Results

and

Discussion

4. RESULTS AND DISCUSSION

This part of the current study involves six sites contaminated with heavy metals due to the different anthropogenic sources. Except for soils of Toukh whose main source of contamination is the vehicle exhausts and soils of Shobra Al- Khema whose source of contamination is the smelters emissions, the main source of contamination of the other four soils is the irrigation with wastewaters.

Quality control data for the analysis of soil and plant samples using the ICP-MS were quite good. The mean recoveries for the reference soil and plant materials are shown in Table (8).

4.1. Evaluation of the investigated waters for heavy metals toxicities:

Data presented in Table (9) show that the nutritive heavy metals (Cu, Fe, Mn and Zn) were, in general, found in concentrations exceeding the corresponding ones of the non-nutritive ones (Cd, Co, Ni and Pb). However, Pb and Ni were detected in relatively higher values in the sewage waters of Al-Gabal Al-Asfar and Arab Abou Saed. In general, Cd and Co recorded the lowest concentration of heavy metals in the studied waters, according to the recommended maximum concentrations of heavy metals in irrigation water given by FAO (1994), concentrations of Mn, Zn, Cu, Cd, Ni and Pb in Al-Gabal Al-Asfar sewage water; Mn, Zn, Cu, Co, Cd, Ni and Pb in Arab Abou Saed

Table (8): Determination of Mn, Zn, Cu, Co, Cd and Pb $\mu g\ g^{-1}$ in standard reference materials, Rice Flour 1568a and Soil reference material NCSZC 73007 using ICP-MS.

Reference materials		Found values ($\mu g\ g^{-1}$)	Certificate values ($\mu g\ g^{-1}$)
Rice Flour 1568a	Mn	18.9	20±1.6
	Zn	16.43	19.4±0.5
	Cu	2.126	2.4±0.3
	Cd	0.021	0.022±0.016
Soil reference material NCSZC 73007	Mn	423.58	441±20
	Zn	106.14	100±8
	Cu	30.28	32±2
	Co	13.300	13.6±0.6
	Cd	0.263	0.25±0.02
	Pb	62.266	61±2

Table (9): Total heavy metal contents of the waters used for irrigating the investigated soils.

Source of the irrigation water	Total heavy metal contents mgL^{-1}							
	Cd	Co	Ni	Pb	Cu	Fe	Mn	Zn
Al- Gabal Al-Asfar treated sewage water	0.03	0.02	4.74	12.00	4.26	4.88	6.96	12.22
Arab Abou- Saed treated sewage water	0.10	0.38	4.80	8.78	6.22	4.92	6.88	12.30
Bahr Al-Baqar drain	0.16	0.13	4.58	6.04	9.88	4.85	1.00	6.25
The Nile fresh water (Toukh region)	0.01	n.d	0.02	0.04	0.04	0.52	0.06	0.20
Namoul drain	0.11	0.38	3.74	7.96	4.66	78.28	29.59	25.99
The Nile fresh water (Shobra Al- Khema region)	0.008	0.002	0.02	0.03	0.04	0.46	0.12	0.14

n.d = not detected

sewage water; Mn, Zn, Cu, Co, Cd, Ni and Pb in Bahr Al- Baqar drain's water and all the investigated heavy metals in Namoul drain's water exceed the recommended maximum concentrations for waters used continuously on all soils which are 5, 0.2, 2, 0.2, 0.05, 0.01, 0.2 and 0.5 mg L^{-1} for Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb respectively. This means that these heavy metals will cause growth reduction due to toxicities.

4.2. Heavy metal concentrations in the investigated soils:

Data presented in Table (10) show values of total and DTPA- extractable heavy metal contents as well as availability indexes of these heavy metals in the studied soils. Values of total Cd ranged from 0.2 mg kg^{-1} in Toukh soil to 2.7 mg kg^{-1} in Al-Gabal Al-Asfar soil. This occurred although Cd content in the sewage water used for irrigating Al-Gabal Al-Asfar soil was as low as 0.03 mg L^{-1}. The pollution of soil with Cd found as a contaminant in the phosphatic fertilizers beside of the variation in water content of the heavy metals and its suspended organic matter may account for the high content of Cd in Al-Gabal Al-Asfar soil. Total Co in soil flocculated between 12.17 mg kg^{-1} in Arab Abu Saed soil and 27.14 mg kg^{-1} in Bahr El-Baqar soil. Total Ni content was the highest in Al-Gabal Al-Asfar soil (98.33 mg kg^{-1}), the lowest in Toukh soil (20.8 mg kg^{-1}) and came in between in the other soils.

The corresponding highest value of total Pb was attained in Al-Gabal Al-Asfar soil, while the lowest one was attained in Arab Abou-Saed (83.47 and 10.91 mg kg^{-1}, respectively). Values of total Cu and Zn were highest in Al-Gabal Al-Asfar soil (137.5 and 417.09 mg kg^{-1}, respectively. The corresponding lowest values were detected in Bahr Al-Baqar and Shobra Al-Khema soils, respectively (47.8 mg kg^{-1} for copper and 62.65 mg kg^{-1} for Zn). Total content of Fe and Mn were the highest in Namoul and Bahr Al Baqar soils (30000 mg kg^{-1} for Fe and 475.18 mg kg^{-1} for Mn, respectively) and the lowest in Arab Abou-Saed and Al-Gabal Al-Asfar ones (10675 mg kg^{-1} for Fe and 156.02 mg kg^{-1} mg kg^{-1} for Mn, respectively).

