survey, the average PA content in the whole wheat grains ranged from 0.54 ± 0.16 for HAL to 0.93 ± 0.11 for SSQ locations respectively as shown in (Fig. 6.15). About $63.1 \pm 12.2\%$ of P in the wheat grain was present as PA. The results of the PA content is compared with other countries in Table 6.6. The results show that the PA content in the current survey is within the range of other countries. Moreover, the results mostly indicate that PA content in wheat grain grown in the calcareous soils of Kurdistan positively with PA content in wheat grain from another part of the world as shown in Table 6.6.

The regression analysis indicates that PA in wheat grains was positively correlated ($R^2=0.18$, $p<0.001$) with P_{Olsen} in the surveyed soils (Fig. 6.19). This result indicates that the application of P fertilizer increased PA content in wheat grains.

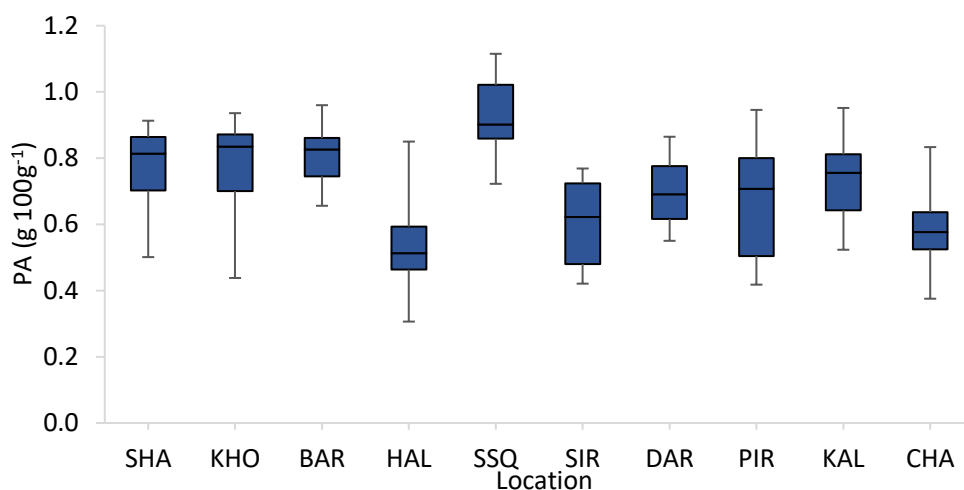


Fig. 6.17: Distribution of phytic acid (PA) in wheat grain samples (n=120), collected at ten different locations in Sulaimanyah province, Kurdistan region, Iraq.

Table 6.6. Comparison of Phytic acid content in wheat grain from several countries.

Phytic (g 100g ⁻¹)	Wheat No.	Country	References
0.42-1.12	120	Kurdistan region	Current study
0.46-0.95	46	Mexico	Magallanes-Lopez et al., (2017)
1.46-1.69	15	Serbia	Brankovic et al., (2015)
0.71-1.11	65	Pakistan	Hussain et al., (2012)
0.52-0.98	186	China	Liu et al., (2006)
0.60-1.00	100	Canary Islands	Febles et al., (2002)

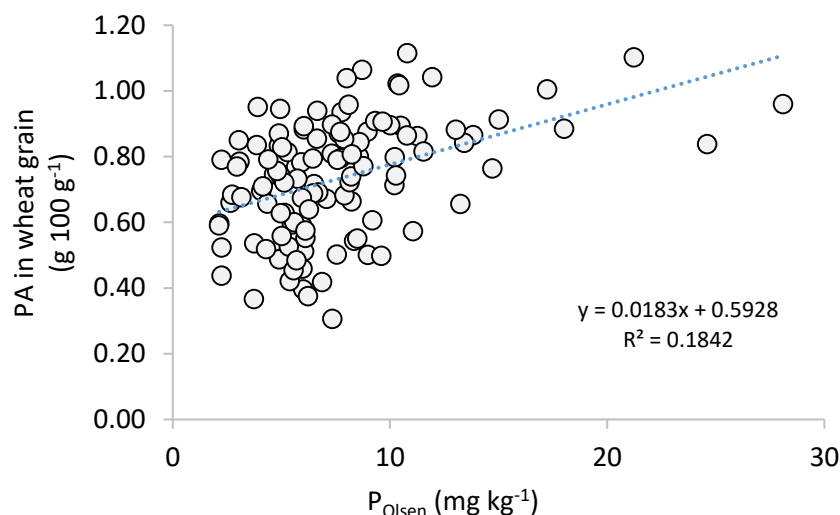


Fig. 6.18: Correlation between PA content in wheat grains and available P in corresponded soil (n=120).

The regional average PA/Zn molar ratio for whole wheat grains ranged between 15.7 ± 5.06 and 30.6 ± 6.18 for HAL and BAR locations respectively (a 2-fold variation) as shown in (Fig. 6.20), with a significant difference was found between locations ($p < 0.05$). Hussain et al. (2012a) and Tavajjoh et al. (2011) reported that PA/Zn molar ratios ranged from 23.9 to 41.4 and 26.5 to 26.9 in 65 and 17 bread wheat and bread wheat genotypes grown in the calcareous soils of Pakistan and Iran respectively. Compared to those reported for wheat grain grown in the calcareous soils, results of the present survey show that PA/Zn molar ratio is within a comparable range.

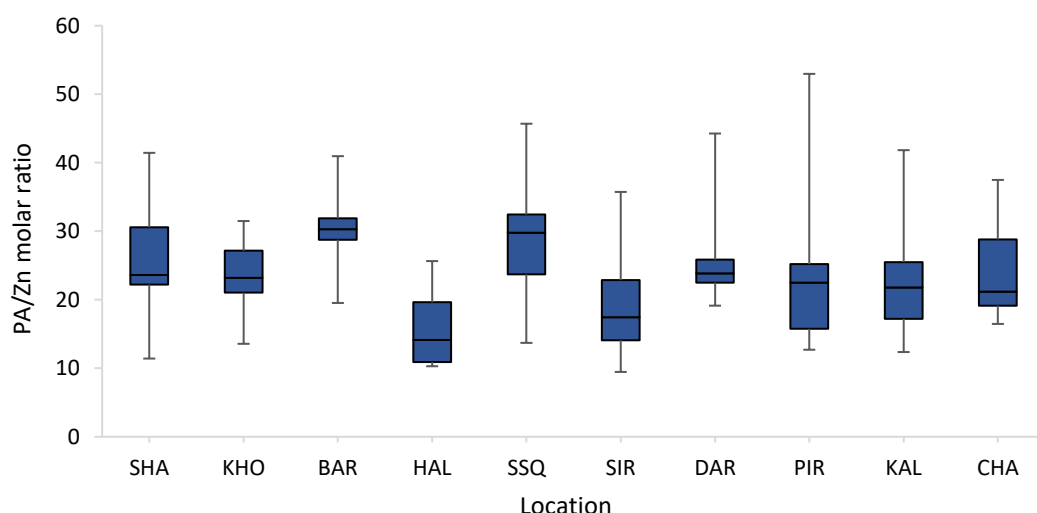


Fig. 6.19: Distribution of phytic acid to zinc molar ratio (PA/Zn) in wheat grain samples (n=120), collected at ten different locations in Sulaimanyah province, Kurdistan region, Iraq.

The main reasons for PA and PA/Zn molar ratio differences appear to be location, soil type, variety, climatic conditions, phosphate fertilizer application and the presence or absence of Zn fertilization. Magallanes-Lopez et al. (2017) studied PA content and PA/Zn molar ratio in a worldwide collection of commercial durum wheat and found that environmental factors caused a difference in wheat grain PA concentration between 0.46 and 0.95 g 100g⁻¹. Erdal et al. (2002) studied PA in twenty wheat cultivars grown with and without Zn application in the calcareous soils of Turkey and wheat grown in 55 different locations in Central Anatolia. They reported that the application of 23 kg Zn ha⁻¹ to soils, reduced the PA contents of wheat grains from 1.07 to 0.91 g 100g⁻¹ and PA/Zn molar ratio from 126 to 55. These were due to a reduction of P concentration in wheat grains from 3900 to 3500 mg kg⁻¹ and increasing Zn concentration from 14 to 23 mg kg⁻¹. In the calcareous soils of Kurdistan, P deficiency occurs due to high calcium content. Therefore, farmers apply phosphate fertilizer to improve yield which causes an increase in PA content in wheat grain and in the PA/Zn ratio.

The PA/Zn molar ratio has been used as an indicator of Zn bioaccessibility by the International Zinc Nutrition Consultative Group (IZiNCG, 2004). The PA/Zn molar ratio is of course affected by the concentrations of both PA and Zn in wheat grains. Considering the PA/Zn molar ratios observed in the current survey, and in previous studies on calcareous soils, it is apparent that ratios are not acceptable when durum wheat is the staple food and principal source of dietary Zn. The relatively high PA/Zn molar ratio in the current survey (average of 24.3 ± 8.59) suggests that wheat biofortification with Zn and decreasing the application of P fertilizer are urgent priorities in the Kurdistan region. Developing cereal cultivars with low PA contents and a high affinity for Zn would also be useful strategies.

6.3.7.2 PA concentration and PA/Zn molar ratio in flour and bread

Table 6.7 presents the PA concentrations and PA/Zn molar ratios of flour and corresponding bread samples consumed in Sulaimanyah province. Hawrami and Tiry bread had, on average, higher PA contents, followed by Lawasha, Mashini and Samoon, whereas the PA/Zn molar ratio in Lawasha bread was on average higher followed by Mashini, Tiry, Hawrami and Samoon bread. No significant differences were found ($p < 0.05$) between flours while in bread there were significant differences. Phytic acid in flour and bread samples ranged between 0.16-0.31 and 0.08-0.16 g 100⁻¹ respectively and the PA/Zn molar ratio ranged between 15.9-22.9 and 8.95-14.3 respectively. A slightly similar range for PA and PA/Zn molar ratio for flour and bread consumed in Iran was reported by Roohani et al. (2012) and Gargari et al. (2007) for bread with 0.252-0.485 g 100g⁻¹ and 18-29 for flatbread and 0.211-0.315 g 100g⁻¹ and 14.9-23.8 for all flour types and 0.094-0.133 g 100g⁻¹ and 7.76-10.1 for all types of Iranian consumed bread respectively. The preparation method for breadmaking in the two societies are similar.

Regarding the average values of PA/Zn ratio, the flour and bread samples showed low-to-moderate Zn bioavailability, meanwhile, the molar ratio for all surveyed samples was in the range 24.4 and 12.1 for flour and bread samples respectively. According to Jambrec et al. (2016) only 55% of the Zn content of diets is estimated to be absorbed if the PA/Zn ratio of diets is less than 5, but only 35% of Zn would be absorbed if the ratio is between (5-15) and less than 15% would be absorbed if the ratio is more than 15. Therefore, Zn bioavailability in Kurdistanian flour is low (less than 15%) whereas, Zn bioavailability in bread is moderate (35%) due to low PA concentrations.

Lower PA and PA/Zn molar ratios in bread than flours can be caused by fermentation during breadmaking the fermentation process decreases PA content and hence the PA/Zn molar ratio. This is probably due to the action of endogenous phytase in flour as well as microbial phytase which together cause hydrolysis of PA (Magala et al., 2015, Ahmed et al., 2014a, Frontela et al., 2011). Regarding bread type, the smallest reductions of PA and PA/Zn molar ratio (15.8 and 17.4%) were observed in Lawasha bread and the highest reductions of 50 and 61% were observed in Samoon bread reflecting differences in fermentation time and activation of yeast phytase during this process. Samoon bread needs overnight fermentation while other flatbreads can be made after just 1 h of fermentation. Pozrl et al. (2009) and Qazi et al. (2003) studied the effect of flour type and the breadmaking procedure on the loss of PA. They found that PA in white flour decreased from 0.37 ± 0.004 to 0.10 ± 0.001 and whole-wheat bread from 0.662 to $0.280 \text{ g } 100\text{g}^{-1}$ when the fermentation process was increased from 1 h to 6 h and 15 to 45 mins respectively. Frontela et al. (2011) found that the baking process decreased PA content from 58.6 to $9.4 \text{ mg } 100\text{g}^{-1}$ in white bread and from 7.2 to $4.4 \text{ mg } 100\text{g}^{-1}$ in muffins. The use of yeast also affects the decrease in PA content. Chhabra and Sidh (1988) studied the fate of PA during breadmaking and found that increasing yeast addition to dough from 1.5% to 3% increased PA loss from 46.2 to 51.7% in white bread.

Table 6.7: Phytic acid and phytic acid to Zn molar ratio in 50 flour and corresponding bread samples collected in Sulaimanyah province, Kurdistan region, Iraq.

PA (g 100 g ⁻¹)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	0.32	-	0.14	-	-	-	-	0.48	0.31	0.17
	Bread	0.2	-	0.05	-	-	-	-	0.29	0.18	0.12
Tiry	Flour	0.32	-	0.14	0.45	-	-	-	0.27	0.30	0.13
	Bread	0.1	-	0.1	0.2	-	-	-	0.3	0.18	0.10
Lawasha	Flour	0.13	0.26	0.15	0.11	0.16	0.12	0.17	0.61	0.21	0.17
	Bread	0.11	0.28	0.14	0.14	0.17	0.08	0.11	0.3	0.17	0.08
Mashini	Flour	0.15	-	0.13	0.2	0.23	0.14	-	-	0.17	0.04
	Bread	0.1	-	0.09	0.13	0.08	0.16	-	-	0.11	0.03
Samoon	Flour	0.16	0.15	0.16	0.18	0.15	0.12	0.17	-	0.16	0.02
	Bread	0.07	0.1	0.09	0.1	0.07	0.07	0.08	-	0.08	0.01

PA/Zn molar ratio		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	14.4	-	14.4	-	-	-	-	18.8	15.9	2.54
	Bread	4.15	-	4.15	-	-	-	-	19.2	9.17	8.69
Tiry	Flour	14.4	-	14.4	26.9	-	-	-	32.2	22.0	9.01
	Bread	5.66	-	5.66	13.6	-	-	-	15.6	10.1	5.23
Lawasha	Flour	17.5	19.1	17.4	14.7	17.6	13.9	19.3	20.1	17.5	2.18
	Bread	15.1	20.8	14.6	16	18.7	7.93	11.3	9.68	14.3	4.41
Mashini	Flour	16.5	-	23.3	20.1	27.7	17.8	-	-	21.1	4.51
	Bread	12.1	-	14.2	12.1	7.99	18.4	-	-	13.0	3.79
Samoon	Flour	20.3	20	23.1	20.8	22.5	22.5	31.1	-	22.9	3.81
	Bread	7.83	9.62	8.89	9.51	7.45	9.37	10	-	8.95	0.96

6.3.8 Estimated Zn bioavailability

Considering dietary Zn intake from wheat, it is not just the concentration of Zn in grain that is important, but the proportion that is available for absorption. Magallanes-Lopez et al., (2017) reported that the PA in wheat grain is considered to be an anti-nutrient because it chelates Zn during digestion and prevents its systemic absorption. Zinc bioavailability decreases due to this chelation reaction between PA and soluble Zn (Khaniki, 2005; Lonnerdal, 2000).

6.3.8.1 Estimated Zn bioavailability in wheat grain

Estimated Zn bioavailability of wheat grain in (mg d^{-1}) ranged from 1.84 ± 0.08 for BAR to 2.65 ± 0.05 for HAL locations respectively, when the Imran et al., (2016) parameters were used. The equivalent values were 1.52 ± 0.08 - 2.29 ± 0.05 for the Miller et al., (2007) values and 1.65 ± 0.08 - 2.46 ± 0.05 for equation values proposed by Brawn et al., (2010), as shown in Fig. 6.21; a significant difference ($p < 0.05$) was found between locations. The results indicate that in all the wheat grain samples, bioavailable Zn is lower than the optimum level of 3 mg for daily consumption of 300 g of wheat (Hussain et al., 2013) for communities dependent on wheat grain for Zn intake. Maqsood et al. (2014) determined estimated Zn bioavailability in 58 wheat cultivars grown in the wheat-cotton zone in calcareous soils in Punjab, Pakistan using a trivariate model. They reported that estimated bioavailable Zn ranged from 0.8 to 2.4 $\text{mg } 300 \text{ g}^{-1}$ with an average value of 1.5. Hussain et al. (2012a) also analysed 65 wheat grains grown in calcareous soils of Pakistan and found that estimated Zn bioavailability ranged from 1.52 to 2.25 $\text{mg } 300 \text{ g}^{-1}$. Compared to these reports, the result of the Kurdistan survey shows that the estimated bioavailable Zn in wheat grain is in the range of Zn bioavailability reported globally for wheat grown in the calcareous soils.

On average, about 23.3 ± 3.48 , 19.6 ± 2.99 and $21.2 \pm 2.91\%$ of grain Zn was actually bioavailable depending on Imran et al. (2016), Miller et al. (2010) and Brown et al.

(2007) parameters respectively. Low bioavailability of Zn in the wheat was probably due to high PA/Zn molar ratio caused by a high concentration of P in wheat grains and low Zn concentrations. A field experiment conducted by Hussain et al. (2013) showed that application of 18 kg Zn ha⁻¹ as ZnSO₄·7H₂O, increased estimated Zn bioavailability by 47% in 300 g wheat flour. Li et al. (2015) conducted a two-year field experiment to investigate the effects of P and Zn fertilization on Zn bioavailability in wheat grain. They found that estimated Zn bioavailability in whole grain was more in the Zn-alone treatments than in combined P+Zn treatments.

