

## Studying the Uptake of Toxic Metals by Plants using New Mathematical Modeling

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### Abstract

Two plants species were used to study the uptake of heavy metals by plants; these were *Lolium perenne* and Cock's Foot. The species were grown in Hoagland's solution with double concentration of phosphate for one week; the roots species were cut and grown in Hoagland's solution without phosphate to prevent precipitation of any metal phosphate. Four experiments were run with a composite rotatable design with three variables and at five levels (-1.68,-1,0,+1,+1.68) was used. The metals were added as metal nitrate to Hoagland's solution in which the plants are grown on a logarithmic scale and after one week of grown the roots and shoots of plant were cut and digested with concentrated nitric acid and examined for metals by atomic absorption spectrophotometer. This research proved that some elements increased the absorption of other elements in growth solution to the shoot through root while other elements interfere the absorption phenomena for example zinc effects on absorption of copper

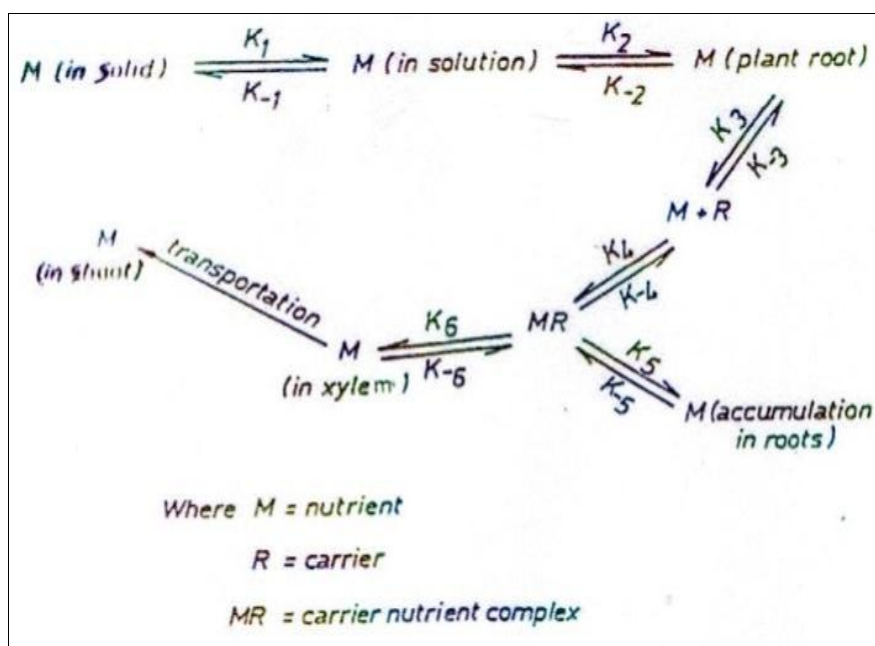
### Introduction

To date, the mechanism of uptake of heavy metals by plant is not known, however it has been proposed that:

1. Plant root excrete hydronium ion at their surface so that exchange with cat-ion can occur<sup>(1)</sup>.

2. Metal ions move to the root surface by diffusion so that adsorption of ion on the root surface occurs<sup>(2)</sup>.

3. The movement of metal ions from soil to shoots via roots in plants may occur in the way suggested below<sup>(3)</sup>.



Scheme (1) The Movement of Metal Ion from Soil

Heavy metals in the environment can reach toxic levels in plant by accumulation<sup>(4)</sup>. Although trace of heavy metals are essential as key of components of enzyme system, other inhibit the system<sup>(5)</sup>. There are many factors influencing the toxic heavy metals to plants, such as ion form, complex, chelate, molecule, colloidal, precipitated, adsorbed, charge and ionic radius of metal<sup>(6)</sup>. The studying of particular method

for assessing the toxicity of metals on plants depend upon yield of plants and the concentration of metals in plant tissue<sup>(7)</sup> fig (2). It was shown that the yield curve two lines when the concentration is plotted in a logarithmic form against the yield of plant, one line is horizontal and the other a sloping regression line fig (3) which meet at the upper critical level<sup>(8)</sup>.