The aforementioned results reveal that total heavy metal contents of the investigated soils were in general not a function of their contents in the waters used for irrigating them since other factors such as the mineral composition of the parent material and agricultural management practices and the aerosols fall on soil may affect these contents. These findings stand in well agreement with those of Abdellah (1995) and Matter (1999). Considering values of DTPA- extractable heavy metals data in Table (10) reveal that Al-Gabal Al-Asfar soil was characterized by the highest content of Cd (0.22 mg kg^{-1}), Co (1.97mg kg^{-1}), Ni (6.48 mg kg^{-1}), Pb (6.22 mg kg^{-1}), Cu (11.03 mg kg^{-1}) and Zn (21.75 mg kg^{-1}) while Namoul soil attained the highest concentrations of DTPA- extractable Fe (43.7 mg kg^{-1}) whereas Shobra Al- Khema soil showed the highest concentration of DTPA- extractable Mn (22.11g^{-1}).

Table (10): Total and DTPA extractable contents of some heavy metals in the investigated soils.

Soil Location	Heavy metal contents mg kg^{-1}															
	Cd		Co		Ni		Pb		Cu		Fe		Mn		Zn	
	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
Al-Gabal Al-Asfar	2.695	0.221	25.15	1.967	98.33	6.48	83.469	6.218	137.5	11.03	28375	39.05	156.021	6.787	417.09	21.75
Arab Abou-saed	2.288	0.139	12.17	0.713	28.73	1.21	10.908	0.563	48.96	3.344	10675	11.74	336.055	14.114	282.65	14.443
Bahr Al-Baqar	0.195	0.013	27.14	1.631	49.23	2.46	40.326	2.46	47.8	3.37	22675	24.9	475.18	20.243	324.08	16.625
Toukh	0.191	0.013	26.54	1.863	20.8	1.05	15.842	1.107	59.22	4.453	21800	28.3	361.7	16.277	69.8485	3.835
Namoul	0.299	0.018	26.1	1.762	46.83	3.04	12.243	0.857	57.66	4.215	30000	43.7	375.58	18.704	227.48	12.625
Shobra Al-Khema	0.306	0.021	24.08	1.705	23.35	1.63	19.5733	1.219	109.71	7.91	27100	35.23	442.28	22.114	62.65	3.44

T: Total A: DTPA- extractable

On the other hand, the lowest values of DTPA-extractable heavy metals were attained by Bahr Al-Baqar and Toukh soils for Cd (0.013 mg kg^{-1}), Arab Abou-Saed for Co (0.71 mg kg^{-1}), Toukh soil for Ni (1.05 mg kg^{-1}), Arab Abou-Saed soil for Pb (0.56 mg kg^{-1}) and Cu (3.34 mg kg^{-1}), Fe (11.74 mg kg^{-1}), Al Gabal Al-Asfar for Mn (6.79 mg kg^{-1}) whereas Shobra Al-Khema soil showed the lowest concentration of DTPA- extractable Zn (3.44 mg kg^{-1}).

Aboulroos et al. (1996) reported that the background level of DTPA - extractable Cd in the non- polluted soils of Egypt is 0.014-0.022 mg kg^{-1} soil. The comparison between these results and those obtained herein indicates that concentration of DTPA - extractable Cd in Al gabal Al-Asfar and Arab Abu-saed soils exceeded that of the non- polluted soils.

Also, values of DTPA – extractable Pb in Al Gabal Al-Asfar and Bahr Al-Baqar exceeded the back ground levels in non-polluted soils in Egypt which are 1.17- 1.61 mg kg^{-1} for Pb.

Likewise, values of DTPA – extractable Co, Ni, Pb, Cu and Zn exceeded those reported as background levels for these metal ions in non- polluted soils of Egypt which are 0.17- 0.21 mg kg^{-1} for Co, 0.54- 0.74 mg kg^{-1} for Ni, 1.86- 2.50 mg kg^{-1} for Cu and 1.56- 2.46 mg kg^{-1} for Zn.

Accordingly, one should be cautious of using such investigated soils for cropping because such high concentrations of metals might result in toxicity to all but adapted plants.

Data presented in Table (14) show that DTPA-extractable forms of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb were significantly correlated with their total concentrations in soil and the correlation coefficient values were 0.976, 0.967, 0.999, 0.996, 0.937, 0.822, 0.940 and 0.996, respectively. The relationship between DTPA extractable amount of heavy metals and the factors affecting their availability were calculated in the regression equations are presented in (Table 11).

4.3. Heavy metals phytoextraction by maize

This part of study focuses on heavy metals phytoextraction by maize (*Zea mays* L.) plants grown on the aforementioned soils which are polluted with heavy metals to different extents. Such a situation provides an in situ nodded of phytoremediation of soils polluted with heavy metals.

Data presented in Tables (12) and (13) reveal that plants differed in their contents of the concerned metal ions from a soil site to another. Also, the pattern of accumulation of all these metal ions was different as far as plant parts are concerned. The concentrations of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb in maize roots were significantly correlated with the DTPA extractable forms and their correlation coefficients were -0.945, 0.983, 0.976, 0.993, 0.942, 0.997, 0.934 and 0.995, respectively (Table, 14). Concentrations of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb in leaves were also significantly correlated with their concentrations in roots and their correlation coefficients were 0.981, 0.942, 0.937, 0.975, 0.973, 0.946, 0.998 for Fe, Mn, Zn, Cu, Co, Cd and Pb, respectively, while insignificant relationship was found between Ni concentrations in leaves and roots.