Fig. 6.21 shows correlations between estimated bioavailable Zn and measured PA concentration for all the surveyed wheat grains. As expected from the trivariate models used, the correlations were negative ($r=-0.46$, -0.47 and -0.42 , $p<0.001$) (Fig. 6.22). According to the data, the estimated bioavailable Zn decreased by 19.7%, 15.7% and 15.2% when the concentration of PA intake increased from 885.6 to 3526 mg 300g⁻¹ in wheat grains (the full range of measured PA data). Khoshgoftarmanesh et al. (2017) reported that the amount of dietary Zn intake mainly depends on PA and decreases with increasing PA concentration. Miller et al. (2015) studied the effect of PA on Zn absorption in children using stable-isotope studies and found that the absorption of Zn decreased by 0.04 mg d⁻¹ for every increment of 500 mg d⁻¹ intake of PA.

Present survey indicates that estimated Zn bioavailability, estimated from the trivariate models, in wheat grain grown in different locations of Kurdistan, is sub-optimum for human health.

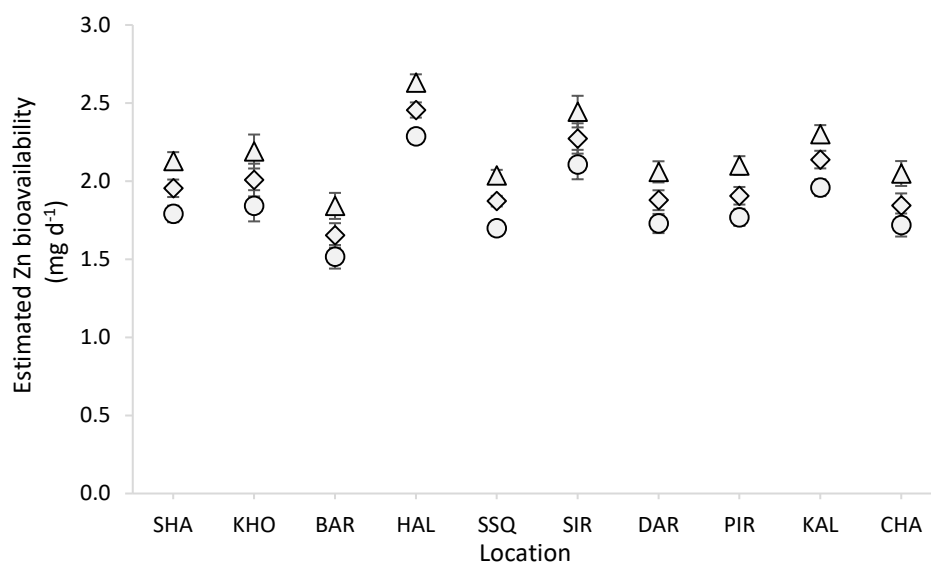


Fig.6.20: Estimated Zn bioavailability in mg d^{-1} for consumption of 300 g wheat grain. Samples were collected from ten different locations in Sulaimanyah province; Zn bioavailability was estimated using trivariate models with three different values for K_{MAX} , K_{P} and K_{R} reported by (Δ) Imran et al., (2016), (O) Brown et al., (2010) and ($\diamond$) Miller et al., (2007).

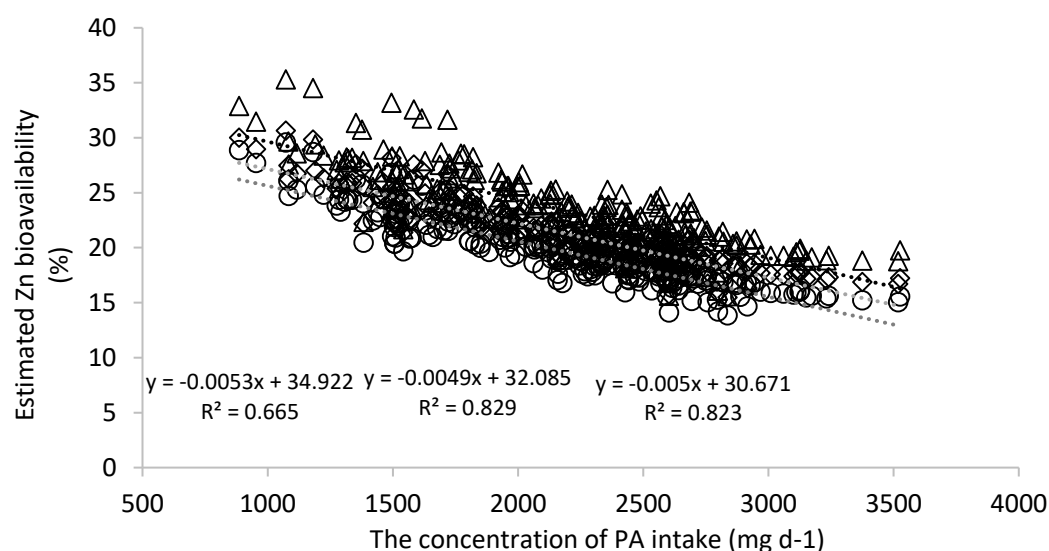


Fig. 6.21: Correlations between estimated Zn bioavailability (%) and PA intake from consumption of 300 g day^{-1} of wheat grain. Samples were collected from ten different locations in Sulaimanyah province; human Zn bioavailability was calculated using trivariate models with different A_{MAX} , K_{R}

and K_p values reported by (Δ ; $R^2= 0.6647$) Imran et al. (2016), (O ; $R^2=0.8288$) Brown et al. (2010) and ($\diamond$; $R^2=0.823$) Miller et al. (2007).

6.3.8.2 Estimated Zn bioavailability in flour and bread

Average estimated Zn bioavailability in flours and bread (mg d^{-1}) showed significant ($p<0.05$) variation among bread types but not between locations. The lowest estimated Zn bioavailability was observed in Samoon flour bread and the highest was in Hawrami (Table 6.8). As discussed previously the difference in estimated Zn bioavailability between bread types is probably due to the use of different flour extraction rates and times of fermentation.

As seen previously for PA concentrations, the estimated Zn bioavailability was higher in bread than in corresponding flour. Estimated bioavailable Zn in bread increased by 4.19-29.3, 3.75-31.4 and 3.07-31.6% over flour, when Imran et al. (2016), Miller et al. (2007) and Brown et al. (2010) parameters were employed respectively. As discussed previously, this is due to the degradation of PA during the bread making process. The regression analysis showed strong negative correlations ($r=-0.66$, $p<0.001$ and $r=-0.29$, $p<0.003$) between estimated Zn bioavailability in flours and bread with the concentration of PA intake as shown in (Fig. 6.23a and b). The percentage of estimated bioavailable Zn, decreased by 29% and 10% when PA increased from 232 to 1900 and 116 to 1000 mg d^{-1} for flour and bread respectively. The highest increase in the estimated Zn bioavailability between flour and bread was observed in Tiry and Samoon bread, while the lowest was in Mashini bread. The results in this survey indicate that Zn bioavailability in consumed bread is acceptable with bioaccessibility of about 43 ± 6 and $47\pm4.6\%$ of Zn indicating moderate Zn bioavailability.

The regression analysis showed that the estimated bioavailable Zn in bread was positively ($r=0.60$, $p<0.001$) correlated to estimated bioavailable Zn in flours (Fig. 6.24). Among all bread types, the highest correlation was found in Samoon and lowest in Hawrami. Also, regression analysis showed that PA concentrations were strongly ($r=0.86$, $p<0.001$) correlated to inorganic P in flour samples while in bread samples, this correlation was not significant ($r=0.16$, $p<0.12$) (Fig. 6.25), probably due to changes in P_i and PA concentrations in the bread during the baking process.

Table 6.8: Estimated Zn bioavailability contents for flour and corresponding bread samples collected from seven different locations in Sulaimanyah province. The estimated values of Zn bioavailability were calculated using trivariate models with different A_{MAX} , K_R and K_P .

Imran et al. (2016)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	1.78	-	1.49	-	-	-	-	2.22	1.83	0.3
	bread	1.91	-	2.06	-	-	-	-	1.76	1.91	0.74
Tiry	Flour	1.54	-	1.49	1.64	-	-	-	1.11	1.45	0.2
	bread	2.13	-	2.09	1.89	-	-	-	2.07	2.05	0.09
Lawasha	Flour	1.19	1.51	1.30	1.28	1.38	1.37	1.33	2.27	1.45	0.32
	bread	1.25	1.63	1.46	1.33	1.32	1.73	1.65	2.93	1.66	0.51
Mashini	Flour	1.44	-	0.90	1.39	1.16	1.24	-	-	1.23	0.19
	bread	1.41	-	1.38	1.50	0.67	1.30	-	-	1.25	0.3
Samoon	Flour	1.44	1.15	1.10	1.37	1.10	0.92	0.86	-	1.13	0.2
	bread	1.56	1.71	1.69	1.64	1.58	1.39	1.38	-	1.56	0.12

Miller et al. (2007)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	1.54	-	1.22	1.41	-	-	-	1.99	1.54	0.28
	bread	1.62	-	1.67	-	-	-	-	1.5	1.6	0.07
Tiry	Flour	1.54	-	1.03	-	-	-	-	0.91	1.16	0.27
	bread	1.66	-	1.72	1.59	-	-	-	1.79	1.69	0.07
Lawasha	Flour	0.96	1.27	1.06	1.03	1.13	1.11	1.09	2.07	1.22	0.33
	bread	1	1.37	1.19	1.33	1.08	1.41	1.35	2.7	1.43	0.5
Mashini	Flour	1.17	-	0.72	1.14	0.95	1	-	-	1	0.16
	bread	1.14	-	0.91	1.34	1.3	1.06	-	-	1.15	0.16
Samoon	Flour	0.97	0.93	0.89	1.13	0.88	0.73	0.69	-	0.89	0.14
	bread	1.25	1.39	1.35	1.33	1.28	1.11	1.11	-	1.26	0.1

Brown et al. (2010)		HAL	KHO	SSQ	SUL	PIR	CHA	KAL	HMD	Average	Standard deviation
Hawrami	Flour	1.49	-	1.25	-	-	-	-	1.99	1.58	0.31
	bread	1.62	-	1.78	-	-	-	-	1.5	1.63	0.11
Tiry	Flour	1.49	-	1.25	1.35	-	-	-	0.91	1.25	0.21
	bread	1.68	-	1.8	1.6	-	-	-	1.79	1.72	0.08
Lawasha	Flour	1	1.26	1.09	1.08	1.16	1.15	1.11	2.07	1.24	0.32
	bread	1.05	1.35	1.23	1.36	1.1	1.48	1.39	2.7	1.46	0.49
Mashini	Flour	1.2	-	0.75	1.16	0.95	1.03	-	-	1.02	0.16
	bread	1.19	-	0.95	1.38	1.34	1.09	-	-	1.19	0.16
Samoon	Flour	0.99	0.96	0.91	1.14	0.91	0.76	0.71	-	0.91	0.13
	bread	1.33	1.45	1.41	1.39	1.35	1.18	1.17	-	1.33	0.1

P-value= 0.001 for both pieces of bread type.

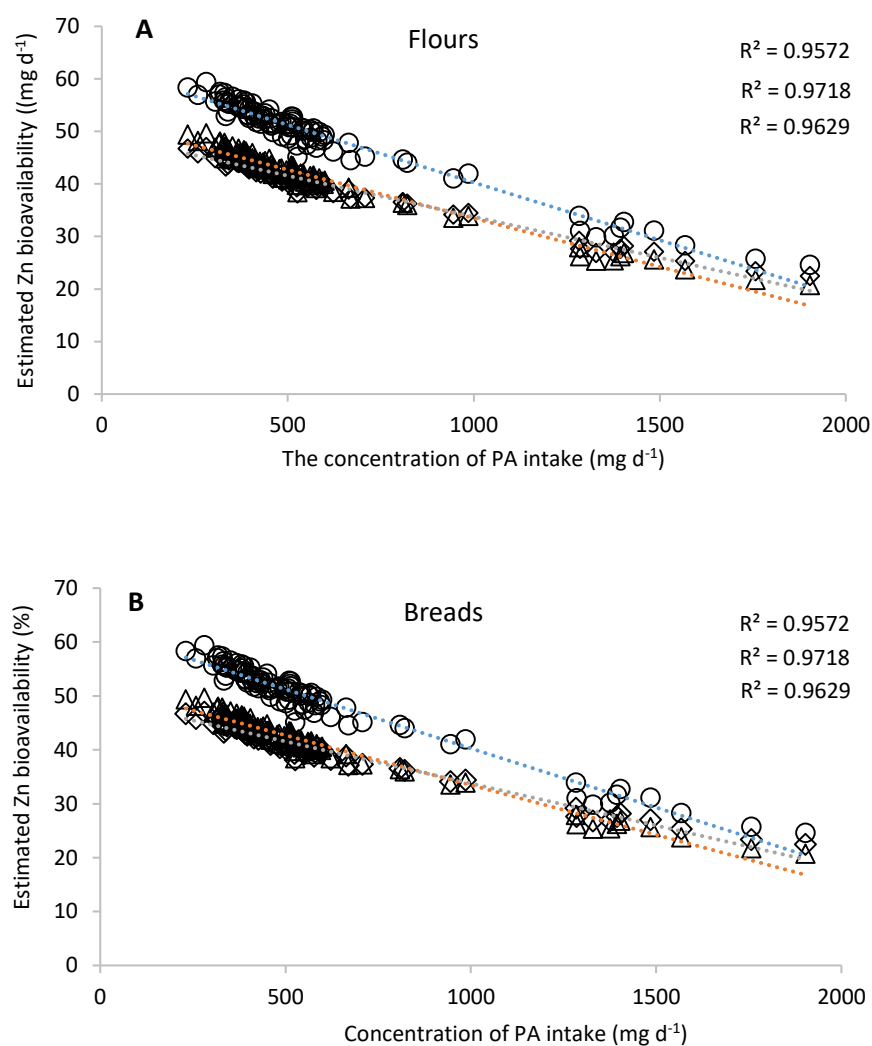


Fig. 6.22: Estimated Zn bioavailability and measured PA intakes from 300g day^{-1} of flour or bread. Samples were collected from seven different locations in Sulaimanyah province; estimated human Zn bioavailability was calculated using trivariate models with different A_{MAX} , K_R and K_P values reported by (O) Imran et al. (2016), (Δ) Brown et al. (2010) and ($\diamond$) Miller et al. (2007).

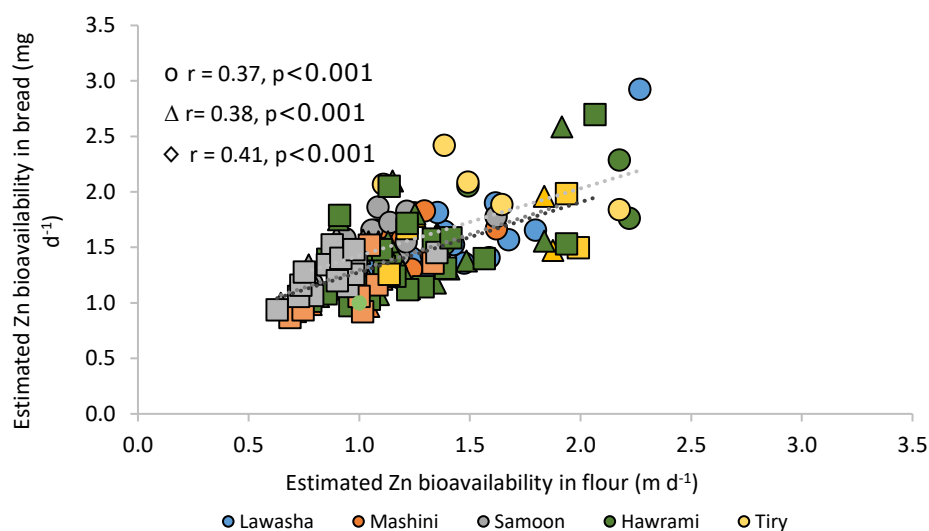


Fig. 6.23: Correlation between estimated Zn bioavailability from consumption of 300g day⁻¹ of bread or corresponding flours. Values were estimated from trivariate models with different A_{MAX} , K_R and K_P values reported by (O) Imran et al. (2016), (Δ) Brown et al. (2010) and ($\diamond$) Miller et al. (2007).

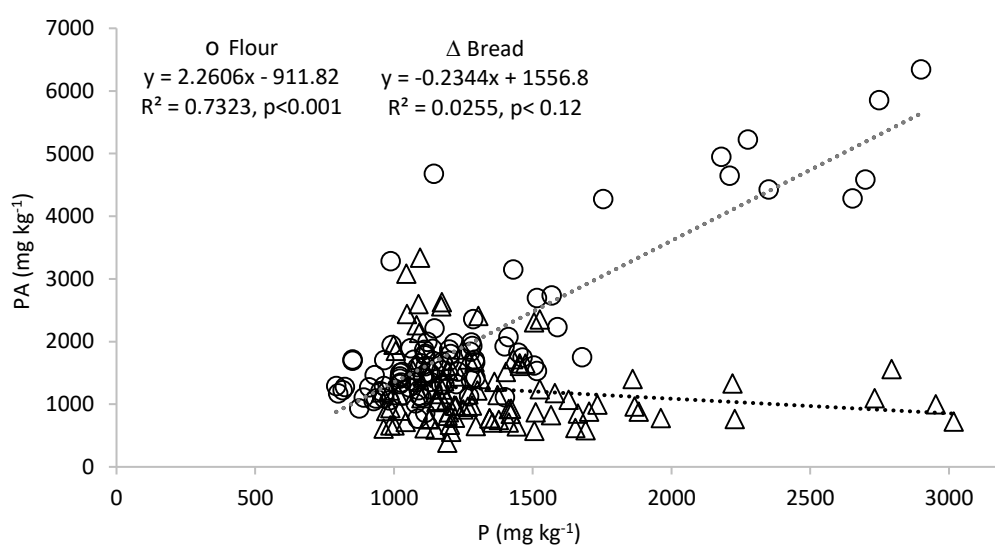


Fig. 6.24: Correlation between phytic acid and the concentration of P in flour (O) and bread (Δ) samples collected from seven different locations in Sulaimanyah province.