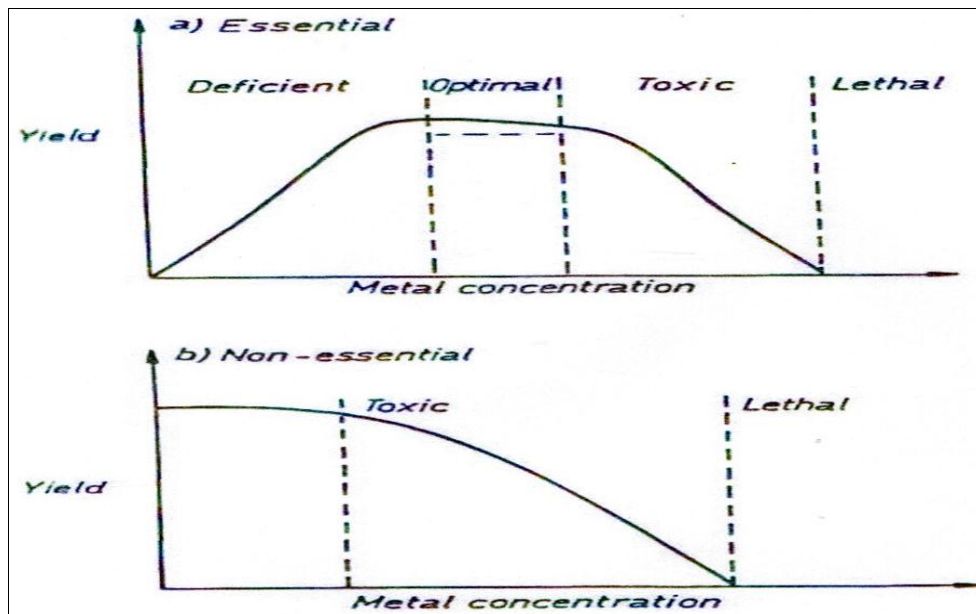


Figure (1) Deficiency and Toxicity of Heavy Metals

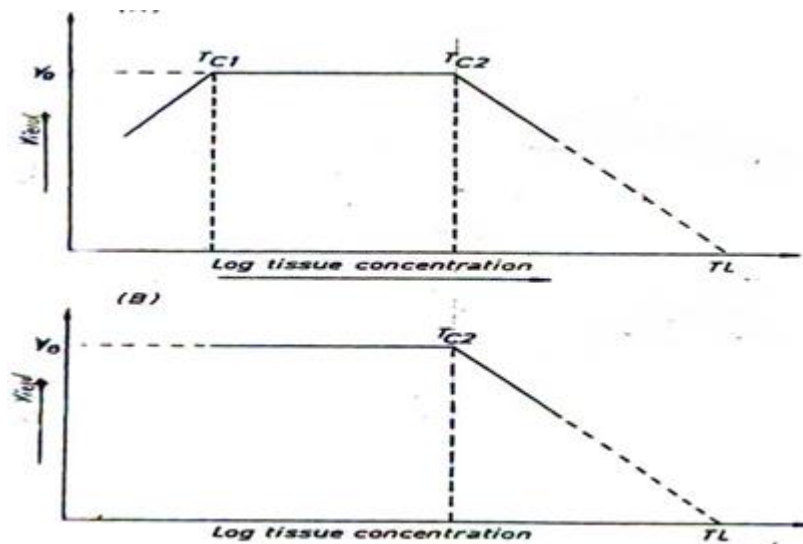


Figure (2) Yield Cure for

- (A) Essential elements
- (B) Non-essential elements
- $Y_0$  Yield unaffected by toxicity
- $TC_1$  Lower critical level
- $TC_2$  Upper critical level
- $TL$  Lethal concentration

Response Surface Methodology (RSM) is a technique used in this work to study the relationships between one or more measured responses on the one hand and a number of input factors on the other<sup>(9)</sup>.

RSM can be represented in three dimensions showing the relationship between response and factors<sup>(10)</sup>.

The estimated response in the composite rotatable design at a given point has a value which is dependent only on the distance of that point from the center of the design and not on the direction<sup>(11)</sup> fig

(3). The previous design was used to study the interactive effects of elements upon plant growth; there are two major questions to be considered. How far does the presence of one of this element in:

1. In the roots of plant modify the uptake of other elements and their translocation to the shoot<sup>(12)</sup>.
2. In the tissue of shoot modify the toxicity of the other elements in same tissue<sup>(13)</sup>.