Table (11): Regression equations for available heavy metal contents in soil in relation to the factors controlling their availability

Heavy metal	Regression equation	R^2
Cd	0.077 + 0.0588 total Cd + 0.00004 Clay % - 0.00404 Avail. Mn	98.1
Co	1.11 + 0.0232 total Co + 0.00983 Avail. Fe - 0.0358 CaCO3 % - 0.00471 [Na]	99
Ni	- 0.634 + 0.019 Pb + 0.0582 total Ni + 0.314 OM %	97.8
Pb	- 0.500 + 0.0687 total Pb + 0.013 Avail. Ni + 0.217 OM %	99.8
Fe	7.2 - 7.16 Co - 1.06 CaCO3 % + 0.00166 total Fe	96.3
Mn	- 62.8 + 0.0077 total Mn + 9.81 pH + 0.120 Clay %	98.7
Zn	1.11 + 0.0460 Zn total	98.8
Cu	- 0.588 + 0.0821 total Cu	99.1

Table (12): Soil available contents of Fe, Mn, Zn and Cu and their corresponding concentrations in the different organs of the maize plants (µg g^{-1}), transfer factor and translocation factor.

Heavy metals	Site	Heavy metals content (µg g-1)			Soil-Root transfer factor	Root- Leaves translocation factor
		Roots	stems	leaves		
Fe	Al- Gabal Al-Asfar	125.500	57.2100	37.948	3.214	0.302
	Arab Abou- saed	360.700	55.250	100.600	30.723	0.279
	Bahr Al-Baqar	200.200	50.570	48.060	8.041	0.2407
	Toukh	158.500	66.000	51.310	5.600	0.324
	Namoul	108.000	85.480	35.490	2.472	0.329
	Shobra Al- Khema	120.100	75.580	36.340	3.409	0.303
Mn	Al- Gabal Al-Asfar	26.181	1.929	47.305	3.858	1.807
	Arab Abou- saed	45.809	10.454	73.826	3.271	1.612
	Bahr Al-Baqar	79.949	26.607	117.176	3.950	1.466
	Toukh	64.977	20.798	72.543	3.991	1.116
	Namoul	77.080	12.861	109.323	4.118	1.418
	Shobra Al- Khema	87.832	30.442	132.783	3.973	1.512
Zn	Al- Gabal Al-Asfar	72.564	39.105	82.044	3.336	1.131
	Arab Abou- saed	53.476	22.570	65.712	3.703	1.229
	Bahr Al-Baqar	64.576	15.310	76.692	3.884	1.188
	Toukh	25.640	30.791	43.198	6.686	1.685
	Namoul	42.402	35.631	39.216	3.359	0.925
	Shobra Al- Khema	28.940	37.579	32.372	8.415	1.119
Cu	Al- Gabal Al-Asfar	25.130	8.234	43.994	2.279	1.751
	Arab Abou- saed	13.934	5.046	7.511	4.167	0.539
	Bahr Al-Baqar	14.565	4.451	4.817	4.322	0.331
	Toukh	16.764	7.608	11.468	3.765	0.684
	Namoul	16.353	4.962	5.558	3.880	0.340
	Shobra Al- Khema	20.499	13.561	26.685	2.592	1.302

Table (13): Soil available contents of Co, Cd, Ni and Pb and their corresponding concentrations in the different organs of the maize plants (µg g^{-1}), transfer factor and translocation factor.

Heavy metals	Site	Heavy metal content (µg g-1)			Soil-Root transfer factor	Root- Leaves translo-cation factor
		Roots	Stems	Leaves		
Co	Al- Gabal Al-Asfar	1.674	0.0971	0.494	0.851	0.295
	Arab Abou- saed	0.912	0.072	0.342	1.279	0.375
	Bahr Al-Baqar	1.644	0.089	0.457	1.008	0.278
	Toukh	1.937	0.093	0.499	1.040	0.258
	Namoul	1.621	0.095	0.461	0.920	0.285
	Shobra Al- Khema	1.706	0.091	0.467	1.000	0.274
Cd	Al- Gabal Al-Asfar	0.278	0.023	0.140	1.251	0.504
	Arab Abou- saed	0.191	0.023	0.099	1.367	0.520
	Bahr Al-Baqar	0.039	0.013	0.031	3.080	0.789
	Toukh	0.051	0.014	0.029	3.976	0.579
	Namoul	0.0318	0.012	0.023	1.768	0.712
	Shobra Al- Khema	0.048	0.013	0.034	2.326	0.712
Ni	Al- Gabal Al-Asfar	5.60	4.71	4.09	0.86	0.73
	Arab Abou- saed	4.44	3.32	1.90	3.67	0.43
	Bahr Al-Baqar	4.77	4.41	3.90	1.94	0.82
	Toukh	4.57	3.72	2.61	4.36	0.57
	Namoul	4.82	2.48	2.67	1.58	0.55
	Shobra Al- Khema	4.18	2.98	2.67	2.57	0.64
Pb	Al- Gabal Al-Asfar	12.062	0.706	13.303	1.940	1.103
	Arab Abou- saed	1.380	0.764	1.522	2.451	1.103
	Bahr Al-Baqar	4.433	1.824	4.976	1.802	1.123
	Toukh	1.160	0.880	2.044	1.047	1.762
	Namoul	0.947	0.549	1.031	1.105	1.088
	Shobra Al- Khema	1.938	0.959	2.261	1.589	1.167

Dube et al. (2004) suggested that the ratio between metal concentration in plant parts and soil is an important criterion for selecting plant species for phytoremediation of soils contaminated with high levels of heavy metals. They added that higher concentration in plant parts than soil might identify accumulator plant.