6.4 Conclusions

Concentrations of P and Zn in calcareous soil samples from Kurdistan differed significantly between sample locations. There was only limited evidence of pH control over 'available' indices of Zn and P but this may be because of the restricted range of soil pH values in the samples. In the majority of soil samples 87% of P_{Olsen} , 78% of Zn_{DTPA} and 98% of Zn_{Sol} were less than the critical levels for optimum plant growth while in about 47% of the soil samples, P_{Sol} exceeded the critical level. Log K_d values between 1.76-3.51 for Zn, expressed as $Zn_{\text{DTPA}}/Zn_{\text{Sol}}$, were approximately two orders of magnitude greater than similar values between 0.12-2.13 ($P_{\text{Olsen}}/P_{\text{Sol}}$) for P. This appears to reflect the strong adsorption of low concentrations of Zn in these soils in contrast to the effects of large annual phosphate fertilizer additions which create a supply of available P despite the calcareous conditions.

The solubility of phosphate across the range of soils investigated was controlled by DCPD, OCP, TCP or HA minerals depending on the location of the soils. There was evidence of consistent local Ca phosphate mineralogy in the soils. For specific areas, trends in phosphate and lime potentials clearly followed specific pure Ca phosphate minerals or fell on a similar slope between individual pure minerals. The soils were markedly undersaturated with respect to Smithsonite ($ZnCO_3$) in all cases. A more conservative solubility pattern was seen for Zn with almost all the soils following the empirical 'Soil Zn' solubility trend proposed by Lindsay. The difference between Zn and P probably reflects diverse P management regimes across the region in contrast to the absence of any Zn management.

In the majority of samples about 88%, the concentration of Zn in wheat grain was lower than the recommend value, as expected from the low availability of Zn in the regional soils. By contrast the P concentrations were higher than the recommended level in about half of the samples. The composition of the wheat grain, flour and bread could reasonably

be explained as a consequence of high annual applications of P fertilizer, the low availability of Zn in Kurdistan calcareous soils and the absence of any tradition of Zn fertilization. Thus the available P in soils and P concentration in wheat grain samples.

The PA in ($\text{g } 100\text{g}^{-1}$) and PA/Zn molar ratio on average were 0.54-0.93 and 15.7-30.6 in wheat grain, 0.08-0.31 and 8.96-22.9 in flour and 0.08-0.18 and 8.96-14.5 in bread samples, which were in the range reported globally. However, the bioavailability of Zn in wheat grain to human populations was low of about $23.3 \pm 3.48\%$ due to high PA concentrations and PA/Zn molar ratios. By contrast, in flour and bread samples were 'moderate' about 43 ± 6 and $47 \pm 4.6\%$ due to a significant reduction of PA in the refined flour and in bread resulting from flour manufacturing and bread-making processes respectively.

Results of this survey suggest that foliar application of Zn in wheat farms has a significant positive effect on cereal-based food quality grown in the calcareous soils of Kurdistan region.

Similar to several countries around the world, fertilizer use in Kurdistan region is high especially NPK fertilizers without micronutrient application. The absence of methodical information on fertilizers and how they might improve yield have made the farmers trust on personal decision and assistance from fertilizer traders and friends to make decision in fertilizer applications. In general, farmers have been individual in fertilizer application with incomplete approval of better fertilizer application technologies. Therefore, policies and programs needed to (i) realize the risk of fertilizer use specifically in the case of P fertilizer, (ii) promote the use of micronutrients particularly Zn fertilizer, (iii) improve yield by practicing experiments to determines micronutrient effects on yield and quality of agricultural products and (iv) encourage farmers education so as to improve environmentally soil fertility. Enhanced understanding of micronutrient fertilizer use and

agricultural product quality can help to increase the amount of bioavailable micronutrients, particularly Zn, in the final edible products in the region.

7 SUMMARY, CONCLUSION AND FUTURE WORK

SUMMARY

Phosphorus and Zn bioavailability in soils and crops, their PA/Zn molar ratio in the crop edible parts, and estimated human Zn bioavailability have been investigated in the current project using soils and crops from the Iraqi Kurdistan region. There was already limited information about the bioavailability of P in the arable soils of the region, but an understanding of Zn status was limited with virtually no published information or available data. Surveys of soil and wheat grain from arable vegetable and wheat farms were undertaken and the status of P and Zn and factors controlling their bioavailability to crops and estimated human Zn intake from crops were investigated. The interaction between P and Zn, the effect of Zn biofortification on crops, and estimated human Zn bioavailability were examined in a field trial on calcareous soils which were representative of the region. Laboratory experiments were also conducted to determine factors controlling P and Zn solubility and available pools. The capacity of the calcareous soil to adsorb applied P and Zn, the stability of these nutrients over time and evidence for time-dependent fixation were also investigated. The main findings of the project are reviewed in the results sections.

7.1 Phosphorus status in soils and crops

The calcareous soils of Iraqi Kurdistan had P_T concentrations in the globally acceptable range, with some variability depending on locations and agricultural activity due to parent materials, weathering processes, and application of phosphate fertilizers. The P_T concentration (mg kg^{-1}) in vegetable farm soils ranged from 622 to 1210, while in the wheat grain farm soils ranged from 645 to 2090. The total P content in the soils does not predict the availability of P and plant P concentrations were not correlated with P_T in the soils. The available forms of P uptake by plants are the reservoir of available P in the solid phase (P_{Olsen}) and the concentration in the solution phase of a soil suspension

in a dilute neutral salt solution (P_{sol}). In the calcareous soils, these two forms are low due to precipitation and adsorption processes caused by the high calcium carbonate content and high pH value of Kurdistan soils. In the surveyed soils, P_{Olsen} and P_{sol} constitutes about 8% and 0.01% of P_T in the arable vegetable soils and 1% and 0.1% of P_T in the wheat grain farm soils respectively. The P availability was lower in the wheat grain farm soils due to higher $CaCO_3$ content, higher pH value and low organic matter compared to the vegetable farm soils. Approximately, 83% and 53% of soils collected from the wheat grain farms had P_{Olsen} and P_{sol} values below the recommended levels of 10 mg kg^{-1} and 0.5 mg L^{-1} for optimum plant growth. High negative correlations were observed between soil inorganic carbon and pH with available P in the soils. The result of solid-liquid partition coefficient of P (as $\log K_d$ P formulated by P_{Olsen}/P_{sol}) showed low adsorption of P in the calcareous soils of Kurdistan probably due to the presence of low amounts of available P in the soils. Furthermore, the results of the solubility measurements showed that in most soils in the region, the solubility of P is controlled by OCP and TCP minerals and solubility increased with increase in P_{Olsen} . Soluble P was also positively correlated with P_{Olsen} . The results of the adsorption experiment showed that a significant amount of applied P adsorbed by the calcareous soils of Kurdistan after 84 days of incubation. The availability of P (P_{Olsen} and P_{sol}) decreased with increasing (contact) time of incubation. A single (soil specific) Langmuir-Freundlich model described *available* P adsorption (P_{Olsen} adsorbed) very well; the data followed a single trend for all contact times. By contrast, the total adsorbed P progressively increased with contact time, causing a systematic decrease in P_{sol} due to P fixation and creating a range of apparent (time-dependent) adsorption isotherms.

Phosphorus concentration in wheat grains grown in the calcareous soils of Kurdistan ranged between 2200 and 4120 mg kg^{-1} with an average of 3290 mg kg^{-1} . A large variation of P concentration in wheat grains was observed, related to location and potentially differences in the application of phosphate fertilizers. Furthermore, the P

concentrations across flours, bread types and locations (mg kg^{-1}) ranged between 800 and 2800 with an average of 1290 for flours and 970-2760 with an average of 1330 for bread. Approximately, 40% of collected wheat grain samples had higher P levels than the recommended concentration of 3570 mg kg^{-1} . This is probably due to excessive application of phosphate fertilizers by farmers attempting to improve yield production.

In a field trial undertaken in Kurdistan the concentration of P in roots, shoots, grain, seeds and fruits of maize, cowpea and tomato increased with application of P fertilizer application rates as TSP (20% P). Different concentrations of P was measured in maize, cowpea, tomatoes roots, shoots, seeds and fruits. The concentration of P was increased by 5.4%, 4.1% and 13.3% in maize grain and cowpea seed and tomato fruit, respectively.

7.2 Zinc status in the soil and crop plants

The total Zn concentrations in the calcareous soils (Zn_T) of the Kurdistan region were within the range reported globally. The concentration of Zn was different between farms and locations, presumably mainly due to variation in parent material and weathering processes, in the absence of any Zn fertilizer programme. The Zn_T concentration in the vegetable farms ranged between $44\text{-}209 \text{ mg kg}^{-1}$ and in wheat farms ranged between $48.5\text{-}121 \text{ mg kg}^{-1}$, indicating higher Zn_T in vegetable farms than wheat farms. This difference may be due to the application of organic fertilizers and irrigation with sewage wastewater in vegetable farms. A high correlation was found between Zn_T and LOI%. Nevertheless, Zn_T in the soils was not related to plant uptake. The available forms of Zn to plants were determined as Zn_{DTPA} and Zn_{sol} . In the vegetable farm soils, 19 out of 21 soil samples had Zn_{DTPA} values above the recommended value of 0.5 mg kg^{-1} , while, Zn_{sol} was below the recommended range of $4\text{-}270 \mu\text{g L}^{-1}$ in all soil samples. However, in the wheat grain farms, about 78% and 98% of soils had Zn_{DTPA} and Zn_{sol} below the recommended values of 0.5 mg kg^{-1} and range of $4\text{-}270 \mu\text{g L}^{-1}$. The low values in the

cereal farm soils can be ascribed to the presence of large carbonate concentrations, high pH value and the absence of Zn fertilization in cereals (wheat). Approximately, 3% and 1% of Zn_T was in the form of Zn_{DTPA} and Zn_{sol} in vegetable farm soils, while about 0.1% and 0.01% of Zn_T was in the form of Zn_{DTPA} and Zn_{sol} in wheat grain farms respectively. The solid-liquid partition coefficient of Zn (as $\log K_d$ Zn) showed high adsorption of Zn particularly in the case of both the vegetable farm soils ($\log_{10} K_d = 3.25-4.10$) and wheat grain farms (2.03-3.30). The value of $\log K_d$ Zn decreased with increase in the soil organic carbon but was not correlated with $CaCO_3$ content and pH which might be due to the relatively high $CaCO_3$ contents in soils and narrow range of pH values. The results of solubility measurements showed that in most soils in the region, the solubility of Zn was controlled by smithsonite or, more commonly, adsorption reactions with Zn^{2+} ion activities which were undersaturated with respect to smithsonite. Solubility increased with increase in Zn_{DTPA} . Zn_{sol} was significantly correlated with Zn_{DTPA} , while both Zn_{DTPA} and Zn_{sol} were negatively correlated with soil pH value. The results of an adsorption experiment showed that more than 95% of applied ^{70}Zn was adsorbed by the calcareous soils after one day and adsorption reached 99.5% after 84 days of incubation. The Freundlich isotherm model described Zn_{DTPA} adsorption adequately. A single (soil-dependent) adsorption trend was observed between Zn_{DTPA} and Zn_{sol} , in contrast to (total) adsorbed Zn against Zn_{sol} which showed a marked time-dependence indicating prolonged adsorption in response to Zn fixation. The availability of Zn (Zn_{DTPA} and Zn_{sol}) decreased with increasing time of incubation and increased with application rates. The apparent Zn_E value increased with increasing Zn rates and time of incubation –again indicating time-dependent fixation of the added ^{70}Zn as it progressively mixed with less labile soil Zn fractions. Values of $\%Zn_E$ ranged between 70 and 91% of DTPA-extracted ^{70}Zn and 13.8 and 8.86% of total applied Zn at day 84.

The Zn concentrations measured in a survey of wheat grain grown in the calcareous soils of Kurdistan were very low. There was wide variation in grain Zn concentration among the surveyed fields and locations which ranged between 12.3-63.2 mg kg⁻¹. Furthermore, the total Zn concentration (mg kg⁻¹) across flours, bread types and locations ranged between 30-5.45 with an average of 9.76 for flours and 30.5-5.48 with an average of 10.8 for bread. Approximately, 88% of collected wheat grain samples had Zn concentrations below the recommended value of 41.6 mg kg⁻¹. This is probably due to the low availability of Zn and absence of Zn fertilization in the region. However, the Zn concentration in shoots, seeds and fruit of maize, cowpea and tomatoes increased with biofortification of Zn-EDTA fertilizer (5% Zn) and with increasing the applied rates. For example, the biofortification of 1.2 kg ha⁻¹ of Zn fertilizer to maize and 0.8 kg ha⁻¹ to cowpea and tomatoes are grown in the field caused small increases in the Zn concentration of maize, cowpea and tomatoes when compared with control; the Zn concentration was increased by 9%, 6.9%, and 4.5% in maize and cowpea seeds and tomatoes fruit, respectively. However, the effect of Zn biofortification was greater in shoots than in seeds and fruits which may be due to the interaction of Zn with P in the crop plants. The Zn concentration in shoots was increased by 67%, 44% and 55% for maize, cowpea and tomato as compared to the control, respectively.

7.3 Phosphorus interactions with Zn

The results in chapter 5 indicated that the concentration of Zn in crop plants increased with increasing Zn rates but decreased with increasing P rates in shoots, grain, seed and fruit of maize, cowpea and tomatoes. The application of P fertilizer reduced Zn concentration in plant tissue due to enhancing P uptake by plant and shoot growth which diluted the Zn concentration. Furthermore, the combined application of P and Zn to crops decreased Zn concentration in crops due to an apparent antagonistic effect. In general, seeds and fruit P concentrations decreased with an increased rate of applied Zn. The lowest Zn concentrations in shoots and seeds were recorded along with the

highest P concentrations in maize. The results suggest that P-Zn interactions occurred inside crops because Zn fertilizer was applied to the shoot system as a foliar application. However, no interaction between P and Zn in wheat grain samples was observed which may be because farmers apply N fertilizer with P fertilizer each growing season which may reduce the effect of P on Zn uptake due to a synergistic effect of N on Zn uptake.

7.4 Phytic acid content and PA to Zn molar ratio

Phytic acid is considered to be an anti-nutrient because PA chelates Zn during digestion and prevents its systemic absorption. The PA concentrations and PA to Zn molar ratios were determined in crops, wheat grains, flour and bread. The values of PA and PA to Zn molar ratio concentrations in wheat grains ranged between 0.31-1.12 g 100g⁻¹ and 9.47-53 respectively, indicating that 7.3% of the wheat grain total weight was PA and about 63.1±12.2% of P was present as PA. Regression analysis showed that the PA contents of wheat grain samples were positively correlated with P_{Olsen} in soils. The results suggest that the concentration of PA and ratio of PA to Zn were high due to the application of P fertilizer but differed between locations. Moreover, about 88% of wheat grains contained PA to Zn molar ratios higher than 15, indicating low bioavailability of Zn. A crop breeding program combined with enhanced agronomic management methods is needed to enhance both Zn uptake and bioavailability in wheat grain.

Values of PA concentration and PA to Zn molar ratio in flours and bread were lower than in whole wheat grain flours. This is probably due to the removal of part of the PA during flour milling processes of flour and also fermentation during the bread making process. The reduction of PA during fermentation is the result of the action of endogenous phytase in flour as well as microbial (yeast) phytase. The values of PA concentration and PA to Zn molar ratio in flours and bread were 0.08-0.63 and 0.05-0.30 g 100g⁻¹ and 9.72-42.6 and 4.15-22.2, respectively, indicating that 1.1 and 0.7% of flour and bread total weight were PA and about 42 and 28% of P was present as PA, respectively.

Considering the different bread types produced in Kurdistan, Samoon had the lowest PA concentration $0.08 \text{ g } 100\text{g}^{-1}$ and Hawrami had highest PA content $0.30 \text{ g } 100\text{g}^{-1}$ this is due to the time of fermentation during the breadmaking process. A high correlation was found between PA concentration in flour and the corresponding bread but the bread making process caused a reduction in both PA concentration and PA to Zn molar ratio. Large reductions in PA and PA/Zn molar ratio levels of 32% and 41% were observed in bread during the bread making process. Samoon showed the greatest reduction and but Hawrami had the smallest reduction due to differences in the time of fermentation during the bread making process.

The application of Zn fertilizer as Zn-EDTA on maize, cowpea and tomato crops decreased PA and PA to Zn molar ratio due to increased Zn concentration in their grain, seed and fruit. Zinc applications of 1.2 kg ha^{-1} to maize decreased PA and PA/Zn molar ratio in maize grain by 5 and 7%. And also, biofortification of 0.8 kg ha^{-1} to cowpea and tomato crops decreased PA and PA to Zn molar ratio by 1 and 7% in cowpea seed and 9, and 8.5% and in tomato fruit respectively as compared to control. The PA and PA to Zn ratio varied between crops grains, seed and fruit. However, the application of phosphate fertilizer at a rate of 150 kg ha^{-1} for maize crops increased PA and PA to Zn molar ratio by 15, 4% in maize grain. While application of 120 kg ha^{-1} for cowpea and tomato increased PA and PA/Zn molar ration by 19 and 23% in cowpea seed and by 3 and 12% in tomato fruit as compared to controls. The greatest response to Zn biofortification was found with tomatoes and the lowest with cowpea. However, the highest response for phosphate fertilizer was found with maize and the lowest with cowpea.