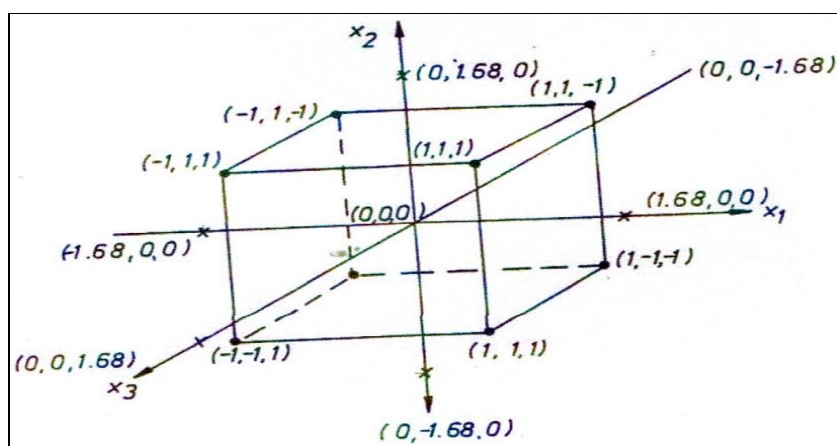


Figure (3) Central Composite Rotatable design in the three X variables with Five levels

### Experimental Work and Results

Lolium perenne and Cock's foot which are relatively hardy species of grass were chosen for study of uptake of heavy metals by plants table (1) they are very quick growing and hence ideal for this type of study using RSM. The plant was germinated and after ten days of development, five seedlings were taken and left to grow in Hoagland's nutrient solution with double concentration of phosphate for one week then. The root of the plant was cut and the fresh weight of each bunch was recorded. The design of experiment was composite rotatable with three variables;  $X_1$  (trivalent metals  $Al^{+3}$  and  $La^{+3}$ ),  $X_2$  (divalent metals  $Pb^{+2}$ ), and  $X_3$  (monovalent metals  $Tl^{+1}$  and  $Ag^{+1}$ ). The metals were added as metal nitrate and the addition was on a logarithmic scale on a level from (-1.68, -1, 0, +1, +1.68) table (2).

The plant left to grow again for one week in Hoagland's solution without phosphate, after that roots separated from shoots, the root length was recorded and dry weight of root was also recorded as yield of plant. The roots and shoots were digested with concentrated nitric acid and examined for metals by atomic absorption spectrophotometer. The results obtain given in tables (3-6).

The 3-D plot of measured response with two metals was drawn up by SAS and GINO while other metals kept constant. The results obtained are shown in fig (4-7).

Table (1) Heavy Metals Ione Added to Nutrient Solution

Experiment Number	Species	Ions Added
1	Cock's Foot	$La^{3+}$ , $Pb^{2+}$ , $Tl^{+}$
2	Cock's Foot	$La^{3+}$ , $Pb^{2+}$ , $Ag^{+}$
3	Lolium Perenne	$Al^{3+}$ , $Pb^{2+}$ , $Tl^{+}$
4	Lolium Perenne	$Al^{3+}$ , $Pb^{2+}$ , $Ag^{+}$

Table (2) The Coded Value with Corresponding Concentration of Metal Ions ( $\mu\text{g/ml}$ )

Coded Value	Al	Ag	La	Tl	Pb
-1.68	$5 \times 10^{-4}$	$5 \times 10^{-4}$	$2.5 \times 10^{-3}$	$1 \times 10^{-4}$	$5 \times 10^{-4}$
-1.0	$3.2 \times 10^{-3}$	$1.28 \times 10^{-3}$	0.016	$6.4 \times 10^{-4}$	$3.2 \times 10^{-3}$
0.00	0.55	0.02	0.250	0.01	0.05
+1.0	0.755	0.3	3.876	0.155	0.775
+1.68	5.00	200	25	1.5	5.00

Table (3) Tissue Concentrations of La, pb, Tl, Cu, Zn, Fe, Mg, and Mn in Roots of Cock's Foot Seedlings