Soil-to-plant transfer factors were calculated for roots according to Ban-Nai et al. (1999) and Jamali et al. (2006) and the obtained values ranged between 2.47-30.72, 3.27-4.12, 3.34-8.42, 2.28-4.32, 0.85-1.28, 1.25 – 3.98, 0.86-4.36, 1.1-2.45 for Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb, respectively.

Root-to-leaves translocation factors ranged between 0.24-0.33, 1.12-1.81, 0.93-1.69, 0.33-1.75, 0.258-0.375, 0.504-0.789, 0.43-0.82 and 1.09-1.76 for Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb, respectively. A wide range in the calculated transfer factor values was found for Cu. Cu concentrations, generally, tended to be highest in the plant roots because Cu may be strongly chelated in plant roots. Eissa and El-Kasssa (1999) and Zein et al. (2002) went to similar findings. High translocation of Cu was found to be accompanied by high availability and high accumulation in roots , where Cu may be preferably chelated in roots and the excess is translocated to the leaves.

The highest translocation factors were recorded for Mn, Zn and Pb and these values indicate high translocation of these heavy metals from root to leaves and also suggest that maize plants could be suitable for the phytoextraction of these heavy metals in soil. Fitz and Wenzel (2002) mentioned that the plants exhibiting biological absorption coefficients (BAC) values >1 are candidates for hyperaccumulators (BAC is defined as the total element concentration in shoots with respect to total element concentration in soil). The concentrations of Mn, Zn and Pb in maize stem and leaves were much lower than the total concentration in soil.

Accordingly maize plants are not considered hyperaccumulators for these heavy metals but can be used in the induced phytoremediation process of Mn, Zn and Pb. The lowest translocation factors were detected for Fe, Cu and Co, which indicates higher retention of these heavy metals in maize roots.

Data presented in Table (12) illustrate that Fe concentrations were, generally within the normal range of Fe (20- 300mg kg^{-1}) in plant leaves which was reported by Brown et al. (1983). On the other hand, it didn't exceed 250 mg kg^{-1} which is the toxic limit of Fe reported by Jones (1972). Mn concentrations in the studied maize plant organs did not achieve the toxic level (500- 2000 mg kg^{-1}) reported by the National Research Council (1973).

Boawn and Rosmussen (1971) reported that normal values for normal Zn content in leaves ranging from 15- 150 mg kg^{-1}. They added that the toxic level is > 500 mg kg^{-1}. Also, Kabata Pendias and Pendias (2001) reported that levels of Zn concentration in plants range from 10 to 100 mg kg^{-1} for most agricultural crops and toxic limits range from 100to 400 mg kg^{-1}. Accordingly, it can be said that concentrations of Zn in the maize plants grown on the studied soils were mostly within the normal range.

Gupta (1979) reported that the normal Cu concentration in plants is 4- 15 mg kg^{-1} whereas the toxic level in the leaves of plants is more than 20 mg kg^{-1}. Accordingly, it can be deduced that Cu concentration reached the toxic level in Al Gabal Al-Asfar and Shobra Al-Khema soils.

Pinkerton (1982) reported that the normal range of Co in plant leaves (on dry weight basis) is 0.01 to 0.30 mg kg^{-1} and indicated that the toxic concentration is more than 20 mg kg^{-1}. Consequently, it can be deduced that Co concentration in all organs of the maize plants grown on the different studied soils did not reach the toxic levels.

Brown et al. (1983) reported that 0.02- 0.80 mg kg^{-1} Cd in plant leaves is a normal range but 5- 700 mg kg^{-1} is a toxic one. On the other hand, Kabata Pendias and Pendias (2001) reported a toxic limit for Cd ranging from 5 to 30 mg Cd kg^{-1} plant. The comparison between these limits and the results obtained herein illustrate that Cd concentrations didn't exceed the normal range.

Ni seemed to be concentrated in root than stem or leaves. Such a distribution of Ni within the maize plant is quite different from the corresponding ones reported by Halstead et al. (1969) and Welch and Gary (1975) who stated that Ni is mobile in plant and is likely to be accumulated in both leaves and seeds. On the other hand, the results obtained herein stand in a well agreement with those of Gendy (2004) who found that Ni was accumulated in roots of sunflower plant in concentrations obviously

higher than in shoots. Such a contradiction may be due to transport and storage of Ni seems to be metabolically controlled. Ni, like other divalent cations (Co^{2+}, Cu^{2+} and Zn^{2+}) is known to form organic compounds and complexes (Kabata Pendias and Pendias, 2001).

Brown et al. (1983) indicated that the normal range of Ni in plant leaves is 0.1- 1.0 mg kg^{-1}, while the toxic one is 50-- 200 mg kg^{-1}. Kabata Pendias and Pendias (2001) reported that the toxic limit of Ni is 10- 100 mg kg^{-1}. The comparison between these limits and the results obtained herein illustrate that Ni concentrations didn't exceeded the normal range.

Brown (1983) indicated that the normal range of Pb in plant leaves is from 0.1- 5.0 mg kg^{-1}. Kabata Pendias and Pendias (2001) stated that normal Pb concentration in plants grown in uncontaminated and unmineralized areas appears to be quite constant, ranging from 0.1 to 10 mg kg^{-1} and averaging 5 mg kg^{-1}. Accordingly, it can be concluded that the maize plants grown in Al Gabal Al Asfar soil accumulated Pb in concentration higher than those considered in the normal range, while the maize plants grown on the other studied soils were mostly within the normal range.