7.5 Estimated human Zn bioavailability

The estimated human Zn bioavailability in crops grown in the calcareous soils of the Kurdistan region was low, particularly in wheat grain. Variation in estimated human Zn bioavailability was observed in wheat grain samples (300 g) collected from different locations. Some locations had estimated human Zn bioavailability higher than reported by previous studies, while in other locations measured values were lower. Consideration of the PA concentration and average daily Zn intake suggest that only $21.5 \pm 2.9\%$ of grain Zn was bioavailable so that the *effective* Zn intake from wheat is only $1.84\text{--}2.63 \text{ mg d}^{-1}$ for the local population. Overall results suggest that the available dietary Zn was below the recommended levels (11 mg d^{-1}). This is partly due to low uptake by wheat but also due to the presence of large concentrations of PA in wheat grains aggravated by high phosphate fertilizer applications by farmers. A high negative correlation was found between estimated human Zn bioavailability in wheat grain and P_{Olsen} in corresponding soils.

The bioaccessibility of Zn in flour and bread samples was higher than in whole wheat grain flour. This is due to the removal of a significant amount of PA throughout the flour manufacturing and bread making processes. A high dependency was observed between locations and bread types. Regarding bread types, the Zn intake results suggest that only $43 \pm 6\%$ and $47 \pm 5\%$ were bioavailable so that the effective Zn intake was only $0.79\text{--}2.27$ and $1.09\text{--}2.93 \text{ mg d}^{-1}$ for flour and bread respectively, for the local population. The results suggest that dietary Zn was below the daily recommended levels of 3 mg d^{-1} from bread, but was in the range of bioavailable Zn in bread suggested by previous studies. The bread making process increases the bioavailable Zn due to the reduction of PA and PA/Zn molar ratio because during the fermentation process; the phytase enzyme hydrolyses PA.

The biofortification of Zn fertilizer (Zn-EDTA) increased bioavailable Zn in maize, cowpea and tomato crops and increased the bioavailable Zn. Biofortification of maize, cowpea and tomato crops with 1.2 and 0.8 kg Zn ha⁻¹ increased bioavailable Zn by 10.8%, 3.35% and 5.80% for maize grain, cowpea seed and tomato fruit. However, the application of P fertilizer decreased the bioavailable Zn by 12%, 1.36% and 4.83% in maize grain, cowpea seed and tomato fruit, respectively, when compared to controls. Only in maize grain was the effect of applied P fertilizer on bioavailable Zn significant. This was due to the increase of the concentration of PA and PA to Zn molar ratio in crops grain, seed and fruit.

7.6 Future work and wider considerations

The major research themes that could be considered essential to improve our understanding of soil phosphorus and zinc availability, plant uptake and estimated human zinc bioavailability in Iraqi Kurdistan are listed below.

- Further identification of phosphorus and zinc fractionation and speciation in the arable calcareous soils of Kurdistan and quantification of the time dependence of Zn and P liability following application of fertilizer. It may be possible to resolve a simple kinetic model based on the apparent 'half-life' of labile Zn and P which could improve fertilizer efficiency in the region.
- A more detailed investigation of the solubility of phosphate and zinc under fluctuating (controlled) CO₂ partial pressures to simulate variable rhizosphere conditions under fluctuating water regimes. Given the calcareous nature of the soils, it is likely that CO₂ partial pressure may have a profound effect on local Zn and P solubility. It may be possible to translate the results from such studies into practical recommendations regarding organic manures and irrigation approach intended to maximise re-mobilization of adsorbed/fixed Zn and P.
- Investigation of the effect of biofortification of zinc fertilizers on bioavailable Zn in wheat grain grown in the region. There is a need to improve the Zn nutrition of the

edible parts of cereal and vegetable crops – especially with respect to human health rather than plant health considerations. There is a pressing need for better information on timing and amounts of Zn foliar applications.

- Determine Zn deficiency in the Kurdistan region population by dietary questioners and biomarker samples (blood) survey. It is vital that more information is made available on the status of Zn in the population of the region. Alongside improved Zn biofortification of crops, other options may include dietary diversity information disseminated through existing extension channels.

REFERENCES

- ABDOLI, M., ESFANDIARI, E., SADEHGZADEH, B. & MOUSAVI, S., B. 2016. Zinc Application Methods Affect Agronomy Traits and Grain Micronutrients in Bread and Durum Wheat under Zinc-Deficient Calcareous Soil. *YYU J AGR SC*, 26, 202-214.
- ABDOLZADEH, A., WANG, X., VENEKLAAS, E. J. & LAMBERS, H. 2010. Effects of phosphorus supply on growth, phosphate concentration and cluster-root formation in three *Lupinus* species. *Ann Bot*, 105, 365-74.
- ABEBE, Y., BOGALE, A., HAMBIDGE, K. M., STOECKER, B. J., BAILEY, K. & GIBSON, R. S. 2007. Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. *Journal of Food Composition and Analysis*, 20, 161-168.
- ABID, M., AHMED, N., QAYYUM, M. F., SHAABAN, M. & RASHID, A. 2013. Residual and cumulative effect of fertilizer zinc applied in wheat-cotton production system in an irrigated aridisol. *Plant Soil Environ.*, 59, 505-510.
- ABOLFAZLI, F., FORGHANI, A. & NOROUZI, M. 2012. Effects of phosphorus and organic fertilizers on phosphorus fractions in submerged soil. *Journal of Soil Science and Plant Nutrition*, 12, 349-362.
- ADHAMI, E., MAFTOUN, M., RONAGHI, A., KARIMIAN, N., YASREBI, J. & ASSAD, M., T. 2006. Inorganic Phosphorus Fractionation of Highly Calcareous Soils of Iran. *Communications in Soil Science and Plant Analysis*, 37.
- AHMAD, W., WATTS, M. J., IMTIAZ, M., AHMED, I. & ZIA, M. H. 2012. Zinc deficiency in soils, crops and humans. *Agrochimica*, 5, 65-97.
- AHMED, A., RANDHAWA, M. A. & SAJID, M. W. 2014a. Bioavailability of Calcium, Iron, and Zinc in Whole Wheat Flour. *Wheat and Rice in Disease Prevention and Health*.
- AHMED, H., YOUNG, S. D. & SHAW, G. 2014b. Factors affecting uranium and thorium fractionation and profile distribution in contrasting arable and woodland soils. *Journal of Geochemical Exploration*, 145, 98-105.
- AHMED, O. A. 2013. The Effect of Organic Waste Addition on Biochemical Factors and Availability of Phosphorus in Desert Soils. *Journal of Environment and Earth Science*, 3, 67-74.
- AHMED, Y., WATTS, M., J., IMTIAZ, M., AHMED, I. & ZIA, M., H. 2012. Zinc deficiency in soils, crops and humans. *Agrochimica*, LVI, 65-97.
- AKABATA-PENDIAS, A. & MUKHERJEE, A., B. 2008. Trace Elements from Soil to human.
- AKHTAR, M., YAQUB, M., NAEEM, A., ASHRAF, M. & HERNANDEZ, V. E. 2016. Improving phosphorus uptake and wheat productivity by phosphoric acid application in alkaline calcareous soils. *J Sci Food Agric*, 96, 3701-7.
- AKINREMI, O. O. & CHO, C. M. 1991. Phosphate and Accompanying Cation Transport in a Calcareous Cation-Exchange Resin System. *Soil Science Society of America Journal*, 55, 959-964.
- AKRAM, M., A., DEPAR, N. & MEMON, M., Y. 2017. Synergistic use of nitrogen and zinc to bio-fortify zinc in wheat grains. *Eurasian Journal of Soil Science (Ejss)*, 6, 319-326.
- AL-ODUD, S. S. 2011. IMPROVING PHOSPHORUS AVAILABILITY FROM PHOSPHATE ROCK IN CALCAREOUS SOILS BY AMENDING WITH: ORGANIC ACID, SULFUR, AND/ OR ORGANIC MANURE. *Ozean Journal of Applied Sciences* 4.
- AL-ROHILY, K. M., GHONEIM, A. M., MODAIHSH, A. S. & MAHJOUB, M. O. 2013. Phosphorus Availability in Calcareous Soils Amend with Chemical Phosphorus fertilizer, Cattle Manure Compost and Sludge manure. *International journal of soil Science*, 8, 17-24.
- AL-TAMEEMI, H. J., ASHOOR, N. I. & I, O. L. 2015. Solubility and Movement of Calcium Phosphate Minerals in Some Calcareous Soils as Influenced by Application of Phosphate Fertilizer and Water Potential. *Merit Research Journal of Agricultural Science and Soil Sciences*, 3, 098-105.

- AL-TAMIMI, R. A. 2006. Zinc sorption by some Torrifluvents soil of Sub-Saharan region South of Libya. *Emir. J. Agric. Sci.*, 18, 01-10.
- AL HARBI, S., F., GHONAIM, A., M., MODAIHSH, A., S. & MAHJOUB, M. O. 2013. Effect of Foliar and Soil Application of Phosphorus on Phosphorus Uptake, Use Efficiency and Wheat Grain Yield in Calcareous Soil. *Journal of Applied Sciences*, 13 188-192.
- ALAM, S., M. & AZAM SHAH, S. 2002. Phosphorus Uptake and yield of Wheat as Influenced by Intergrated Use of Phosphatic fertilizers *Pak. J. Agri. sa*, 39, 265-268.
- ALI, F., SADIQ, A., ALI, I., AMIN, M. & AMIR, M. 2014a. Effect of Applied Phosphorus on the Availability of Micronutrients in Alkaline-Calcareous Soil. *Journal of Environment and Earth Science*, 4, 143-147.
- ALI, M. S., SUTRADHAR, A., EDANO, M. L., EDWARDS, J. T. & GIRMA, K. 2014b. Response of Winter Wheat Grain Yield and Phosphorus Uptake to Foliar Phosphite Fertilization. *International Journal of Agronomy*, 2014, 1-8.
- ALLEN-DURRANCE, A. E. 2017. A Quick Reference on Phosphorus. *Vet Clin North Am Small Anim Pract*, 47, 257-262.
- ALLOWAY, B. J. 2009. Soil factors associated with zinc deficiency in crops and humans. *Environ Geochem Health*, 31, 537-48.
- ALLOWAY, J., B. 2008. Zinc in Soils and Crop Nutrition. *Second edition, published by IZA and IFA Brussels, Belgium and Paris, France*.
- ALVAREZ, J. M., ALMENDROS, P. & GONZALEZ, D. 2008. Residual effects of natural Zn chelates on navy bean response, Zn leaching and soil Zn status. *Plant and Soil*, 317, 277-291.
- ALVAREZ, J. M. & MARIA, I. R. 2003. Effects of Zinc Complexes on the Distribution of Zinc in Calcareous Soil and Zinc Uptake by Maize. *J. Agric. Food Chem*, 51.
- ANNAHEIM, K. E., DOOLETTE, A. L., SMERNIK, R. J., MAYER, J., OBERSON, A., FROSSARD, E. & BÜNEMANN, E. K. 2015. Long-term addition of organic fertilizers has little effect on soil organic phosphorus as characterized by ³¹P NMR spectroscopy and enzyme additions. *Geoderma*, 257-258, 67-77.
- ANTONIADIS, V., SHAHEEN, S., M., TSADILAS, C., D., SELIM, M., H. & RINKLEBE, J. 2017. Zinc sorption by different soils as affected by selective removal of carbonates and hydrous oxides. *Applied Geochemistry xxx*, 1.
- ARMSTRONG, D. L. 1999. Important Factors Affecting Crop Response to Phosphorus. *Better Crops* 83, 16-19.
- ASHRAF, M. S., RANJHA, A. M., YASEEN, M., AHMAD, N. & HANNAN, A. 2008. Zinc Adsorption Behavior of Different Textured Calcareous Soils Using Freundlich and Langmuir Models *Pak. J. Agri. Sci.*, 45, 6-10.
- ASHWORTH, D. J. & ALLOWAY, B. J. 2004. Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. *Environmental Pollution*, 127, 137-144.
- ASLAM, M., ZIA, R., M., ULLAH, S. & YASIN, M. 2000. Application of freundlich adsorption isotherm to determine phosphorus requirement of several rice soils. *Int. J. Agric. Biol.*, 2.
- AUDETTE, Y., O'HALLORAN, I. P. & PAUL VORONEY, R. 2016. Kinetics of phosphorus forms applied as inorganic and organic amendments to a calcareous soil. *Geoderma*, 262, 119-124.
- AYOUB, S., MCGAW, B., A., SHAND, C., A. & MIDWOOD, A., J. 2003. Phytoavailability of Cd and Zn in soil estimated by stable isotope exchange and chemical extraction Ahmed *Plant and Soil* 252, 291-300.
- BAI, J., YE, X., JIA, J., ZHANG, G., ZHAO, Q., CUI, B. & LIU, X. 2017. Phosphorus sorption-desorption and effects of temperature, pH and salinity on phosphorus sorption in marsh soils from coastal wetlands with different flooding conditions. *Chemosphere*, 188, 677-688.
- BARANIMOTLAGH, M. & GHOLAMI, M. 2013. Time-Dependent Zinc Desorption in Some Calcareous Soils of Iran. *Pedosphere*, 23, 185-193.

- BARRAMEDA-MEDINA, Y., LENTINI, M., ESPOSITO, S., RUIZ, J. M. & BLASCO, B. 2017. Zn-biofortification enhanced nitrogen metabolism and photorespiration process in green leafy vegetable *Lactuca sativa* L. *J Sci Food Agric*, 97, 1828-1836.
- BEAL, T., MASSIOT, E., ARSENAULT, J. E., SMITH, M. R. & HIJMANS, R. J. 2017. Global trends in dietary micronutrient supplies and estimated prevalence of inadequate intakes. *PLoS One*, 12, e0175554.
- BERGMAN, C., GRAY-SCOTT, D., CHEN, J. J. & MEACHAM, S. 2009. What is next for the Dietary Reference Intakes for bone metabolism related nutrients beyond calcium: phosphorus, magnesium, vitamin D, and fluoride? *Crit Rev Food Sci Nutr*, 49, 136-44.
- BHARTI, K., PANDEY, N., SHANKHDHAR, D., SRIVASTAVA, P. C. & SHANKHDHAR, S. C. 2013. Improving nutritional quality of wheat through soil and foliar zinc application. *Plant Soil Environ.*, 59, 348-352.
- BHATTACHARYYA, P., NAYAK, A. K., SHAHID, M., TRIPATHI, R., MOHANTY, S., KUMAR, A., RAJA, R., PANDA, B., LAL, B., GAUTAM, P., SWAIN, C. K., ROY, K. S. & DASH, P. K. 2015. Effects of 42-year long-term fertilizer management on soil phosphorus availability, fractionation, adsorption-desorption isotherm and plant uptake in flooded tropical rice. *The Crop Journal*, 3, 387-395.
- BLACK, R. E. 2003. Zinc Deficiency, Infectious Disease and Mortality in the Developing World. *J. Nutr.*, 133, 1485-1489.
- BOGDANOVIC, D., UBAVIC, M. & CUVARDIC, M. 1999. Effect of phosphorus fertilization on Zn and Cd contents in soil and corn plants. *Nutrient Cycling in Agroecosystems*, 54, 49-56.
- BOUAIN, N., SHAHZAD, Z., ROUACHED, A., KHAN, G. A., BERTHOMIEU, P., ABDELLELY, C., POIRIER, Y. & ROUACHED, H. 2014. Phosphate and zinc transport and signalling in plants: toward a better understanding of their homeostasis interaction. *J Exp Bot*, 65, 5725-41.
- BOUIS, H. E., HOTZ, C., MCCLAFFERTY, B., MEENAKSHI, J. V. & PFEIFFER, W. H. 2011. Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin*, 32, 31-40.
- BOUKHALFA-DERAOUI, N., HANIFI-MEKLIECHE, L., MEKLIECHE, A., MIHOUB, A. & DEDDIBOUHOUN, M. 2015 Effect of Phosphorus Application on Durum Wheat in Alkaline Sandy Soil in Arid Condition in Southern Algeria. *Asian Journal of Crop Science*, 7 61-71.
- BRADL, H. B. 2004. Adsorption of heavy metal ions on soils and soils constituents. *J Colloid Interface Sci*, 277, 1-18.
- BRANKOVIC, G., DRAGICEVIĆ, V., DODIG, D., KNEŽEVIĆ, D., KANDIĆ, D., ŠURLAN-MOMIROVIĆ, G. & SEČANSKI, M. 2015. Phytic acid, inorganic phosphorus, antioxidants in bread and durum wheat and their associations with agronomic traits. *AGRICULTURAL AND FOOD SCIENCE*, 24, 183-194.
- BRASCHI, I., CIAVATTA, C., GIOVANNINI, C. & GESSA, C. 2003. Combined effect of water and organic matter on phosphorus availability in calcareous soils. *Nutrient Cycling in Agroecosystems*, 67, 67-74.
- BROADLEY, M. R., WHITE, P. J., HAMMOND, J. P., ZELKO, I. & LUX, A. 2007. Zinc in plants. *New Phytol*, 173, 677-702.
- BROWN, K. H., HAMBIDGE, K. M. & RANUM, P. 2010. Zinc fortification of cereal flours: Current recommendations and research needs. *Food and Nutrition Bulletin*, 31, 62-74.
- BUEKERS, J., VAN LAER, L., AMERY, F., VAN BUGGENHOUT, S., MAES, A. & SMOLDERS, E. 2007. Role of soil constituents in fixation of soluble Zn, Cu, Ni and Cd added to soils. *European Journal of Soil Science*, 58, 1514-1524.
- CAKMAK, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil*, 302, 1-17.
- CAKMAK, I. 2009. Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *J Trace Elem Med Biol*, 23, 281-9.