Trials	Independent variables			Dependent variables									
	$X_1(La)$	$X_2(pb)$	$X_3(Tl)$	La	pb	Tl	Cu	Zn	Fe	Mg	Mn	DWOM	R.L
1	-1	-1	-1	381	520	2.0	3333	612	15000	26984	555	0.0063	2.04
2	+1	-1	-1	2736	305	3.5	2722	527	10000	2444	750	0.009	1.70
3	-1	+1	-1	412	6392	1.0	600	109	6571	3428	250	0.0357	0.46
4	+1	+1	-1	974	841	144	2978	1617	5000	19117	2128	0.0068	2.16
5	-1	-1	+1	1957	1110	3.0	79	154	3157	3092	585	0.0387	3.50
6	+1	-1	+1	3030	935	44	880	251	5194	2500	934	0.0231	4.50
7	-1	+1	+1	261	2186	69	426	684	4195	3575	111	0.6451	9.16
8	+1	+1	+1	3867	1830	73	80	115	7933	4482	81	0.0375	1.34
9	+1.68	0	0	4138	94	13	103	152	6534	4741	112	0.0290	0.82
10	-1.68	0	0	304	842	12	477	372	3630	2826	184	0.1150	16.18
11	0	+1.68	0	1274	5520	5	5312	3771	10937	7812	4464	0.0084	0.56
12	0	-1.68	0	1331	57	15	306	93	5562	4000	118	0.402	3.78
13	0	0	+1.68	385	196	58	159	42	1850	2240	58	0.0779	0.94
14	0	0	-1.68	522	308	2	52	167	2254	1964	125	0.1638	13.10
15	0	0	0	1484	892	14	811	30	6000	3166	157	0.0302	9.30
16	0	0	0	1152	1013	10	197	96	11912	3500	175	0.0408	10.10
17	0	0	0	1102	643	11	183	45	4177	3285	300	0.0767	15.20
18	0	0	0	1692	872	13	170	538	6206	6250	81	0.0594	13.80
19	0	0	0	1159	682	10	165	211	1171	7500	537	0.0414	11.80
20	0	0	0	860	846	5	181	231	7314	5724	104	0.605	10.80

DWOM= Dry weight of plant per treatment (g)

R.L= Root length (cm)

=significant from control

Concentration of metals = $\mu\text{g/g}$  per dry weight of plant

Table (4) Tissue Concentrations of La, pb, Tl, Cu, Zn, Fe, Mg, and Mn in Shoots of Cock's Foot Seedlings

Trials	Independent variables			Dependent variables									
	$X_1(La)$	$X_2(pb)$	$X_3(Tl)$	La	pb	Tl	Cu	Zn	Fe	Mg	Mn	DWOM	
1	-1	-1	-1	16	54	0.085	20	26	156	100	45	0.14314	
2	+1	-1	-1	277	17	0.290	11	12	105	81	18	0.17202	
3	-1	+1	-1	18	591	2.33	26	33	206	108	45	0.12553	
4	+1	+1	-1	14	43	1.77	16	22	119	63	38	0.20485	
5	-1	-1	+1	378	270	0.260	13	31	116	87	32	0.16496	
6	+1	-1	+1	593	61	0.297	15	19	237	136	31	0.09365	
7	-1	+1	+1	12	391	0.517	17	29	116	57	17	0.11698	
8	+1	+1	+1	518	405	0.410	25	17	193	111	42	0.10978	
9	+1.68	0	0	1057	26	1.890	6	26	257	110	38	0.18057	
10	-1.68	0	0	9.0	30	3.35	24	22	130	78	66	0.17057	
11	0	+1.68	0	18	1410	0.2663	23	21	135	76	45	0.1708	
12	0	-1.68	0	96	10	1.030	23	32	119	59	39	0.20261	
13	0	0	+1.68	113	59	2.797	20	14	244	132	43	0.08605	
14	0	0	-1.68	102	39	0.5103	19	53	122	66	52	0.19713	
15	0	0	0	153	54	2.54	22	19	211	134	44	0.10434	
16	0	0	0	92	58	1.269	27	24	154	83	35	0.16181	
17	0	0	0	7	0.0	0.625	26	35	130	75	37	0.19473	
18	0	0	0	85	34	2.083	45	23	167	146	52	0.21009	
19	0	0	0	77	30	2.895	52	23	130	82	38	0.17034	
20	0	0	0	88	35	1.1651	22	42	130	87	32	0.18495	