The elevated concentrations of Pb in the different organs of the maize plants grown on Toukh soil reflect the high level of DTPA- extractable Pb in Toukh soil. The high atmospheric deposition of Pb due to car exhausts is apparently a significant source of this metal in above- earth parts of the maize plants grown in Toukh soil. Moreover, Miller and Koeppe (1971) demonstrated that *Zea mays* L. plants could translocate and accumulate significant quantities of Pb in the leaves.

Table (14): Correlation coefficients of heavy metal concentrations in the different organs of maize plants in relation to the total and available concentrations of heavy metals in soil.

Heavy metal	Plant organ	Roots		tem		Leaves		Total conc.	
		CC	P	CC	P	CC	P	CC	P
Fe	Stem	-0.590	0.218						
	Leaves	0.981	0.001	-0.488	0.326				
	Soil	-0.971	0.001	0.571	0.237	-0.974	0.001		
	Available form	-0.945	0.004	0.671	0.145	-0.912	0.011	0.976	0.001
Mn	Stem	0.885	0.019						
	Leaves	0.942	0.005	0.823	0.044				
	Soil	0.926	0.008	0.900	0.015	0.882	0.020		
	Available form	0.983	<0.001	0.897	0.015	0.944	0.005	0.967	0.002
Zn	Stem	-0.284	0.585						
	Leaves	0.937	0.006	-0.428	0.397				
	Soil	0.984	<0.001	-0.222	0.673	0.900	0.015		
	Available form	0.976	0.001	-0.191	0.717	0.880	0.021	0.999	<0.001
Cu	Stem	0.616	0.193						
	Leaves	0.975	0.001	0.623	0.186				
	Soil	0.982	<0.001	0.694	0.126	0.984	<0.001		
	Available form	0.993	<0.001	0.637	0.174	0.991	<0.001	0.996	<0.001
Co	Stem	0.881	0.021						
	Leaves	0.973	0.001	0.956	0.003				
	Soil	0.936	0.006	0.896	0.016	0.929	0.007		
	Available form	0.942	<0.001	0.986	<0.001	0.989	<0.001	0.937	0.006
Cd	Stem	0.966	0.002						
	Leaves	0.946	0.004	0.830	0.041				
	Soil	0.803	0.055	0.622	0.187	0.949	0.004		
	Available form	0.997	<0.001	0.951	0.004	0.959	0.002	0.822	0.045
Ni	Stem	0.630	0.180						
	Leaves	0.740	0.093	0.770	0.073				
	Soil	0.957	0.003	0.615	0.194	0.784	0.065		
	Available form	0.934	0.006	0.523	0.287	0.750	0.086	0.940	<0.001
Pb	Stem	0.031	0.954						
	Leaves	0.998	<0.001	0.034	0.948				
	Soil	0.993	<0.001	0.119	0.822	0.994	<0.001		
	Available form	0.995	<0.001	0.033	0.951	0.997	<0.001	0.996	<0.001

CC: correlation coefficient P: Probability

4.4. Phytoremediation of the heavy metals- contaminated soils using geranium plants

This part of study deals with usage of geranium (*Pelargonium zonale* L.) plants for accumulating heavy metals from the contaminated soils. These plants are well known as hyperaccumulators for heavy metals and their successes in remediating soils polluted with heavy metals hav been proven by numerous investigators, (Pena et al., 2006; Meers et al., 2005 and Liphadzi et al., 2003).

Different treatments were undertaken herein i.e. application of sulfur, organic compost or EDTA to increase the bio-availability of the contaminants found in soil and hence to enhance their uptake by the grown plants and consequently their removal out of soil.

4.4.1. Effects of the different applied amendments on dry matter yield

Data presented in Table (15) show the effects of the different applied amendments on dry matter yield of geranium (*Pelargonium zonale* L) grown on different polluted soils. Dry matter yield varied from one soil to another and among the different plant organs. Taking into account the control treatment, Al-Gabal Al-Asfar soil produced the highest dry matter yield of roots (2.66g pot^{-1}), stems (3.01 g pot^{-1}) and leaves (5.18 g pot^{-1}) whereas Toukh soil gave the lowest dry matter yield of roots (1.03 g pot^{-1}), stems (1.22 g pot^{-1}) and leaves (2.11 g pot^{-1}). This indicates that geranium growth was enhanced by the heavy metal contamination found in soil.

Table (15): Effects of elemental sulfur, the Nile compost and EDTA on dry matter yield of geranium plants grown in different polluted soils.

Soil Location	Treatment	Dry matter yield (g pot^{-1})			
		Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	**Control**	2.66	3.008	5.183	3.617
	Elemental S*	2.678	2.845	4.668	3.397
	Compost ©	2.605	2.898	4.875	3.459
	EDTA	2.62	2.335	2.74	2.565
Arab Abou- Saed	**Control**	2.081	2.383	2.846	2.437
	Elemental S*	1.913	2.245	2.498	2.219
	Compost ©	2.18	2.034	2.121	2.112
	EDTA	0.824	0.996	1.019	0.946
Bahr Al-Baqar	**Control**	2.367	2.626	3.544	2.846
	Elemental S*	2.28	2.476	3.154	2.637
	Compost ©	1.407	2.08	3.003	2.163
	EDTA	1.038	1.113	1.033	1.061
Toukh	**Control**	1.033	1.215	2.108	1.452
	Elemental S*	1.163	1.578	2.573	1.771
	Compost ©	0.982	1.153	1.594	1.243
	EDTA	0.986	1.293	1.663	1.314
Namoul	**Control**	2.443	2.595	3.558	2.865
	Elemental S*	2.413	2.765	3.773	2.984
	Compost ©	2.449	2.67	3.608	2.909
	EDTA	2.345	2.62	2.69	2.552
Shobra Al- Khema	**Control**	2.645	2.938	4.29	3.291
	Elemental S*	2.46	2.755	3.455	2.890
	Compost ©	2.545	2.738	3.66	2.981
	EDTA	2.445	2.805	2.9	2.717

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	0.100	0.148	0.392
Treat. X Soil	0.245	0.363	0.959

Table (16): Effects of elemental sulfur, the Nile compost and EDTA on Fe accumulation in geranium plants grown in different polluted soils.