- CAKMAK, I. & KUTMAN, U. B. 2017. Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science*.
- CAKMAK, I. & MARSCHNER, H. 1993. Effect of zinc nutritional status on activities of superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. *Plant and Soil*, 155, 127-130.
- CALVO, M. S. & URIBARRI, J. 2013. Public health impact of dietary phosphorus excess on bone and cardiovascular health in the general population. *Am J Clin Nutr*, 98, 6-15.
- CAREY, A., MOORE, A. & LEYTEM, A. 2011. Phosphorus in the Calcareous Soils of Southern Idaho A Literature Review with Implications for Crop Production Manure Management, and Water Quality. *the University of Idaho*.
- CATLETT, K. M., HEIL, D. M., LINDSAY, W. L. & EBINGER, M. H. 2002. Soil Chemical Properties Controlling Zinc²⁺ Activity in 18 Colorado Soils. *Soil Sci. Soc. Am. J.*, 66, 1182-1189.
- CHEN, K., JIANG, W. D., WU, P., LIU, Y., KUANG, S. Y., TANG, L., TANG, W. N., ZHANG, Y. A., ZHOU, X. Q. & FENG, L. 2017a. Effect of dietary phosphorus deficiency on the growth, immune function and structural integrity of head kidney, spleen and skin in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol*, 63, 103-126.
- CHEN, L., TIAN, F. & SU, Z. 2016. Phosphorus Nutrition and Health: Utilization of Phytase-producing Bifidobacteria in Food Industry. *Probiotics and Prebiotics in Human Nutrition and Health*.
- CHEN, W., LI, L. L., CHANG, A. C., WU, L., CHANEY, R. L., SMITH, R. & AJWA, H. 2009. Characterizing the solid-solution partitioning coefficient and plant uptake factor of As, Cd, and Pb in California croplands. *Agriculture, Ecosystems & Environment*, 129, 212-220.
- CHEN, X. P., ZHANG, Y. Q., TONG, Y. P., XUE, Y. F., LIU, D. Y., ZHANG, W., DENG, Y., MENG, Q. F., YUE, S. C., YAN, P., CUI, Z. L., SHI, X. J., GUO, S. W., SUN, Y. X., YE, Y. L., WANG, Z. H., JIA, L. L., MA, W. Q., HE, M. R., ZHANG, X. Y., KOU, C. L., LI, Y. T., TAN, D. S., CAKMAK, I., ZHANG, F. S. & ZOU, C. Q. 2017b. Harvesting more grain zinc of wheat for human health. *Sci Rep*, 7, 7016.
- CHERIF, J., MADIOUNI, C., AMMAR, W. B. & JEMAL, F. 2011. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solanum lycopersicum*). *Journal of Environmental Sciences*, 23, 837-844.
- CHHABRA, P. & SIDH, J. S. 1988. Fate of phytic acid during bread making. *Die Nahrung*, 32, 15-19.
- CHILDERS, D. L., CORMAN, J., EDWARDS, M. & ELSER, J. J. 2011. Sustainability Challenges of Phosphorus and Food: Solutions from Closing the Human Phosphorus Cycle. *BioScience*, 61, 117-124.
- CHOWANIAK, M. & GONDEK, K. 2009. Changes in the Available Magnesium and Zinc Contents of Soils After the Application of Sewage Slugges and Sewage Sulagges-Peat mixtures *journal of central Eroupian Agriculture*, 10, 79-88.
- CLEMENTE, R., ESCOLAR, A. & BERNAL, M. P. 2006. Heavy metals fractionation and organic matter mineralisation in contaminated calcareous soil amended with organic materials. *Bioresour Technol*, 97, 1894-901.
- CORDELL, D., ROSEMARIN, A., SCHRODER, J. J. & SMIT, A. L. 2011. Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84, 747-58.
- DAHIYA, S., SHANWAL, A. V. & HEGDE, A. G. 2005. Studies on the sorption and desorption characteristics of Zn(II) on the surface soils of nuclear power plant sites in India using a radiotracer technique. *Chemosphere*, 60, 1253-61.
- DANDANMOZD, F. & HOSSEINPUR, A. R. 2010. Thermodynamic parameters of zinc sorption in some calcareous soils. *Journal of American Science*, 6, 298-304.
- DANYAGRI, G. & DANG, Q.-L. 2014. Soil Temperature and Phosphorus Supply Interactively Affect Physiological Responses of White Birch to CO₂ Elevation. *American Journal of Plant Sciences*, 05, 219-229.

- DAS, S. & GREEN, A. 2013. Importance of zinc in crops and human health. *SAT eJournal* 11.
- DATABASES, U. F. C. 2018.
- DE MATOS, A. T., FONTES, M. P. F., DA COSTA, L. M. & MARTINEZ, M. A. 2001. Mobility of heavy metals as related to soil chemical and mineralogical characteristics and mobility of Brazilian soils. *Environmental Pollution* 111, 429-435.
- DEGRYSE, F., VOEGELIN, A., JACQUAT, O., KRETZSCHMAR, R. & SMOLDERS, E. 2011. Characterization of zinc in contaminated soils: complementary insights from isotopic exchange, batch extractions and XAFS spectroscopy. *European Journal of Soil Science*, 62, 318-330.
- DELGADO, A., MADRID, A., KASSEM, S., ANDREU, L. & DEL CAMPILLO, M. D. C. 2002. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. *Plant and Soil*, 245.
- DERAOUI, N. B., MEKLIKE, L. H. & MIHOUB, A. 2015. Effect of Incubation Period of Phosphorus Fertilizer on some Properties of Sandy Soil with Low Calcareous Content, Southern Algeria. *Asian Journal of Agricultural Research*, 9, 123-131.
- DESALEGN, T., ALEMU, G., ADELLA, A., DEBELE, T. & GONZALO J, J. 2016. Effect of Lime and Phosphorus Fertilizer on Acid Soils and Barley (*Hordeum Vulgare* L.) Performance in the Central Highlands of Ethiopia. *Experimental Agriculture*, 53, 432-444.
- DEVAU, N., HINSINGER, P., LE CADRE, E., COLOMB, B. & GÉRARD, F. 2011. Fertilization and pH effects on processes and mechanisms controlling dissolved inorganic phosphorus in soils. *Geochimica et Cosmochimica Acta*, 75, 2980-2996.
- DIBARTOLA, S. P. & WILLARD, M. D. 2012. Disorders of Phosphorus. *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice*.
- DICKINSON, N., GULLIVER, J., MACPHERSON, G., ATKINSON, J., RANKIN, J., CUMMINGS, M., NISBET, Z., HURSTHOUSE, A., TAYLOR, A., ROBERTSON, C. & BURGHARDT, W. 2009. A framework to explore micronutrient deficiency in maternal and child health in Malawi, Southern Africa. *Environ Health*, 8 Suppl 1, S13.
- DONATE-CORREA, J., MUROS-DE-FUENTES, M., MORA-FERNANDEZ, C. & NAVARRO-GONZALEZ, J. F. 2012. FGF23/Klotho axis: phosphorus, mineral metabolism and beyond. *Cytokine Growth Factor Rev*, 23, 37-46.
- DRISSI, S., AÏT HOUSSA, A., BAMOUH, A., DAOUDI, E. M. & BENBELLA, M. 2015a. Efficacy of Dairy Cattle Slurry in Preventing Zinc Deficiency of a Silage Corn (*Zea mays* L.) Grown on a Sandy Soil. *Journal of Agricultural Science*, 7.
- DRISSI, S., HOUSSA, A., BAMOUH, A., COQUANT, J.-M. & BENBELLA, M. 2015b. Effect of Zinc-Phosphorus Interaction on Corn Silage Grown on Sandy Soil. *Agriculture*, 5, 1047-1059.
- DU, Y., LI, P., NGUYEN, A. V., XU, Z. P., MULLIGAN, D. & HUANG, L. 2015. Zinc uptake and distribution in tomato plants in response to foliar supply of Zn hydroxide-nitrate nanocrystal suspension with controlled Zn solubility. *Journal of Plant Nutrition and Soil Science*, 178, 722-731.
- DUFFNER, A., HOFFLAND, E. & TEMMINGHOFF, E. J. M. 2012. Bioavailability of zinc and phosphorus in calcareous soils as affected by citrate exudation. *Plant and Soil*, 361, 165-175.
- DVORAK, P., TLUSTO, P., SZÁKOVÁ, J., ÈERNÝ, J. & BALÍK, J. 2003. Distribution of soil fractions of zinc and its uptake by potatoes, maize, wheat and barley after soil amendment by sludge and inorganic Zn salt. *LANT SOIL ENVIRON*, 49, 203-212.
- EIII, K. 2017. Nutrients for Bone Structure and Calcification. *Nutritional Pathophysiology of Obesity and its Comorbidities*.
- ERDAL, I., KAYA, M. & KÜÇÜKYUMUK, Z. 2014. Effects of Zinc and Nitrogen Fertilization on Grain yield and Some Parameters Effecting Zinc Bioavailability in Lentil Seeds *Legume Res.*, 37, 55-61.
- ERDAL, I., YILMAZ, A., TABAN, S., EKER, S., TORUN, B. & ÇAKMAK, I. 2002. PHYTIC ACID AND PHOSPHORUS CONCENTRATIONS IN SEEDS OF WHEAT CULTIVARS GROWN WITH AND WITHOUT ZINC FERTILIZATION. *Journal of Plant Nutrition*, 25, 113-127.

- ERENOGLU, B., NIKOLIC, M., RÖMHELD, V. & ÇAKMAK, I. 2002. Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency. *Plant and Soil* 241, 251-257.
- ERVIN, R. B., WANG, C.-Y., WRIGHT, J. D., STEPHENSON, J. K. & 2004. Dietary Intake of Selected Minerals for the United States Population: 1999–2000. *U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Centers for Disease Control and Prevention National Center for Health Statistics*.
- FAGERIA, V. D. 2001. Nutrient Interactions in Crop Plants. *Journal of Plant Nutrition*, 24, 1269-1290.
- FAHAD, S., AHMAD, M. K., ANJUM, M. A. & HUSSAIN, S. 2014. The Effect of Micronutrients (B, Zn and Fe) Foliar Application on the Growth, Flowering and Corm Production of Gladiolus (*Gladiolus grandiflorus* L.) in Calcareous Soils. *J. Agr. Sci. Tech.*, 16, 1671-1682.
- FAO DATABASE 2011. Food and Agricultural Organization, online at <http://faostat.fao.org> on February 11, 2011.
- FARMER, A., M. 1993. The Effects of Dust on vegetation a Review. *Environmental Pollution*, 79.
- FAVUS, M. J., BUSHINSKY, D. A. & LEMANN, J. J. 2006. Regulation of Calcium, Magnesium, and Phosphate Metabolism. *American Society for Bone and Mineral Research*.
- FAYAZ, A., SADIQ, A., ALI, I., AMIN, M. & AMIR, M. 2014. Effect of Applied Phosphorus on the Availability of Micronutrients in Alkaline Calcareous Soil. *Journal of Environment and Earth Science*, 4.
- FEBLES, C. I., ARIAS, A., HARDISSON, A., RODRÍGUEZ-ALVAREZ, C. & SIERRA, A. 2002. Phytic Acid Level in Wheat Flours. *Journal of Cereal Science*, 36, 19-23.
- FEKRI, M., GORGIN, N. & SADEGH, L. 2011. Phosphorus desorption kinetics in two calcareous soils amended with P fertilizer and organic matter. *Environmental Earth Sciences*, 64, 721-729.
- FERNANDES, A. M., SORATTO, R. P. & GONSALES, J. R. 2014. Root morphology and phosphorus uptake by potato cultivars grown under deficient and sufficient phosphorus supply. *Scientia Horticulturae*, 180, 190-198.
- FERNANDEZ-CALVINO, D., PATEIRO-MOURE, M., NOVOA-MUNOZ, J. C., GARRIDO-RODRIGUEZ, B. & ARIAS-ESTEVEZ, M. 2012. Zinc distribution and acid-base mobilisation in vineyard soils and sediments. *Sci Total Environ*, 414, 470-9.
- FERNANDEZ, V. & BROWN, P. H. 2013. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Front Plant Sci*, 4, 289.
- FRONTELA, C., ROS, G. & MARTÍNEZ, C. 2011. Phytic acid content and “in vitro” iron, calcium and zinc bioavailability in bakery products: The effect of processing. *Journal of Cereal Science*, 54, 173-179.
- FURUTANI, J., SEGAWA, H., ARANAMI, F., KUWAHARA, S., SUGANO, M., BANNAI, K., YAMATO, H., ITO, M. & MIYAMOTO, K. 2013. Dietary inorganic phosphorus regulates the intestinal peptide transporter PepT1. *J Ren Nutr*, 23, e11-20.
- GABAZA, M., SHUMOY, H., MUCHUWETI, M., VANDAMME, P. & RAES, K. 2018. Iron and zinc bioaccessibility of fermented maize, sorghum and millets from five locations in Zimbabwe. *Food Research International*, 103, 361-370.
- GAL, N. J. & DAHL, W. J. 2013. Facts about Phosphorus. *Ifas extension, university of Florida*, FSHN13-13.
- GARCIA-ESTEPA, R. M., GUERRA-HERNANDEZ, E. & GARCIA-VILLANOVA, B. 1999. Phytic acid content in milled cereal products and breads. *Food Research International*, 32.
- GARCIA, M., C., DIEZ, J., A., VALLEJO, A., GARCIA, L. & CARTAGENA, M., C. 1997. Effect of Applying Soluble and Coated Phosphate Fertilizers on Phosphate Availability in Calcareous Soils and on P Absorption by a
- Rye-Grass Crop. *J.Agric.FoodChem*, 45, 1931-1036.
- GARG, S. & BAHL, G. S. 2008. Phosphorus availability to maize as influenced by organic manures and fertilizer P associated phosphatase activity in soils. *Bioresour Technol*, 99, 5773-7.

- GARGARI, B. P., MAHBOOB, S. & RAZAVIEH, S. V. 2007. Content of phytic acid and its mole ratio to zinc in flour and breads consumed in Tabriz, Iran. *Food Chemistry*, 100, 1115-1119.
- GIANQUINTO, G., ABU-RAYYAN, A., TOLA, L. D., PICCOTINO, D. & PEZZAROSSA, B. 2000. Interaction effects of phosphorus and zinc on photosynthesis, growth and yield of dwarf bean grown in two environments. *Plant and Soil* 220.
- GIBSON, R., S., BAILEY, K., B., GIBBS, M. & FERGUSON, E., L. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin, The United Nations University*, 3, S134-S146.
- GIRIJA, V., V., RATTAN, R., K. & DATTA, S., P 2013. Adsorption study: A systematic approach to determine zinc availability in soils of divergent characteristics. *International Journal of Agricultural sciences*, 4, 102-105.
- GRANT, C., BITTMAN, S., MONTREAL, M., PLENCHETTE, C. & MOREL, C. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *CANADIAN JOURNAL OF PLANT SCIENCE*.
- GRAOUEB-BACART, M., SAYEN, S. & GUILLON, E. 2015. Adsorption of enrofloxacin in presence of Zn(II) on a calcareous soil. *Ecotoxicol Environ Saf*, 122, 470-6.
- GUPTA, D. K., CHATTERJEE, S., DATTA, S., VEER, V. & WALTHER, C. 2014. Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. *Chemosphere*, 108, 134-44.
- GUPTA, R. K., GANGOLIYA, S. S. & SINGH, N. K. 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *J Food Sci Technol*, 52, 676-84.
- GUSTAFSSON, J. P., MWAMILA, L. B. & KERGOAT, K. 2012. The pH dependence of phosphate sorption and desorption in Swedish agricultural soils. *Geoderma*, 189-190, 304-311.
- GUTTIERI, M. J., BAENZIGER, P. S., FRELS, K., CARVER, B., ARNALL, B., WANG, S., AKHUNOV, E. & WATERS, B. M. 2015. Prospects for Selecting Wheat with Increased Zinc and Decreased Cadmium Concentration in Grain. *Crop Science*, 55.
- HADGU, F., GEBREKIDAN, H., KIBRET, K. & YITAFERU, B. 2014. Study of phosphorus adsorption and its relationship with soil properties, analyzed with Langmuir and Freundlich models. *Agriculture, Forestry and Fisheries*, 3, 40-51.
- HAFEEZ, B., KHANIF, Y. M. & SALEEM, M. 2013. Role of Zinc in Plant Nutrition- A Review. *American Journal of Experimental Agriculture*, 3, 374-391.
- HALAJNIA, A., HAGHNIA, G. H., FOTOVAT, A. & KHORASANI, R. 2009. Phosphorus fractions in calcareous soils amended with P fertilizer and cattle manure. *Geoderma*, 150, 209-213.
- HANRAHAN, G., SALMASSI, T. M., KHACHIKIAN, C. S. & FOSTER, K. L. 2005. Reduced inorganic phosphorus in the natural environment: significance, speciation and determination. *Talanta*, 66, 435-44.
- HARARAH, M. A., AL-NASIR, F., EL-HASAN, T. & AL-MUHTASEB, A. A. H. 2011. Zinc adsorption-desorption isotherms: possible effects on the calcareous vertisol soils from Jordan. *Environmental Earth Sciences*, 65, 2079-2085.
- HART, J. J., NORVELL, W. A., WELCH, R. M., SULLIVAN, L. A. & KOCHIAN, L. V. 1998. Characterization of Zinc Uptake, Binding, and Translocation in Intact Seedlings of Bread and Durum Wheat Cultivars. *Plant Physiol.*, 118.
- HASHEMI, S. S. & BAGHERNEJAD, M. 2009. Zinc Sorption by Acid, Calcareous and Gypsiferous Soils as Related to Soil Mineralogy. *Iran Agricultural Research*, 28.
- HAWKINS, H.-J., HETTASCH, H., MESJASZ-PRZYBYLOWICZ, J., PRZYBYLOWICZ, W. & CRAMER, M. D. 2008. Phosphorus toxicity in the Proteaceae: A problem in post-agricultural lands. *Scientia Horticulturae*, 117, 357-365.
- HAYNES, R., J. & MOKOLOBATE, M., S. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient Cycling in Agroecosystems*, 59, 47-63.