DWOM= Dry weight of plant per treatment (g)

Concentration of metal=  $\mu\text{g/g}$  per dry weight of plant

=signification from control

Table (5) Tissue Concentrations of La, pb, TI, and Fe in Roots of Lolium perenne Seedlings

Trials	Independent variables			Dependent variables					
	$X_1(La)$	$X_2(pb)$	$X_3(TI)$	La	pb	TI	Fe	DWOM	R.L
1	-1	-1	-1	83	747	100	1889	0.2159	18.86
2	+1	-1	-1	126	59	91	1063	0.4198	21.36
3	-1	+1	-1	61	91	190	1855	0.1270	20.50
4	+1	+1	-1	413	2703	150	876	0.5019	22.20
5	-1	-1	+1	20	291	210	1357	0.3083	20.90
6	+1	-1	+1	703	90	180	1581	0.2334	16.20
7	-1	+1	+1	98	9003	120	3673	0.1148	12.80
8	+1	+1	+1	1329	429	100	1376	0.1563	8.900
9	+1.68	0	0	3272	1147	20	1627	0.0603	3.00
10	-1.68	0	0	40	956	70	829	0.2145	19.00
11	0	+1.68	0	534	2261	60	955	0.0387	1.500
12	0	-1.68	0	63	3065	880	827	0.5300	21.40
13	0	0	+1.68	1010	1612	1050	5788	0.0619	7.70
14	0	0	-1.68	19	198	5	656	0.6113	24.20
15	0	0	0	421	361	60	1401	0.2026	24.80
16	0	0	0	314	169	40	920	0.1728	25.20
17	0	0	0	247	344	50	688	0.6024	25.80
18	0	0	0	136	29	10	207	0.5994	26.0
19	0	0	0	153	217	70	1474	0.2887	25.62
20	0	0	0	33	512	50	782	0.5314	24.40

DWOM= Dry weight of plant per treatment (g)

R.L= Root length (cm)

=significant from control

Concentration of metals = $\mu\text{g/g}$  per dry weight of plant

Table (6) Tissue Concentrations of Al, Pb, Ag, and Fe in Shoots of Lolium perenne Seedlings

Trials	Independent variables			Dependent variables				
	$x_1(Al)$	$x_2(Pb)$	$x_3(Ag)$	Al	Pb	Ag	Fe	DWOM
1	-1	-1	-1	9	4	0.50	82	1.0961
2	+1	-1	-1	10	4	0.45	87	1.087
3	-1	+1	-1	6	121	5.0	82	0.9008
4	+1	+1	-1	41	35	9.8	97	0.7410
5	-1	-1	+1	7	95	2.0	50	1.1652
6	+1	-1	+1	4	14	35.0	305	0.8868
7	-1	+1	+1	10	153	4.0	143	0.7251
8	+1	+1	+1	4	106	0.50	48	0.92471
9	+1.68	0	0	9	23	12.0	52	1.3430
10	-1.68	0	0	5	7	0.39	36	1.5828
11	0	+1.68	0	4	232	1.50	50	1.7152
12	0	-1.68	0	15	0.8	0.50	23	1.2870
13	0	0	+1.68	13	8.0	7.0	36	1.47870
14	0	0	-1.68	12	3.2	3.0	60	0.7550
15	0	0	0	2	8.0	0.39	56	1.1451
16	0	0	0	23	0.89	2.0	25	1.5322
17	0	0	0	5	2.0	3.0	41	1.8812
18	0	0	0	5	2.0	2.0	56	1.3292
19	0	0	0	5	4.2	2.0	42	1.5265
20	0	0	0	5	6.0	4.0	31	1.5621

DWOM = Dry weight of plants per treatment (g)