Soil Location	Treatment	Fe concentration ($\mu g\ g^{-1}$)			
Al- Gabal Al-Asfar	Control	1163	1262	1211	1212.00
	Elemental S*	3218	2098	2122	2479.33
	Compost ©	3430	2193	2282	2635.00
	EDTA	3802	2657	2633	3030.67
Arab Abou- Saed	Control	761	654	638	684.33
	Elemental S*	1669	1088	1100	1285.67
	Compost ©	1817	1154	1184	1385.00
	EDTA	2128	1498	1441	1689.00
Bahr Al-Baqar	Control	909	825	775	836.33
	Elemental S*	2081	1341	1454	1625.33
	Compost ©	2176	1401	1475	1684.00
	EDTA	2469	1698	1672	1946.33
Toukh	Control	1058	949	911	972.67
	Elemental S*	2420	1578	1672	1890.00
	Compost ©	2560	1649	1716	1975.00
	EDTA	2838	1998	1922	2252.67
Namoul	Control	1639	1432	1421	1497.33
	Elemental S*	3577	2430	2591	2866.00
	Compost ©	3798	2503	2676	2992.33
	EDTA	4091	3117	3037	3415.00
Shobra Al- Khema	Control	1265	1119	1120	1168.00
	Elemental S*	2904	1877	1989	2256.67
	Compost ©	3064	1929	2067	2353.33
	EDTA	3462	2438	2383	2761.00

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	43.47	21.51	26.92
Treat. X Soil	106.48	52.68	65.94

Table (17): Effects of elemental sulfur, the Nile compost and EDTA on Mn accumulation in geranium plants grown in different polluted soils.

Soil Location	Treatment	Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	Control	718	613	675	668.67
	Elemental S*	1070	638	764	824.00
	Compost ©	1180	723	817	906.67
	EDTA	1415	933	885	1077.67
Arab Abou- Saed	Control	728	596	662	662.00
	Elemental S*	1156	694	698	849.33
	Compost ©	1239	707	721	889.00
	EDTA	1399	799	885	1027.67
Bahr Al-Baqar	Control	1114	886	1055	1018.33
	Elemental S*	1639	1009	1111	1253.00
	Compost ©	1822	1058	1378	1419.33
	EDTA	2005	1390	1492	1629.00
Toukh	Control	899	682	844	808.33
	Elemental S*	1322	789	889	1000.00
	Compost ©	1458	795	919	1057.33
	EDTA	1555	993	995	1181.00
Namoul	Control	1123	853	1055	1010.33
	Elemental S*	1652	986	1111	1249.67
	Compost ©	1822	993	1249	1354.67
	EDTA	1943	1241	1443	1542.33
Shobra Al- Khema	Control	1222	937	1181	1113.33
	Elemental S*	1817	1084	1253	1384.67
	Compost ©	2004	1293	1304	1533.67
	EDTA	2144	1465	1422	1677.00

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	12.25	8.75	10.04
Treat. X Soil	30.01	21.43	25.58

Table (18): **Effects of elemental sulfur, the Nile compost and EDTA on Zn accumulation in geranium plants grown in different polluted soils.**

Soil Location	Treatment	Zn concentration ($\mu g\ g^{-1}$)			
		Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	Control	642	691	979	770.67
	Elemental S*	681	777	1050	836.00
	Compost ©	729	855	1290	958.00
	EDTA	900	1013	1346	1086.33
Arab Abou- Saed	Control	630	691	876	732.33
	Elemental S*	686	777	950	804.33
	Compost ©	734	861	1179	924.67
	EDTA	907	1029	1246	1060.67
Bahr Al-Baqar	Control	587	633	886	702.00
	Elemental S*	628	711	961	766.67
	Compost ©	672	788	1170	876.67
	EDTA	830	942	1238	1003.33
Toukh	Control	498	532	751	593.67
	Elemental S*	528	598	809	645.00
	Compost ©	565	663	984	737.33
	EDTA	698	792	1036	842.00
Namoul	Control	547	585	826	652.67
	Elemental S*	580	657	978	738.33
	Compost ©	622	729	1082	811.00
	EDTA	768	871	1139	926.00
Shobra Al- Khema	Control	448	473	661	527.33
	Elemental S*	485	526	743	584.67
	Compost ©	525	590	885	666.67
	EDTA	646	720	942	769.33

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	5.37	5.70	7.21
Treat. X Soil	13.15	13.96	17.67

Table (19): Effects of elemental sulfur, the Nile compost and EDTA on Cu accumulation in geranium plants grown in different polluted soils.