- HAYNES, R. J., MURTAZA, G. & NAIDU, R. 2009. Chapter 4 Inorganic and Organic Constituents and Contaminants of Biosolids.
- HEANEY, R. P. & GRAEFF-ARMAS, L. A. 2018. Vitamin D Role in the Calcium and Phosphorus Economies. *Vitamin D*.
- HEIDARI, S., REYHANITABAR, A. & OUSTAN, S. 2017. Kinetics of phosphorus desorption from calcareous soils using DGT technique. *Geoderma*, 305, 275-280.
- HEIRI, O., LOTTER, A. F. & LEMCKE, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology*, 25, 101-110.
- HEMALATHA, S., PLATEL, K. & SRINIVASAN, K. 2005. Influence of food acidulants on bioaccessibility of zinc and iron from selected food grains. *Mol Nutr Food Res*, 49, 950-6.
- HEMALATHA, S., PLATEL, K. & SRINIVASAN, K. 2007. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. *Food Chemistry*, 102, 1328-1336.
- HO, E. 2004. Zinc deficiency, DNA damage and cancer risk. *J Nutr Biochem*, 15, 572-8.
- HOLFORD, I. C. R. 1997. Soil phosphorus: its measurement, and its uptake by plants. *Soil Research*, 35, 227-240.
- HOLLIDAY, V. T. & GARTNER, W. G. 2007. Methods of soil P analysis in archaeology. *Journal of Archaeological Science*, 34, 301-333.
- HOLLOWAY, R., E., BERTRAND, I., FRISCHKE, A., J., BRACE, D., M., MCLAUGHLIN, M., J. & SHEPPERD, W. 2001. Improving fertiliser efficiency on calcareous and alkaline soils with fluid sources of P, N and Zn. *Plant and Soil*, 236.
- HOPKINS, B. & ELLSWORTH, J. 2005. Phosphorus availability with alkaline/calcareous soil. *Western Nutrient Management Conference. Salt Lake City, UT.*, 6.
- HUSSAIN, S., MAQSOOD, M., A., RENGE, Z., AZIZ, T. & ABID, M. 2013. Estimated Zinc Bioavailability in Milling Fractions of Biofortified Wheat Grains and in Flours of Different Extraction Rates. *International journal of Agricultur and Biology* 15, 921-926.
- HUSSAIN, S., MAQSOOD, M. & MILLER, L. 2012a. Bioavailable zinc in grains of bread wheat varieties of Pakistan. *Cereal Research Communications*, 40, 62-73.
- HUSSAIN, S., MAQSOOD, M. A. & RAHMATULLAH 2011. Zinc Release Characteristics from Calcareous Soils using Diethylenetriaminepentaacetic Acid and Other Organic Acids. *Communications in Soil Science and Plant Analysis*, 42, 1870-1881.
- HUSSAIN, S., MAQSOOD, M. A., RENGEL, Z. & AZIZ, T. 2012b. Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. *Plant and Soil*, 361, 279-290.
- IMRAN, M., REHIM, A., SARWAR, N. & HUSSAIN, S. 2016. Zinc bioavailability in maize grains in response of phosphorous-zinc interaction. *J. Plant Nutr. Soil Sci*, 179.
- ISLAM, F., YASMEEN, T., RIAZ, M., ARIF, M. S., ALI, S. & RAZA, S. H. 2014. *Proteus mirabilis* alleviates zinc toxicity by preventing oxidative stress in maize (*Zea mays*) plants. *Ecotoxicol Environ Saf*, 110, 143-52.
- JALALI, M. 2007. Phosphorus status and sorption characteristics of some calcareous soils of Hamadan, western Iran. *Environmental Geology*, 53, 365-374.
- JALALI, M. & JALALI, M. 2017. Minerals control phosphorus solubility in long-term-cultivated calcareous soils. *Soil Research*, 55.
- JALALI, M. & KHANLARI, Z. V. 2006. Mobility and Distribution of Zinc, Cadmium and Lead in Calcareous Soils Receiving Spiked Sewage Sludge. *Soil and Sediment Contamination: An International Journal*, 15, 603-620.
- JALALI, M. & MOHARRAMI, S. 2007. Competitive adsorption of trace elements in calcareous soils of western Iran. *Geoderma*, 140, 156-163.
- JALALI, M. & MORADI, F. 2013. Competitive sorption of Cd, Cu, Mn, Ni, Pb and Zn in polluted and unpolluted calcareous soils. *Environ Monit Assess*, 185, 8831-46.
- JALALI, M. & RANJBAR, F. 2010. Aging effects on phosphorus transformation rate and fractionation in some calcareous soils. *Geoderma*, 155, 101-106.

- JALALI, M. & TABAR, S., S. 2011. Chemical fractionation of phosphorus in calcareous soils of Hamedan, western Iran under different land use. *Journal of Plant Nutrition and Soil Science*, 174, 523-531.
- JALALI, M. & ZINLI, N., A., M. 2012. Effects of Common Ions on Zn Sorption in Some Calcareous Soils of Western Iran. *Pedosphere*, 22, 190-200.
- JAMBREC, D., SAKAC, M., JOVANOVIĆ, P., MISAN, A., PESTORIĆ, M., TOMOVIĆ, V. & MANDIĆ, A. 2016. Effect of processing and cooking on mineral and phytic acid content of buckwheat-enriched tagliatelle. *Chemical Industry and Chemical Engineering Quarterly*, 22, 319-326.
- JIA, L., WANG, W., LI, Y. & YANG, L. 2010. Heavy metals in soil and crops of an intensively farmed area: a case study in Yucheng City, Shandong Province, China. *Int J Environ Res Public Health*, 7, 395-412.
- JUN, W., WEN-ZHAO, L., HAN-FENG, M. & TING-HUI, D. 2010. Inorganic Phosphorus Fractions and Phosphorus Availability in a Calcareous Soil Receiving 21-Year Superphosphate Application. *Pedosphere* 20, 304-310.
- KABATA-PENDIAS, A. & MUKHERJEE, A. B. 2007. *Trace elements from soil to human*, Springer Science & Business Media.
- KALANTAR-ZADEH, K., GUTEKUNST, L., MEHROTRA, R., KOVESDY, C. P., BROSS, R., SHINABERGER, C. S., NOORI, N., HIRSCHBERG, R., BENNER, D., NISSENSON, A. R. & KOPPLE, J. D. 2010. Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clin J Am Soc Nephrol*, 5, 519-30.
- KARAMI, M., AFYUNI, M., KHOSHGOFTARMANESH, A. H., PAPRITZ, A. & SCHULIN, R. 2009. Grain zinc, iron, and copper concentrations of wheat grown in central Iran and their relationships with soil and climate variables. *J Agric Food Chem*, 57, 10876-82.
- KARUNARATNE, A. M., AMERASINGHE, P. H., SADAGOPA RAMANUJAM, V. M., SANDSTEAD, H. H. & PERERA, P. A. J. 2008. Zinc, iron and phytic acid levels of some popular foods consumed by rural children in Sri Lanka. *Journal of Food Composition and Analysis*, 21, 481-488.
- KHANIKI, G. R. J. 2005. Determination of Zinc Contents in Iranian Flat Breads. *Pakistan Journal of Nutrition*, 4, 294-297.
- KHARCHE, V., K., DESAI, V., N. & PHARANDE, A., L. 2011. Effect of Sewage Irrigation on Soil Properties, Essential Nutrient and Pollutant Element Status of Soils and Plants in a Vegetable Growing Area around Ahmednagar City in Maharashtra. *Journal of the Indian Society of Soil Science*, 59, 177-184.
- KHOSHGOFTARMANESH, A. H., NOROUZI, M., AFYUNI, M. & SCHULIN, R. 2017. Zinc biofortification of wheat through preceding crop residue incorporation into the soil. *European Journal of Agronomy*, 89, 131-139.
- KHOUZAM, R. B., POHL, P. & LOBINSKI, R. 2011. Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables. *Talanta*, 86, 425-8.
- KIDD, P. S., DOMINGUEZ-RODRIGUEZ, M. J., DIEZ, J. & MONTERROSO, C. 2007. Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere*, 66, 1458-67.
- KIELA, P. R., RADHAKRISHNAN, V. M. & GHISHAN, F. K. 2017. Phosphorus: Basic Nutritional Aspects. *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals*.
- KIM, J. N., HAN, S. N. & KIM, H. K. 2014. Phytic acid and myo-inositol support adipocyte differentiation and improve insulin sensitivity in 3T3-L1 cells. *Nutr Res*, 34, 723-31.
- KULKARNI, S. D., ACHARYA, R., RAJURKAR, N. S. & REDDY, A. V. R. 2007. Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by in vitro digestion method. *Food Chemistry*, 103, 681-688.

- KUMAR, M. & QURESHI, F. M. 2012. Dynamics of Zinc Fractions, Availability to Wheat (*Triticum aestivum* L.) and Residual Effect on Succeeding Maize (*Zea mays* L.) in Inceptisols. *Journal of Agricultural Science*, 4.
- KUMAR, V., SINHA, A. K., MAKKAR, H. P. S. & BECKER, K. 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, 120, 945-959.
- LAMBERS, H., RAVEN, J. A., SHAVER, G. R. & SMITH, S. E. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends Ecol Evol*, 23, 95-103.
- LAMBERT, R., GRANT, C. & SAUVE, S. 2007. Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. *Sci Total Environ*, 378, 293-305.
- LEYTEM, A. B. & MIKKELSEN, R. L. 2005. The Nature of Phosphorus in Calcareous Soils. *Better Crops*, 89, 11-13.
- LI, B.-Y., HUANG, S.-M., WEI, M.-B., ZHANG, H. L., SHEN, A. L., XU, J.-M. & RUAN, X.-L. 2010. Dynamics of Soil and Grain Micronutrients as Affected by Long-Term Fertilization in an Aquic Inceptisol. *Pedosphere*, 20, 725-735.
- LI, B. Y., ZHOU, D. M., CANG, L., ZHANG, H. L., FAN, X. H. & QIN, S. W. 2007. Soil micronutrient availability to crops as affected by long-term inorganic and organic fertilizer applications. *Soil and Tillage Research*, 96, 166-173.
- LI, M., TIAN, X.-H., LI, X.-L. & WANG, S.-X. 2017. Effect of Zn application methods on Zn distribution and bioavailability in wheat pearling fractions of two wheat genotypes. *Journal of Integrative Agriculture*, 16, 1617-1623.
- LI, M., WANG, S., TIAN, X., ZHAO, J., LI, H., GUO, C., CHEN, Y. & ZHAO, A. 2015. Zn distribution and bioavailability in whole grain and grain fractions of winter wheat as affected by applications of soil N and foliar Zn combined with N or P. *Journal of Cereal Science*, 61, 26-32.
- LI, M., WELTI, R. & WANG, X. 2006. Quantitative profiling of Arabidopsis polar glycerolipids in response to phosphorus starvation. Roles of phospholipases D zeta1 and D zeta2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. *Plant Physiol*, 142, 750-61.
- LI YU-YUAN, L., RUI, Y., RU, G., HONG-AN, W., AN-LEI, C. & YONG, L. 2013. Effects of Long-term Phosphorus Fertilization and Straw Incorporation on Phosphorus Fractions in Subtropical Paddy Soil. *Journal of Integrative Agriculture Advance Online Publication*.
- LIN, H. S., LIN, J. R., HU, S. W., KUO, H. C. & YANG, Y. H. 2014. Association of dietary calcium, phosphorus, and magnesium intake with caries status among schoolchildren. *Kaohsiung J Med Sci*, 30, 206-12.
- LINDSAY, W. L. 1979. Chemical equilibria in soils. *John Wiley and Sons Ltd*.
- LIU, B., AI, S., ZHANG, W., HUANG, D. & ZHANG, Y. 2017. Assessment of the bioavailability, bioaccessibility and transfer of heavy metals in the soil-grain-human systems near a mining and smelting area in NW China. *Sci Total Environ*, 609, 822-829.
- LIU, C., MUCHHAL, U. S., UTHAPPA, M., KONONOWICZ, A. K. & RAGHOTHAMA, K. G. 1998. Tomato Phosphate Transporter Genes Are Differentially Regulated in Plant Tissues by Phosphorus. *Plant Physiol*, 116.
- LIU, H., WANG, Z. H., LI, F., LI, K., YANG, N., YANG, Y., HUANG, D., LIANG, D., ZHAO, H., MAO, H., LIU, J. & QIU, W. 2014. Grain iron and zinc concentrations of wheat and their relationships to yield in major wheat production areas in China. *Field Crops Research*, 156, 151-160.
- LIU, Z. 2005. Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content. *Food Chemistry*, 89, 49-52.
- LIU, Z. H., WANG, H. Y., WANG, X. E., ZHANG, G. P., CHEN, P. D. & LIU, D. J. 2006. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, 44, 212-219.
- LOPEZ, H., W., LEENHARDT, F., COUDRAY, C. & REMESY, C. 2002a. Minerals and phytic acid interactions: is it a real problem for human nutrition? *International Journal of Food Science and Technology*, 37, 227-239.

- LOWE, N. M., FEKETE, K. & DECSI, T. 2009. Methods of assessment of zinc status in humans: a systematic review. *Am J Clin Nutr*, 89, 2040S-2051S.
- LUO, Y., W. & XIE, W., H. 2012. Effects of vegetables on iron and zinc availability in cereals and legumes. *International Food Research Journal*, 19, 455-459.
- MA, H., EVANGELOU, M. W. H., VONTOBEL, P. & SCHULIN, R. 2016a. Root growth and Zn uptake of three common crop plants in response to heterogeneity in soil texture and Zn distribution. *Environmental and Experimental Botany*, 127, 45-54.
- MA, J. F., YANG, F., MAHIDA, S. N., ZHAO, L., CHEN, X., ZHANG, M. L., SUN, Z., YAO, Y., ZHANG, Y. X., ZHENG, G. Y., DONG, J., FENG, M. J., ZHANG, R., SUN, J., LI, S., WANG, Q. S., CAO, H., BENJAMIN, E. J., ELLINOR, P. T., LI, Y. G. & TIAN, X. L. 2016b. TBX5 mutations contribute to early-onset atrial fibrillation in Chinese and Caucasians. *Cardiovasc Res*, 109, 442-50.
- MA, Y. B. & UREN, N. C. 1997. The effects of temperature, time and cycles of drying and rewetting on the extractability of zinc added to a calcareous soil. *Geoderma*, 75, 89-97.
- MABESA, R. L., IMPA, S. M., GREWAL, D. & JOHNSON-BEEBOUT, S. E. 2013. Contrasting grain-Zn response of biofortification rice (*Oryza sativa* L.) breeding lines to foliar Zn application. *Field Crops Research*, 149, 223-233.
- MAGALA, M., KOHAJDOVÁ, Z. & KAROVIČOVÁ, J. 2015. Degradation of phytic acid during fermentation of cereal substrates. *Journal of Cereal Science*, 61, 94-96.
- MAGALLANES-LOPEZ, A. M., HERNANDEZ-ESPINOSA, N., VELU, G., POSADAS-ROMANO, G., ORDONEZ-VILLEGAS, V. M. G., CROSSA, J., AMMAR, K. & GUZMAN, C. 2017. Variability in iron, zinc and phytic acid content in a worldwide collection of commercial durum wheat cultivars and the effect of reduced irrigation on these traits. *Food Chem*, 237, 499-505.
- MALLIKARJUN, S. D. & SHASHIKANT, R. M. 2013. A Batch Study of Phosphate Adsorption Characteristics on Clay Soil. *International Journal of Research in Engineering and Technology*.
- MANZEKE, G. M., MTAMBANENGWE, F., NEZOMBA, H. & MAPFUMO, P. 2014. Zinc fertilization influence on maize productivity and grain nutritional quality under integrated soil fertility management in Zimbabwe. *Field Crops Research*, 166, 128-136.
- MAQSOOD, M. A., HUSSAIN, S., NAEEM, M. A., AHMAD, M., AZIZ, T., RAZA, H. M., KANWAL, S. & HUSSAIN, M. 2014. ZINC INDEXING IN WHEAT GRAINS AND ASSOCIATED SOILS OF SOUTHERN PUNJAB. *Pak. J. Agri. Sci.*, 51, 1-8.
- MARET, W. & SANDSTEAD, H. H. 2006. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol*, 20, 3-18.
- MARTINEZ, C. E. & MOTTO, H. L. 2000. Solubility of lead, zinc and copper added to mineral soils. *Environmental Pollution*, 107, 153-158.
- MARUYAMA, H. & WASAKI, J. 2017. Transgenic approaches for improving phosphorus use efficiency in plants. *Plant Macronutrient Use Efficiency*.
- MARZOUK, E. R., CHENERY, S. R. & YOUNG, S. D. 2013. Predicting the solubility and lability of Zn, Cd, and Pb in soils from a minespoil-contaminated catchment by stable isotopic exchange. *Geochimica et Cosmochimica Acta*, 123, 1-16.
- MAZID, A. 2015. Status of Wheat Production in Kurdistan Region of Iraq: Results of Baseline Survey. *Japan International Cooperation Agency (JICA) and International Center for Agricultural Research in Dry Area (ICARDA)*.
- MCDOWELL, R. W., MAHIEU, N., BROOKES, P. C. & POULTON, P. R. 2003. Mechanisms of phosphorus solubilisation in a limed soil as a function of pH. *Chemosphere*, 51, 685-692.
- MCDOWELLA, R. W. & SHARPLEY, A. N. 2003. Phosphorus solubility and release kinetics as a function of soil test P concentration. *Geoderma*, 112, 143-154.
- MERRIKHPOUR, H. & JALALI, M. 2015. Geostatistical assessment of solid-liquid distribution coefficients (K_d) for Cd, Cu, Pb and Zn in surface soils of Hamedan, Iran. *Modeling Earth Systems and Environment*, 1.