Concentration of metals =  $\mu\text{g/g}$  per dry weight of plants

=signification from control

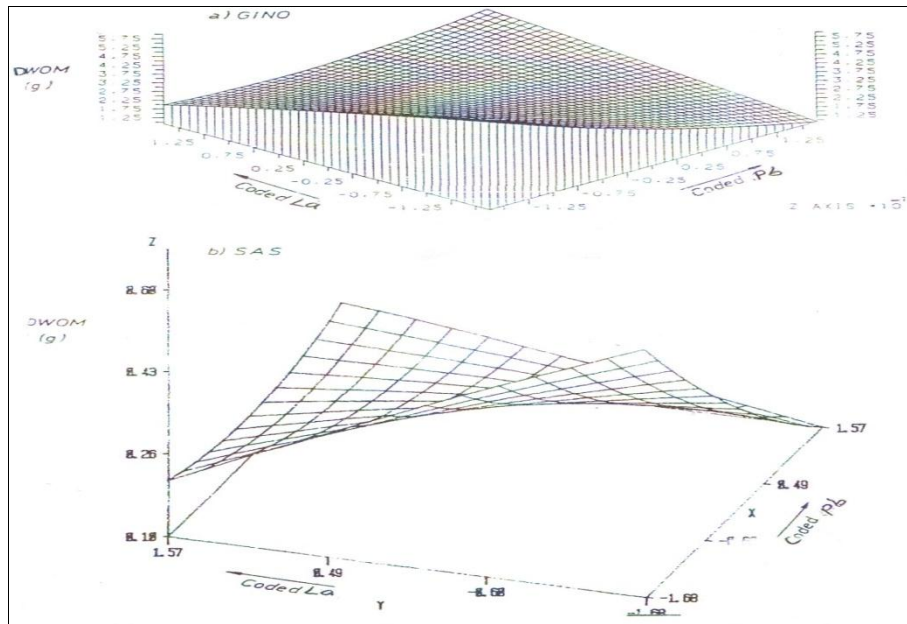


Figure (4) Response Surface for Iron taken up by Roots of Cock's Foot at TI=0

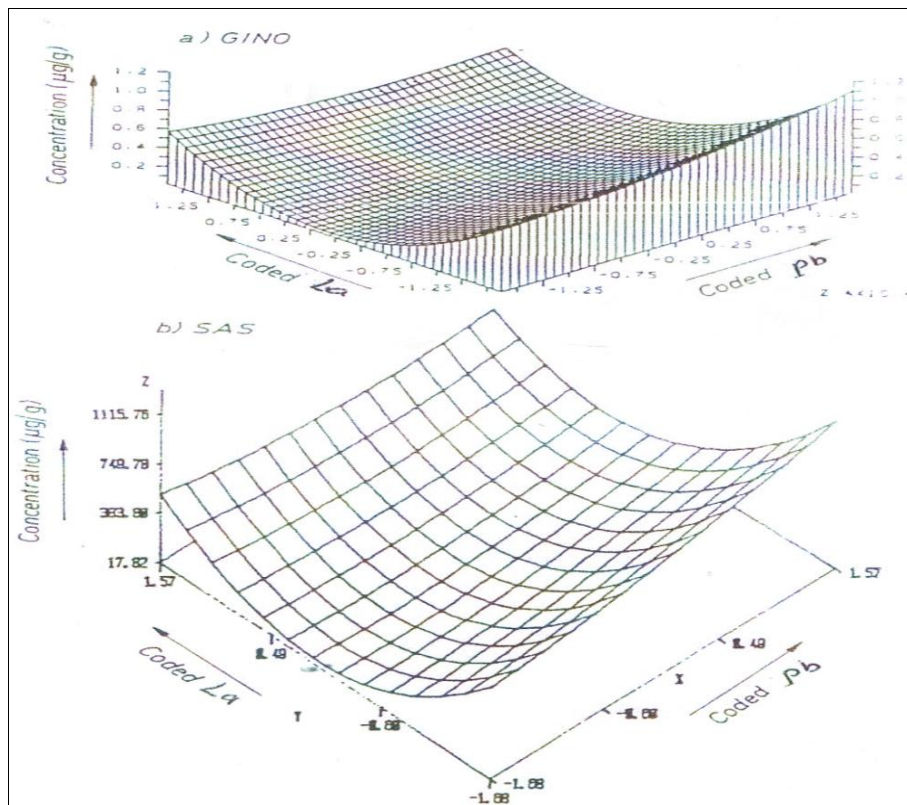