Soil Location	Treatment	Cu concentration ($\mu g\ g^{-1}$)			
		Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	Control	458	374	240	357.33
	Elemental S*	632	401	374	469.00
	Compost ©	721	418	447	528.67
	EDTA	876	443	477	598.67
Arab Abou- Saed	Control	279	216	166	220.33
	Elemental S*	370	234	216	273.33
	Compost ©	432	299	261	330.67
	EDTA	529	321	286	378.67
Bahr Al-Baqar	Control	305	245	167	239.00
	Elemental S*	390	265	244	299.67
	Compost ©	472	343	299	371.33
	EDTA	592	363	319	424.67
Toukh	Control	372	288	185	281.67
	Elemental S*	488	309	288	361.67
	Compost ©	567	399	344	436.67
	EDTA	697	418	367	494.00
Namoul	Control	349	270	173	264.00
	Elemental S*	463	293	244	333.33
	Compost ©	597	383	330	436.67
	EDTA	676	405	355	478.67
Shobra Al- Khema	Control	446	345	236	342.33
	Elemental S*	585	370	360	438.33
	Compost ©	680	479	443	534.00
	EDTA	836	505	473	604.67

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	8.10	5.21	4.64
Treat. X Soil	19.84	12.77	11.37

Table (22): Effects of elemental sulfur, the Nile compost and EDTA on Ni accumulation in geranium plants grown in different polluted soils.

Soil Location	Treatment	Ni concentration (µg g^{-1})			
		Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	Control	245	252	298	265.00
	Elemental S*	312	329	369	336.67
	Compost ©	343	356	463	387.33
	EDTA	376	397	530	434.33
Arab Abou- Saed	Control	53	52	61	55.33
	Elemental S*	65	68	72	68.33
	Compost ©	71	73	77	73.67
	EDTA	77	79	98	84.67
Bahr Al-Baqar	Control	99	103	122	108.00
	Elemental S*	124	135	151	136.67
	Compost ©	130	146	189	155.00
	EDTA	154	164	217	178.33
Toukh	Control	49	51	63	54.33
	Elemental S*	62	66	81	69.67
	Compost ©	69	71	87	75.67
	EDTA	73	87	116	92.00
Namoul	Control	108	112	132	117.33
	Elemental S*	138	146	163	149.00
	Compost ©	152	158	205	171.67
	EDTA	167	176	235	192.67
Shobra Al- Khema	Control	66	70	81	72.33
	Elemental S*	84	89	99	90.67
	Compost ©	92	103	125	106.67
	EDTA	105	111	143	119.67

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	2.08	2.23	2.76
Treat. X Soil	5.084	5.46	6.76

Table (23): Effects of elemental sulfur, the Nile compost and EDTA on Pb accumulation in geranium plants grown in different polluted soils.

Soil Location	Treatment	Pb concentration ($\mu g\ g^{-1}$)			
		Roots	Stems	Leaves	Mean
Al- Gabal Al-Asfar	Control	259	202	192	217.67
	Elemental S*	375	233	263	290.33
	Compost ©	422	241	332	331.67
	EDTA	485	314	368	389.00
Arab Abou- Saed	Control	181	140	133	151.33
	Elemental S*	260	165	183	202.67
	Compost ©	296	168	230	231.33
	EDTA	340	222	269	277.00
Bahr Al-Baqar	Control	197	156	151	168.00
	Elemental S*	292	181	206	226.33
	Compost ©	325	190	262	259.00
	EDTA	373	268	316	319.00
Toukh	Control	251	197	187	211.67
	Elemental S*	361	230	255	282.00
	Compost ©	406	234	320	320.00
	EDTA	466	309	372	382.33
Namoul	Control	244	189	180	204.33
	Elemental S*	355	228	258	280.33
	Compost ©	401	227	323	317.00
	EDTA	461	303	367	377.00
Shobra Al- Khema	Control	221	170	161	184.00
	Elemental S*	316	200	221	245.67
	Compost ©	353	208	265	275.33
	EDTA	406	275	308	329.67

*: Elemental sulfur applied at a rate of 0.5% ©: Compost applied at a rate of 2%

EDTA applied at a rate of 1 mg kg-1

LSD

	Root	Stem	Leaves
Treat.	4.99	3.23	1.78
Treat. X Soil	12.21	7.90	4.37

In general, the application of the elemental sulfur or Nile compost had no significant effect on the plant growth except for the application of the Nile compost to Bahr Al-Baqar soil as it decreased root and stem dry-weights with no significant effect on the dry-weight of leaves. One possible reason is that the application of organic matter to Bahr. Al-Baqar soil increased the maximum water holding capacity of the soil to be more than 90 % and created undesirable conditions for geranium growth.

EDTA Na_2, on the other hand, significantly reduced the dry matter yields of leaves, stems and roots of the geranium plants grown in all the investigated soils except for Toukh. Such a result may be attributed to accumulation of heavy metals in amounts phytotoxic to the plants due to increasing their bio-availability in soil as a result of applying EDTA. These results stand in well agreement with those attained by Blaylock et al. (1997); Chen and Cutright (2001) and Cui et al. (2004) who stated that Indian mustard shoot yield declined due to application of EDTA. They deduced that there is a possible occurrence of direct toxicity for using EDTA or indirect toxicity via increased metal solubility.

4.4.2. Effect of the different applied amendments on the concentrations of heavy metals in plant organs:

Data presented in Tables (16 – 23) reveal that concentrations of the studied heavy metals were generally lowest in the control treatment and highest in EDTA treatment. This occurred in all the studied soils and plant organs.

The concentrations of the studied heavy metals in all the studied organs of geranium plants grown on the heavy metals-contaminated soils followed, generally, the following descending sequence: Fe > Mn > Zn > Cu > Pb > Ni > Co > Cd. Concentrations of Fe were the highest due to the initial total content in soils as well as its relatively available content. Iron concentration in roots of geranium in the control treatment ranged from 761 mg kg^{-1} in Arab Abou Saed soil to 1639 mg kg^{-1} DW of roots in Namoul soil. The corresponding Fe concentrations in stems ranged from 654 mg kg^{-1} DW in Arab Abou Saed soil to 1432 mg kg^{-1} DW in Namoul soil. The concentrations of Fe in leaves of the control treatment were within the range 638 to 1421 mg kg^{-1} DW of leaves with the highest value found in plants grown on Namoul soil whereas the lowest one characterized the plants grown on Arab Abou Saed soil.