- MIHOUB, A. & BOUKHALFA-DERAOUY 2014. Performnace of Difefrent Phosphorus Fertilizer types on Wheat Grown in Calcareous Sandy Soils of El-Menia, Southern Algeria. *Asian Journal of Crop Science*, 6, 383-391.
- MILIVOJEVIC, J., NIKEZIC, D., KRSTIC, D., JELIC, M. & ĐALOVIC, I. 2011. Influence of Physical-Chemical Characteristics of Soil on Zinc Distribution and Availability for Plants in Vertisols of Serbia. *Polish J. of Environ. Stud*, 20, 993-1000.
- MILLER, L. V., HAMBIDGE, K. M. & KREBS, N. F. 2015. Zinc Absorption Is Not Related to Dietary Phytate Intake in Infants and Young Children Based on Modeling Combined Data from Multiple Studies. *J Nutr*, 145, 1763-9.
- MILLER, L. V., KREBS, N. F. & HAMBIDGE, K. M. 2007. A Mathematical Model of Zinc Absorption in Humans As a Function of Dietary Zinc and Phytate. *The Journal of Nutrition*, 135-141.
- MITCHIKPE, E. C. S., DOSSA, R. A. M., ATEGBO, E.-A. D., VAN RAAIJ, J. M. A., HULSHOF, P. J. M. & KOK, F. J. 2008. The supply of bioavailable iron and zinc may be affected by phytate in Beninese children. *Journal of Food Composition and Analysis*, 21, 17-25.
- MKHABELA, M. S. & WARMAN, P. R. 2005. The influence of municipal solid waste compost on yield, soil phosphorus availability and uptake by two vegetable crops grown in a Pugwash sandy loam soil in Nova Scotia. *Agriculture, Ecosystems & Environment*, 106, 57-67.
- MOHASSES, M. M. 2015. Effect of Soil Type, Source of Phosphorus and Application of Sulfur with Thiobacillus on Soil Phosphorus Fractions. *Indian Journal Of Natural Sciences*, 5.
- MOTAVALLI, P. & MILES, R. 2002. Soil phosphorus fractions after 111 years of animal manure and fertilizer applications. *Biology and Fertility of Soils*, 36, 35-42.
- MOUSAVI, S., R., GALAVI, M. & RAZAEI, M. 2013. Zinc (Zn) Importance for Crop Production – A Review. *International journal of Agronomy and Plant Production*, 4, 64-68.
- MOUSAVI, S. R. 2011. Zinc in Crop Production and Interaction with Phosphorus. *Australian Journal of Basic and Applied Sciences* 5, 1503-1509.
- MOUSAVI, S. R. & GALAVI, M. 2012. The interaction of zinc with other elements in plants: A review. *International Journal of Agriculture and Crop Sciences*, 4, 1881-1884.
- MULLINS, G. 2009. PhosPhorus, Agriculture & the environment. *Virginia Polytechnic Institute and State University, Extension specialist, Crop and Soil Environmental Sciences*.
- MURPHY, J. & RILEY, J. P. 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Watere. *Analytical Chemical Acta*, 27, 31-36.
- MUTUCUMARANA, R. K., RAVINDRAN, V., RAVINDRAN, G. & COWIESON, A. J. 2015. Measurement of true ileal phosphorus digestibility in maize and soybean meal for broiler chickens: Comparison of two methodologies. *Animal Feed Science and Technology*, 206, 76-86.
- NADEEM, M., MOLLIER, A., VIVES, A., PRUD´HOMME, L., NIOLETT, S. & PELLERIN, S. 2014. Short Communication. Effect of phosphorus nutrition and grain position within maize cob on grain phosphorus accumulation. *Spanish Journal of Agricultural Research*, 12.
- NAEEM, A., AKHTAR, M. & AHMAD, W. 2013. Optimizing available phosphorus in calcareous soils fertilized with diammonium phosphate and phosphoric acid using Freundlich adsorption isotherm. *ScientificWorldJournal*, 2013, 680257.
- NAME, N. 1999. Functions of Phosphorus in Plants. *Better Crops*, 83.
- NAN, Z., ZHAO, C., LI, J., CHEN, F. & SUN, W. 2002. Relation Between Soil Properties and selected Heavy Metal Concentartions in Spring Wheat (*Triticum Aestivum* L.) Grown in Contaminated Soils. . *Water, Air, and Soil Pollution*, 133.
- NAZIF, W., MARZOUK, E. R., PERVEEN, S., CROUT, N. M. & YOUNG, S. D. 2015. Zinc solubility and fractionation in cultivated calcareous soils irrigated with wastewater. *Sci Total Environ*, 518-519, 310-9.

- NESET, T. S., BADER, H. P., SCHEIDEGGER, R. & LOHM, U. 2008. The flow of phosphorus in food production and consumption -- Linköping, Sweden, 1870-2000. *Sci Total Environ*, 396, 111-20.
- NEUMANN, G. & MARTINOIA, E. 2002. Cluster roots – an underground adaptation for survival in extreme environments. *TRENDS in Plant Science* 7, 162-167.
- NIKOLIC, M., NIKOLIC, N., KOSTIC, L., PAVLOVIC, J., BOSNIC, P., STEVIC, N., SAVIC, J. & HRISTOV, N. 2016. The assessment of soil availability and wheat grain status of zinc and iron in Serbia: Implications for human nutrition. *Sci Total Environ*, 553, 141-148.
- NOULAS, C., TZIOUVALEKAS, M. & KARYOTIS, T. 2018. Zinc in soils, water and food crops. *J Trace Elem Med Biol*.
- NRIAGU, J. 2007. Zinc Toxicity in Humans. *School of Public Health, University of Michigan, Elsevier B.V.*
- NYSTROM, S. D. 2012. thermodynamic parameters of zinc sorption in some calcareous soils. *International Journal of Agr. & Env*, 1, 1-7.
- OBAID, E. A. & AL-HADETHI, M. E. A. 2013. Effect of Foliar Application with Manganese and Zinc on Pomegranate Growth, Yield and Fruit Quality. *Journal of Horticultural Science & Ornamental Plants*, 5, 41-45.
- OBRADOR, A., NOVILLO, J. & M. ALVAREZ, J. 2003. Mobility and Availability to Plants of Two Zinc Sources Applied to a Calcareous Soil A. Obrador, J. Novillo, and J. M. Alvarez*. *Soil Sci. Soc. Am. J*, 67, 564-572.
- OBREZA, T. A., ZEKRI, M. & CALVERT, D. V. 2015. Citrus Fertilizer Management on Calcareous Soils. *Citrus Fertilizer Management on Calcareous Soils*.
- OKAZAKI, Y. & KATAYAMA, T. 2014. Dietary phytic acid modulates characteristics of the colonic luminal environment and reduces serum levels of proinflammatory cytokines in rats fed a high-fat diet. *Nutr Res*, 34, 1085-91.
- OLSEN, S. R., COLE, C. V., WATANABE, F. S. & DEAN, L. A. 1954. Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate. *Circular 939. USDA, Washington, DC, USA*, p. 19.
- ONWONGA, R., N. , LELEI, J., J., FREYER, B., FRIEDEL, J., K., MWONGA, S., M. & WANDHAWA, P. 2008. Low Cost Technologies for Enhancing N and P Availability and Maize (*Zea mays* L.) Performance on Acid Soils. *World Journal of Agricultural Sciences*, 4, 862-873.
- PAPADOPOULOS, P. & ROWELL, D. L. 1989. PAPADOPOULOS The reactions of copper and zinc with calcium carbonate surfaces. *Journal of Soil Science*, 40, 39-48.
- PASRICHA, N., S., BADDESHA, H., S., AULAKH, M., S. & NAYYAR, V., K. 1987. The Zinc Quantity-Intensity Relationships in Four Different Soils as Influenced by phosphorus *Soil Science*, 143.
- PIZZEGHELLO, D., BERTI, A., NARDI, S. & MORARI, F. 2011. Phosphorus forms and P-sorption properties in three alkaline soils after long-term mineral and manure applications in north-eastern Italy. *Agriculture, Ecosystems & Environment*, 141, 58-66.
- PO, A., EGLI, I., OKOTH, M. W., NAIROBI, K., BAHWERE, P., CERCAMONDI, C. I., ZEDER, C., NJAGE, P. M. K. & OWINO, V. O. 2016. Estimated Iron and Zinc Bioavailability in Soybean-Maize-Sorghum Ready to Use Foods: Effect of Soy Protein Concentrate and Added Phytase. *Journal of Food Processing & Technology*, 07.
- POBLACIONES, M. J. & RENGEL, Z. 2016. Soil and foliar zinc biofortification in field pea (*Pisum sativum* L.): Grain accumulation and bioavailability in raw and cooked grains. *Food Chem*, 212, 427-33.
- POZRL, T., KOPJAR, M., KURENT, I., HRIBAR, J., JANES, A. & SIMCLC, M. 2009. Phytate Degradation during Breadmaking: The Influence of Flour Type and Breadmaking Procedures. *Czech J. Food Sci.*, 27, 29–38.
- PRASAD, R., SHIVAY, Y. S. & KUMAR, D. 2016. Interactions of Zinc with Other Nutrients in Soils and Plants - A Review. *Indian Journal of Fertilisers*, 12, 16-26.
- PREETHA, P. S. & STALIN, P. 2014. Different Forms of Soil Zinc - their Relationship with Selected Soil Properties and Contribution towards Plant Availability and Uptake in

- Maize Growing Soils of Erode District, Tamil Nadu. *Indian Journal of Science and Technology*, 7, 1018-1025.
- PRIHA, O. & SMOLANDER, A. 1999. Nitrogen transformations in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. *Soil Biology and Biochemistry*, 31, 965-977.
- PUGGAARD, L., LUND, P., LIESEGANG, A. & SEHESTED, J. 2014. Long term effect of reduced dietary phosphorus on feed intake and milk yield in dry and lactating dairy cows. *Livestock Science*, 159, 18-28.
- QAZI, I. M., WAHAB, S., SHAD, A. A., ZEB, A. & AYUAB, M. 2003. Effect of Different Fermentation Time and Baking on Phytic Acid Content of Whole-wheat Flour Bread. *Asian Journal of Plant Sciences*, 2, 597-601.
- QUEVAUVILLER, P. H. 1998. Operationally defined extraction procedures for soil and sediment analysis I. Standardization. *trends in analytical chemistry*, 17, 289-298.
- RAINA, R., GARG, G., SETHI, S. K., SCHREIBER, M. J., SIMON, J. F. & THOMAS, G. 2012. Phosphorus Metabolism. *Journal of Nephrology & Therapeutics*, S3:008. doi:10.4172/21610959.S3-008.
- RAMESH, S. A., CHOIMES, S. & SCHACHTMAN, D. P. 2004. Over-expression of an Arabidopsis zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Molecular Biology*, 54.
- RAMZAN, S., BHAT, M., A., KIRMANI, N., A. & RASOOL, R. 2014. Fractionation of Zinc and their Association with Soil Properties in Soils of Kashmir Himalayas. *International Invention Journal of Agricultural and Soil Science*, 2, 132-142.
- RASHMI, I., PARAMA, V., R., R. & BISWAS, A., K. 2016. Phosphorus Sorption Parameters in Relation to Soil Properties in Some Major Agricultural Soils of India. *SAARC J. Agri.*, 14, 01-09.
- RATTAN, R. K., DATTA, S. P., CHHONKAR, P. K., SURIBABU, K. & SINGH, A. K. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, Ecosystems & Environment*, 109, 310-322.
- RAWAJFIH, Z., KHRESAT, S. & RYAN, J. 2010. Soil Phosphorus Fractions in Calcareous Vertisols and Aridisols of Northern Jordan. *Jordan Journal of Agricultural Sciences*, 6, 411-422.
- REYHANITABAR, A., ARDALAN, M. M., KARIMIAN, N., SAVAGHEBI, G. R. & GILKES, R. J. 2011. Kinetics of Zinc Sorption by Some Calcareous Soils of Iran. *J. Agr. Sci. Tech*, 13, 263-272.
- REYHANITABAR, A. & GILKES, R. J. 2010. Kinetics of DTPA extraction of zinc from calcareous soils. *Geoderma*, 154, 289-293.
- RICHARDSON, A. E., LYNCH, J. P., RYAN, P. R., DELHAIZE, E., SMITH, F. A., SMITH, S. E., HARVEY, P. R., RYAN, M. H., VENEKLAAS, E. J., LAMBERS, H., OBERSON, A., CULVENOR, R. A. & SIMPSON, R. J. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, 349, 121-156.
- ROMIC, M. & ROMIC, D. 2003. Heavy metals distribution in agricultural topsoils in urban area. *Environmental Geology* 43, 795-805.
- ROOHANI, N., HURRELL, R., WEGMUELLER, R. & SCHULIN, R. 2012. Zinc and phytic acid in major foods consumed by a rural and a suburban population in central Iran. *Journal of Food Composition and Analysis*, 28, 8-15.
- ROWELL, D. L. 1994. Soil Science: Methods and applications. *Longman Scientific and Technical, UK*.
- RYAN, M. H., MCINERNEY, J. K., RECORD, I. R. & ANGUS, J. F. 2008. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *Journal of the Science of Food and Agriculture*, 88, 1208-1216.
- SACHDEV, P., LINDSAY, W. L. & DEB, D. L. 1992. Activity measurements of zinc in soils of different pH using EDTA. *Geoderma*, 55, 247-257.
- SADEGHZADEH, B. 2013. A review of zinc nutrition and plant breeding. *Journal of Soil Science and Plant Nutrition*, 13, 905-927.

- SAHA, S., CHAKRABORTY, M., SARKAR, D., BATABYAL, K., MANDAL, B., MURMU, S., PADHAN, D., HAZRA, G. C. & BELL, R. W. 2017. Rescheduling zinc fertilization and cultivar choice improve zinc sequestration and its bioavailability in wheat grains and flour. *Field Crops Research*, 200, 10-17.
- SAKAI, H., IWAI, T., MATSUBARA, C., USUI, Y., OKAMURA, M., YATOU, O., TERADA, Y., AOKI, N., NISHIDA, S. & YOSHIDA, K. T. 2015. A decrease in phytic acid content substantially affects the distribution of mineral elements within rice seeds. *Plant Sci*, 238, 170-7.
- SALGUEIRO, M. J., ZUBILLAGA, B. M., LYSIONEK, A., MARABIA, M. I., CARE, R., DE PAOLI, T., HAGER, A., WEILL, R., BOCCIO, J. & BIOCH 2000. Zinc as an Essential Micro Nutrient : A Review *Nutrition Research*, 20, 737-755.
- SAMADI, A. 2006. Temporal Changes in Available Phosphorus in Some Calcareous Soils. *J. Agric. Sci. Technol*, 8, 343-348.
- SAMADI, A. & GILKES, R. J. 1999. Phosphorus Transformations and Their Relationships with Calcareous Soil Properties of Southern Western Australia. *Soil Science Society of America Journal*, 63, 809-815.
- SANDSTEAD, H. H. & FREELAND-GRAVES, J. H. 2014. Dietary phytate, zinc and hidden zinc deficiency. *J Trace Elem Med Biol*, 28, 414-7.
- SASTRE, J., SAHUQUILLO, A., VIDAL, M. & RAURET, G. 2002. Determination of Cd, Cu, Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Analytica Chimica Acta*, 462, 59-72.
- SAUNDERS, A. V., CRAIG, W. J. & BAINES, S. K. 2012. Zinc and vegetarian diets. *The Medical Journal of Australia*, 1, 17-21.
- SCHACHTMAN, D. P., REID, R. J. & AYLING, S. M. 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol.*, 116, 447-453.
- SCHIMEL, D., STILLWELL, M. A. & WOODMANSEE, R. G. 1985. BIOGEOCHEMISTRY OF C, N, AND P IN A SOIL CATENA OF THE SHORTGRASS STEPPE. *Ecology*, 66, 276-282.
- SCHRODER, J. J., SMIT, A. L., CORDELL, D. & ROSEMARIN, A. 2011. Improved phosphorus use efficiency in agriculture: a key requirement for its sustainable use. *Chemosphere*, 84, 822-31.
- SEKITA, A., OKAZAKI, Y. & KATAYAMA, T. 2016. Dietary phytic acid prevents fatty liver by reducing expression of hepatic lipogenic enzymes and modulates gut microflora in rats fed a high-sucrose diet. *Nutrition*, 32, 720-2.
- SELIM, H. M. 2017. Sorption-desorption of trace elements in soils: influence of kinetics. *Trace Elements in Waterlogged Soils and Sediments*, 53-74.
- SHAHEEN, S. M., TSADILAS, C. D. & ESKRIDGE, K. M. 2009. Effect Of Common Ions On Phosphorus Sorption And Lability In Greek Alfisols With Different pH. *Soil Science*, 174, 21-26.
- SHAHEEN, S. M., TSADILAS, C. D. & RINKLEBE, J. 2013. A review of the distribution coefficients of trace elements in soils: influence of sorption system, element characteristics, and soil colloidal properties. *Adv Colloid Interface Sci*, 201-202, 43-56.
- SHAHEEN, S. M., TSADILAS, C. D. & STAMATIADIS, S. 2007. Inorganic phosphorus forms in some entisols and aridisols of Egypt. *Geoderma*, 142, 217-225.
- SHAHID, M., DUMAT, C., KHALID, S., SCHRECK, E., XIONG, T. & NIAZI, N. K. 2017. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *J Hazard Mater*, 325, 36-58.
- SHANE, M. W., MCCULLY, M. E. & LAMBERS, H. 2004. Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *J Exp Bot*, 55, 1033-44.
- SHARIATMADARI, H., SHIRVANI, M. & JAFARI, A. 2006. Phosphorus release kinetics and availability in calcareous soils of selected arid and semiarid toposequences. *Geoderma*, 132, 261-272.