Figure (5) Response Surface for Iron taken up by Shoots of Cock's Foot at TI=0

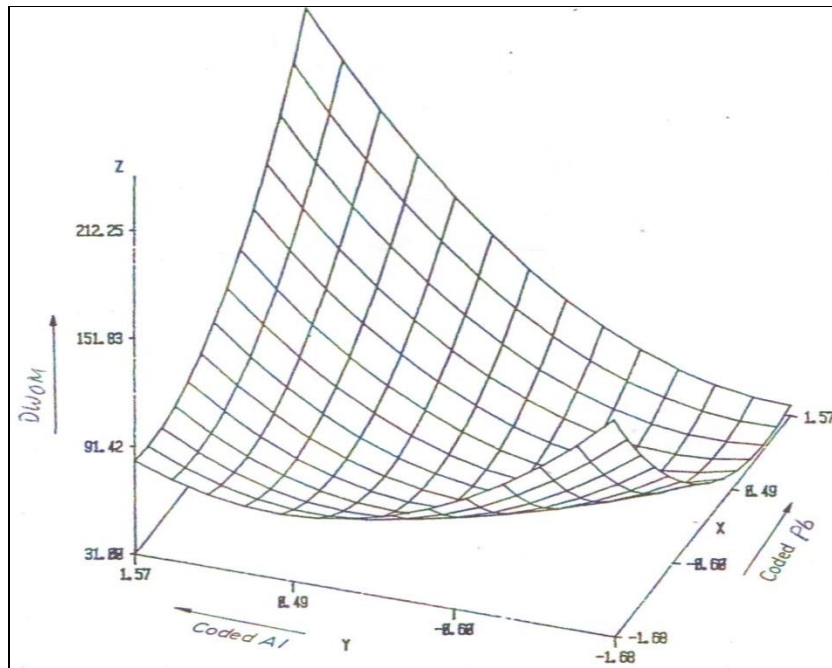


Figure (6) Response Surface for Iron taken up by Roots of Lolium Perenne at TI=0

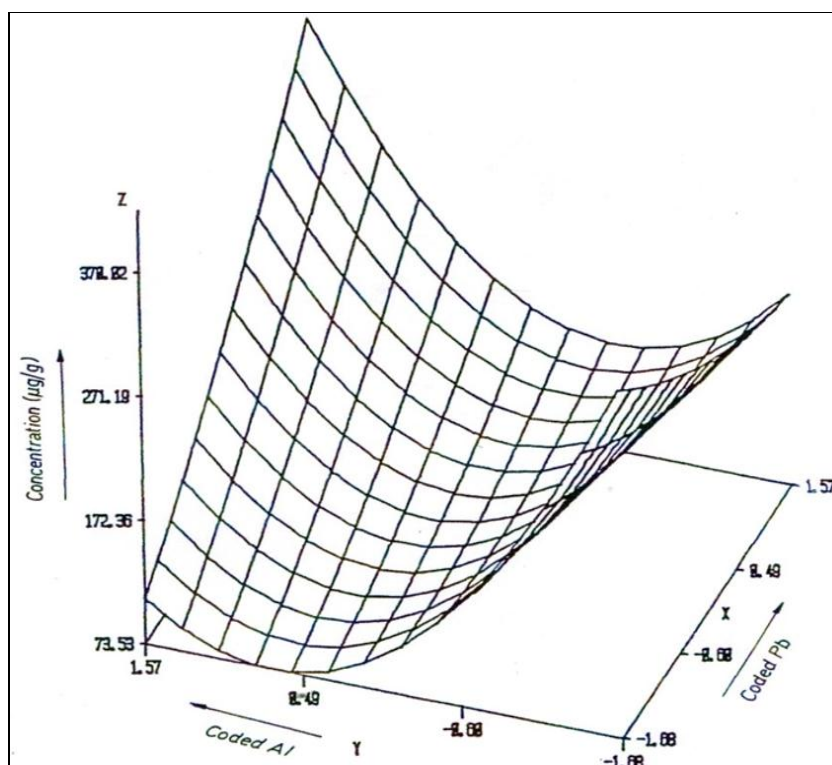


Figure (7) Response Surface for Iron taken up by Shoots of Lolium Perenne at TI=0

**Discussion**

In general, the results indicate that the level of essential metals such as Cu, Zn, Fe, Mg and Mn in roots of Cock's Foot seedling was higher than in the shoot (tables 3 and 4). Copper decrease the absorption of zinc and its translocation from roots to the shoot. Brar et alia<sup>(14)</sup> reported that zinc and copper coordinate to N and S groups of amino acids, so it seems that copper by competing with zinc for the

binding sites of amino acids may affect the translocation of Zinc with the plant. Brar et alia<sup>(15)</sup> also reported that by increasing the iron concentration the zinc uptake by plant was reduced. Wallace and Sag<sup>(16)</sup> reported that nickel decreases the level of manganese in the shoot while it increases the level of cobalt and zinc in the shoot. Competition between such metals, Lead, and zinc with thallium for binding site of sulphur amino acids can affect their

translocation and uptake by Cock's Foot Seedling which were used in the present work. *Lolium Perenne* seedling accumulates more metals in the root than in the shoots ( tables 5 and 6), the interactive effect of La and pb on copper in the roots when TI level is held constant, the surface indicates that when pb is present in the nutrient solution in very low concentration higher level of copper are taken up by roots of *Lolium perenne*. Fig (4) illustrates the effect of La and Pb in nutrient solution on dry weight of plant when TI level is held constant at coded value 0. The surface indicate that when Pb is present in the nutrient solution in very low concentration class than a coded level at 1:00 which equivalent to  $3.2 \times 10^3 \mu\text{g/ml}$  higher level yield at plant produced than when the lead increased in nutrient solution also contained lanthanum.

Fig (5) indicates the interactive at La and Pb in nutrient solution on copper in shoot at TI coded level at 0. The surface indicates that both and decrease the level of copper in shoot lead show negative

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interaction with copper content of roots while lanthanum behave so reduce the copper content, these results agree with Robert et alia<sup>(17)</sup>.

Fig (6) shown the effect of Al and pb in nutrient solution on dry weight of plant when Ag is held constant at coded value 0. The surface indicate that boat and decrease the yield at shoot. Lead shows negative interaction with copper content, these results agree with Mikal and Grad<sup>(18)</sup>. Fig (7) depicts the interactive effect between Al and pb on iron uptake into the shoot of *Lolium Perenne* on the one hand and Al and Ag on the other. The surface indicates that initially Al reduce the level of iron uptake. The effects are due to lead being particularly weak, those due to sliver more pronounced. The observation in these figures are in agreement with Lee Khoo<sup>(19)</sup> . however, when the concentration of the added metals in combination tends toward the maximum experiment levels, then the level of iron taken up increased markedly.

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## دراسة امتصاص العناصر السامة من قبل النباتات باستخدام نظام رياضي جديد

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### الملخص

استخدمت نوعين من النباتات لغرض دراسة امتصاص العناصر الثقيلة من قبل النباتات وهذين النوعين هما Lolium perenne, Cock's Foot وقد تم نمو هذين النوعين من النباتات في محلول هوكلاندر ذو تركيز مضاعف من الفوسفات لمدة اسبوع ثم قطعت الجذور واجري النمو في محلول هوكلاندر مرة ثانية بدون اي تركيز للفوسفات لمنع ترسيب العنصر بصورة فوسفات. وقد اجريت اربع تجارب ضمن تصميم دائري يحتوي على ثلاثة متغيرات وضمن خمسة مجالات (1.68, +1, 0, -1, -1.68). وقد اضيفت العناصر الثقيلة بصيغة نترات للعنصر الى محلول هوكلاندر الذي يجري نمو النباتات فيه وبعد اسبوع من النمو يتم قطع الجذور والسيقان وهضمها باستخدام حامض النتريك المركز ويتم قياس العناصر الثقيلة باستخدام تقنية طيف الامتصاص الذري. ولقد تم التوصل في هذا البحث الى وجود بعض العناصر في محلول النمو يساعد على امتصاص عناصر اخرى الى الساق من خلال الجذر بينما عناصر اخرى تعيق عملية الامتصاص والمثل على ذلك يوترالزك على امتصاص النحاس.