- SHARMA, M. K., SINGH, B. & ARYA, C. K. 2014. Kinetics of zinc transformation in calciorthids soils of western Rajasthan, India. *Journal of Applied and Natural Science* 6, 121-126.
- SHEN, J., YUAN, L., ZHANG, J., LI, H., BAI, Z., CHEN, X., ZHANG, W. & ZHANG, F. 2011. Phosphorus dynamics: from soil to plant. *Plant Physiol*, 156, 997-1005.
- SHENG-LI, G., TING-HUI, D. & MING-DE, H. 2008. Phosphorus Changes and Sorption Characteristics in a Calcareous Soil Under Long-Term Fertilization. *Pedosphere*, 18, 248-256.
- SHIH, C.-Y., KANG, L.-K. & CHANG, J. 2015. Transcriptional responses to phosphorus stress in the marine diatom, *Chaetoceros affinis*, reveal characteristic genes and expression patterns in phosphorus uptake and intracellular recycling. *Journal of Experimental Marine Biology and Ecology*, 470, 43-54.
- SIDDIQUI, S. A. K., R.A. 2010. Trace elements fractionation in calcareous soils of Peshawar Pakistan. *Soil & Environ*, 2, 148-158.
- SINAJ, S., DUBOIS, A. & FROSSARD, E. 2004. Soil isotopically exchangeable zinc: A comparison between E and L values. *Plant and Soil*, 261, 17-28.
- SINEGANI, S. A. A. & SEDRI, S. 2011. Effect of Sterilization and temperature on The decrease kinetic of Phosphorus Bioavailability in Two Different Soil Types. *J. Soil Sci. Plant Nutr*, 11, 110-123.
- SINGH, B., R., KC, D. & RA, A. 2017. Long-Term Effect of Phosphate Fertilization on Cadmium Uptake by Oat and its Accumulation in Soil. *Journal of Environmental & Analytical Toxicology*, 07.
- SINGH, J., P. , KARAMANOS, R., E. & STEWART, J., W., B. 1988. The mechanisms of Phosphorus-Induced Zinc Deficiency in Bean (*Phaseolus vulgaris* L.). *Can. J. Soil Sci*, 68.
- SMIL, V. 2000. PHOSPHORUS IN THE ENVIRONMENT: Natural Flows and Human Interferences. *Annu. Rev. Energy Environ*, 25, 53-88.
- SMITH, F. W., MUDGE, S. R., RAE, A. L. & GLASSOP, D. 2003. Phosphate transport in plants. *Plant and Soil* 241.
- SOETAN, K. O., OLAIYA, C. O. & OYEWOLE, O. E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science* 4, 200-222.
- SOINNE, H. 2009. Extraction methods in soil phosphorus characterisation- Limitations and applications. *Pro Terra*.
- SOLTANGHEISI, A., ISHAK, C. F., MUSA, H. M., ZAKIKHAN, H. & ABDUL RAHMAN, Z. 2013. Phosphorus and Zinc Uptake and their Interaction on Dry Matter and Chlorophyll content of Sweet Corn (Zer mayz var. Saccarata). *journal of Agronomy* 12, 187-192.
- SONG, K., XUE, Y., ZHENG, X., LV, W., QIAO, H., QIN, Q. & YANG, J. 2017. Effects of the continuous use of organic manure and chemical fertilizer on soil inorganic phosphorus fractions in calcareous soil. *Sci Rep*, 7, 1164.
- STACEY, S. P., MCLAUGHLIN, M. J., ÇAKMAK, I., HETTIARACHCHI, G. M., SCHECKEL, K. G. & KARKKAINEN, M. 2008. Root Uptake of Lipophilic Zinc-Rhamnolipid Complexes. *J. Agric. Food Chem*, 56, 2112-2117.
- STEINER, T., MOSENTHIN, R., ZIMMERMANN, B., GREINER, R. & ROTH, S. 2007. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Animal Feed Science and Technology*, 133, 320-334.
- STOYANOVA, Z. & DONCHEVA, S. 2002. The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant. *Braz. J. Plant Physiol*, 14, 111-116.
- STUTTER, M. I., SHAND, C. A., GEORGE, T. S., BLACKWELL, M. S. A., DIXON, L., BOL, R., MACKAY, R. L., RICHARDSON, A. E., CONDRON, L. M. & HAYGARTH, P. M. 2015. Land use and soil factors affecting accumulation of phosphorus species in temperate soils. *Geoderma*, 257-258, 29-39.

- SUH, S. & YEE, S. 2011. Phosphorus use-efficiency of agriculture and food system in the US. *Chemosphere*, 84, 806-13.
- SVECNJAKA, Z., JENEL, M., BUJANA, M., VITALIB, D. & DRAGOJEVICB, I., V. 2013. Trace element concentrations in the grain of wheat cultivars as affected by nitrogen fertilization. *AGRICULTURAL AND FOOD SCIENCE*, 23, 445-451.
- SWIETLIK, D. 2002. Zinc Nutrition of Fruit Crops. *Horttechnology*, 12, 45-50.
- TAKEDA, E., YAMAMOTO, H., YAMANAKA-OKUMURA, H. & TAKETANI, Y. 2012. Dietary phosphorus in bone health and quality of life. *Nutr Rev*, 70, 311-21.
- TAKEDA, E., YAMAMOTO, H., YAMANAKA-OKUMURA, H. & TAKETANI, Y. 2014. Increasing dietary phosphorus intake from food additives: potential for negative impact on bone health. *Adv Nutr*, 5, 92-7.
- TANI, F. H. & BARRINGTON, S. 2005. Zinc and copper uptake by plants under two transpiration rates. Part I. Wheat (*Triticum aestivum* L.). *Environ Pollut*, 138, 538-47.
- TAVAJJOH, M., YASREBI, J., KARIMIAN, N. & OLAMA, V. 2011. Phytic Acid Concentration and Phytic Acid: Zinc Molar Ratio in Wheat Cultivars and Bread Flours, Fars Province, Iran. *J. Agr. Sci. Tech*, 13, 743-755.
- THOMAS, E. & OMUETI, J. 2012. The effect of phosphate fertilizer on heavy metal in soils and *Amaranthus caudatus*. *Agriculture and Biology Journal of North America*, 3, 145-149.
- TRAME, S., WESSELS, I., HAASE, H. & RINK, L. 2018. A short 18 items food frequency questionnaire biochemically validated to estimate zinc status in humans. *J Trace Elem Med Biol*.
- TRAN, H. T., HURLEY, B. A. & PLAXTON, W. C. 2010. Feeding hungry plants: The role of purple acid phosphatases in phosphate nutrition. *Plant Science*, 179, 14-27.
- TUNESI, S., POGGI, V. & GESSA, C. 1999. Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. *Nutrient Cycling in Agroecosystems* 53.
- UDDIN, R., BALOCH, P., A., IQBAL, S., BHUTTO, M., A., NIZAMANI, F., K., SOLANGI, A., H. & SIDIQI, A., A. 2014. Phosphorus Sorption Characteristics of Flour Soil Series *The Journal of Animal & Plant Sciences*, 24, 1547-1553.
- URGUN, V. & RIMMER, D. L. 2000. Reactions of zinc with iron-oxide coated calcite surfaces at alkaline pH. *European Journal of Soil Science*, 51, 511-516.
- VAFAEI, G. & SARRAF, A. 2014. Effect of Phosphorus and Zinc Fertilizer Application to Increasing the Quality of Nourishment in Winter Wheat. *International Journal of Biosciences (IJB)*, 5, 82-87.
- VANCE, C. P., UHDE-STONE, C. & ALLAN, D. L. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource: Tansley review. *New Phytologist*, 157, 423-447.
- WADU, M. C. W. M. 2015a. Phosphorus Solubility and Solid-State Speciation in Fertilizer Bands Applied to Calcareous Soil Systems. *PhD thesis, the University of Manitoba, Winnipeg, Canada*.
- WADU, M. C. W. M. 2015b. Phosphorus Solubility and Solid - State Speciation in Fertilizer Bands Applied to Calcareous Soil Systems
- WANDRUSZKA, V. R. 2006. Phosphorus retention in calcareous soils and the effect of organic matter on its mobility. *Geochem Trans*, 7, 6.
- WANG, B., XIE, Z., CHEN, J., JIANG, J. & SU, Q. 2008a. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. *Journal of Environmental Sciences*, 20, 1109-1117.
- WANG, B., XIE, Z., CHEN, J., JIANG, J. & SU, Q. 2008b. Effects of field application of phosphate fertilizers on the availability and uptake of lead, zinc and cadmium by cabbage (*Brassica chinensis* L.) in a mining tailing contaminated soil. *Journal of Environmental Sciences*, 20, 1109-1117.

- WANG, J., MAO, H., ZHAO, H., HUANG, D. & WANG, Z. 2012. Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau, China. *Field Crops Research*, 135, 89-96.
- WANG, R., GUO, S., LI, N., LI, R., ZHANG, Y., JIANG, J., WANG, Z., LIU, Q., WU, D., SUN, Q., DU, L. & ZHAO, M. 2015a. Phosphorus Accumulation and Sorption in Calcareous Soil under Long-Term Fertilization. *PLoS One*, 10, e0135160.
- WANG, S., WANG, Z., GAO, Y., LIU, L., YU, R., JIN, J., LUO, L., HUI, X., LI, F. & LI, M. 2017. EDTA alone enhanced soil zinc availability and winter wheat grain Zn concentration on calcareous soil. *Environmental and Experimental Botany*, 141, 19-27.
- WANG, Z., LIU, Q., PAN, F., YUAN, L. & YIN, X. 2015b. Effects of increasing rates of zinc fertilization on phytic acid and phytic acid/zinc molar ratio in zinc bio-fortified wheat. *Field Crops Research*, 184, 58-64.
- WATTS-WILLIAMS, S. J., TURNEY, T. W., PATTI, A. F. & CAVAGNARO, T. R. 2013. Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. *Plant and Soil*, 376, 165-175.
- WEISANY, W., SOHRABI, Y., HEIDARI, G., SIOSEMARDEH, A. & BADA KHSHAN, H. 2014. Effects of Zinc Application on Growth, Absorption and Distribution of Mineral Nutrients Under Salinity Stress in Soybean (Glycine MaxL.). *Journal of Plant Nutrition*, 37, 2255-2269.
- WENMING, D., ZHIJUN, G., JINZHOU, D., LIYING, Z. & ZUYI, T. 2001. Sorption characteristics of zinc(II) by calcareous soil± radiotracer study. *Applied Radiation and Isotopes*, 54, 371-375.
- WU, P., CUI, P. X., FANG, G. D., WANG, Y., WANG, S. Q., ZHOU, D. M., ZHANG, W. & WANG, Y. J. 2017. Biochar decreased the bioavailability of Zn to rice and wheat grains: Insights from microscopic to macroscopic scales. *Sci Total Environ*, 621, 160-167.
- WU, P., ZHAO, T. & TIAN, J. 2010. Phytic Acid Contents of Wheat Flours from Different Mill Streams. *Agricultural Sciences in China*, 9, 1684-1688.
- XIANG, H. F., TANG, H. A. & YING, Q. H. 1995. Transformation and distribution of forms of zinc in acid, neutral and calcareous soils of China. *Geoderma*, 66, 121-135.
- XUE, J. & HUANG, P. M. 1994. Zinc adsorption-desorption on short-range ordered iron oxide as influenced by citric acid during its formation. *Geoderma*, 64, 343-356.
- YADAV, D. S., ANTIL, R. S., KUMAR, V. & SINGH, M. 1985. Effect of Phosphate and Zinc application on dry matter yield and uptake of nutrients in Cowpea (*Vigna unguiculata* (L.) Walp.). *Zeitschrift für Pflanzenernährung und Bodenkunde*, 148, 233-240.
- YANG, J. C., WANG, Z. G., ZHOU, J., JIANG, H. M., ZHANG, J. F., PAN, P., HAN, Z., LU, C., LI, L. L. & GE, C. L. 2012. Inorganic phosphorus fractionation and its translocation dynamics in a low-P soil. *J Environ Radioact*, 112, 64-9.
- YANG, S., ZHANG, Z., CONG, L., WANG, X. & SHI, S. 2013. Effect of fulvic acid on the phosphorus availability in acid soil. *Journal of Soil Science and Plant Nutrition*, 13, 526-533.
- YANG, X. W., TIAN, X. H., LU, X. C., CAO, Y. X. & CHEN, Z. H. 2011. Impacts of phosphorus and zinc levels on phosphorus and zinc nutrition and phytic acid concentration in wheat (*Triticum aestivum* L.). *J Sci Food Agric*, 91, 2322-8.
- ZENG, F., ALI, S., ZHANG, H., OUYANG, Y., QIU, B., WU, F. & ZHANG, G. 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environ Pollut*, 159, 84-91.
- ZHANG, M., LI, C., LI, Y. C. & HARRIS, W. G. 2014. Phosphate minerals and solubility in native and agricultural calcareous soils. *Geoderma*, 232-234, 164-171.
- ZHANG, W., LIU, D., LIU, Y., CHEN, X. & ZOU, C. 2017. Overuse of Phosphorus Fertilizer Reduces the Grain and Flour Protein Contents and Zinc Bioavailability of Winter Wheat (*Triticum aestivum* L.). *J Agric Food Chem*, 65, 1473-1482.
- ZHANG, W., LIU, D., LIU, Y., CUI, Z., CHEN, X. & ZOU, C. 2016. Zinc uptake and accumulation in winter wheat relative to changes in root morphology and

- mycorrhizal colonization following varying phosphorus application on calcareous soil. *Field Crops Research*, 197, 74-82.
- ZHANG, Y.-Q., DENG, Y., CHEN, R.-Y., CUI, Z.-L., CHEN, X.-P., YOST, R., ZHANG, F.-S. & ZOU, C.-Q. 2012a. The reduction in zinc concentration of wheat grain upon increased phosphorus-fertilization and its mitigation by foliar zinc application. *Plant and Soil*, 361, 143-152.
- ZHANG, Y.-Q., SUN, Y.-X., YE, Y.-L., KARIM, M. R., XUE, Y.-F., YAN, P., MENG, Q.-F., CUI, Z.-L., CAKMAK, I., ZHANG, F.-S. & ZOU, C.-Q. 2012b. Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Research*, 125, 1-7.
- ZHANG, Y., SHI, R., REZAUL, K. M., ZHANG, F. & ZOU, C. 2010. Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. *J Agric Food Chem*, 58, 12268-74.
- ZHAO, A. Q., TIAN, X. H., CAO, Y. X., LU, X. C. & LIU, T. 2014. Comparison of soil and foliar zinc application for enhancing grain zinc content of wheat when grown on potentially zinc-deficient calcareous soils. *J Sci Food Agric*, 94, 2016-22.
- ZHAO, A. Q., TIAN, X. H., CHEN, Y. L. & LI, S. 2016. Application of ZnSO₄ or Zn-EDTA fertilizer to a calcareous soil: Zn diffusion in soil and its uptake by wheat plants. *J Sci Food Agric*, 96, 1484-91.
- ZHOU, M. & LI, Y. 2001. Phosphorus-Sorption Characteristics of Calcareous Soils and Limestone from the Southern Everglades and Adjacent Farmlands. *SOIL SCI. SOC. AM. J*, 65, 1404-1412.
- ZHU, J., LI, M. & WHELAN, M. 2018. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci Total Environ*, 612, 522-537.
- ZHU, Y., G., SMITH, S., E. & SMITH, F., A. 2001a. Zinc (Zn)- Phosphorus (P) Interactions in Two Cultivars of Spring Wheat (*Triticum aestivum* L.) Differing in P Uptake Efficiency. *Annual of Botany*, 88, 941-945.
- ZHU, Y. G., SMITH, S. E. & SMITH, F. A. 2001b. Zinc (Zn)-phosphorus (P) Interactions in Two Cultivars of Spring Wheat (*Triticum aestivum* L.) Differing in P Uptake Efficiency. *Annals of Botany*, 88, 941-945.
- ZIADI, N., WHALEN, J. K., MESSIGA, A. J. & MOREL, C. 2013. Assessment and Modeling of Soil Available Phosphorus in Sustainable Cropping Systems.
- ZWONITZER, J. C., GARY, M., PIERZYNSKI. & HETTIARACHCHI, G. M. 2003. Effect of Phosphorus addition on Lead, cadmium, and Zinc Bioavailability in Ametal-contaminated Soil *Water, Air, and Soil Pollution*, 143.