Cadmium concentration, on the other hand, was the lowest probably due to its low total and available contents as a result of the precipitation of its carbonates and hydroxides (Lindsay, 1979) and competition with coated root surface (Anderson and Nilson, 1979). Cadmium concentrations in roots of geranium plants of the control treatment ranged from 34 mg kg^{-1} in Toukh soil to 57 mg kg^{-1} in Al Gabal Al Asfar soil. The corresponding concentrations of stems ranged from 31 mg kg^{-1} in Toukh soil to 46 mg kg^{-1} in Al Gabal Al Asfar soil while those leaves ranged from 21 mg kg^{-1} in Toukh soil to 31 mg kg^{-1} in Al Gabal Al Asfar and Shobra Al-Khema soils.

It is worthy to indicate that in spite of the relatively low total and available Ni contents of the studied soils, the geranium plants grown thereon could hyperaccumulate Ni in their different tissues. The concentrations of Ni in all of the plant organs highly exceeded the toxic levels reported for other plant species. Its concentrations in the geranium roots of the control treatment ranged from 49 mg kg^{-1} in Toukh soil to 245 mg kg^{-1} in Al-Gabal Al-Asfar soil. The corresponding concentrations in the stem ranged from 51mg kg^{-1} in Toukh soil to 252 mg kg^{-1} in Al gabal Al Asfar soil, whereas the corresponding Ni concentrations in the leaves occurred within the ranges from 61 to 298 mg kg^{-1}. The lowest concentration characterized the leaves of the geranium plants grown on Arab Abou-Saed soil whereas the highest one characterized those of the geranium plants grown on Al Gabal Al Asfar soil. Dan et al. (2002) reported that geranium plants grown in artificial soil system and exposed to a metal concentration of 1000 mg Ni L^{-1} in the form of Ni(NO3).6H2O over a period of 14 days could accumulate 1190 mg Ni kg-1 DW of shoot as well as 21000 mg Ni kg^{-1} DW of root tissue. Such a finding indicates that geranium plants can take up and accumulate significant amounts of Ni without any visible changes in their yield.

Regarding concentrations of Mn, Zn, Cu, Co and Pb, data presented in Tables (16-23) reveal that their highest root concentrations were 1222, 642, 458, 113 and 259 mg kg^{-1}, respectively whereas their corresponding lowest ones were 718, 448, 279, 48 and 181 mg kg^{-1}, respectively. Generally, these elevated concentrations illustrate that geranium plants exhibited to environment contaminated with heavy metals could uptake and remove pronounced portions of these metals out of the soil system. However, it was aimed here at enhancing uptake of these metal ions by treating the soil with amendments that thought to be able to increase bio-availability of metal ions and hence their uptake and removal by plant.

4.4.2.1. The enhancing effect of the applied elemental sulfur:

In this concern, application of the elemental sulfur "S" at a rate of 0.5% (on dry weight basis) could significantly increase concentrations of the studied metal ions in the roots, stems and leaves of the geranium plants grown on the heavy metal-contaminated soils.

In the current study, application of the elemental sulfur increased roots concentration of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb to maximum values of 3577, 1817, 686, 632, 164, 87, 312 and 375 mg kg^{-1} in soils of Namoul for Fe, Arab Abu-Saed for Mn and Zn, Al Gabal Al-Asfar, Shubra Al-Khema, Al Gabal Al-Asfar for Cd, Ni and Pb, respectively.

The corresponding maximum concentrations in stem were 2430, 1084, 777, 401, 176, 65, 329 and 230 mg kg^{-1}, respectively, whereas those in leaves were 2591, 1253, 1050, 374, 208, 59, 369 and 263 mg kg^{-1}, respectively. These results stand in well agreement with those of Kayser ėt al. (1999) who postulated that application of elemental sulfur to a contaminated calcareous soil (6.4% $CaCO_3$) increased Cd concentration by 27 fold in Indian mustard plant. Likewise Kayser et al. (2000) found that Cd and Zn concentrations in Tobacco, sunflower and maize plants increased by 1.4 to 2.2 fold due to elemental sulfur application. Cui et al. (2004) found that Pb and Zn concentrations in shoots of Indian mustard increased due to application of elemental sulfur and the increases were obvious with increasing the rate of the applied sulfur from 20-160 mmol kg^{-1}. Salib (2005) in his work on Abou- Rawash contaminated soils, found that *Pelargonium zonale* grown on this soil for 49 days could accumulate Zn, Mn, Co, Pb and Cd at concentrations of 17320, 5420, 3280, 8610 and 1150 mg kg^{-1}, respectively of roots dry weight. The corresponding concentrations of shoot were 2650, 2230, 810, 2700 and 80 mg kg^{-1}, respectively.

Certain groups of acidophilic soil bacteria, predominately the genus *Thiobacillus* sp., can metabolize sulfur in the presence of O_2 and generate H^+ and SO_4^{2-}, leading to soil acidification (Lee et al., 1998). The reduction effect of the generated or released H^+ ions on soil pH leads to more solubility of the metal ions in soil and consequently more uptake of these ions by plant (Tichy et al., 1997 and Kayser et al., 1999 and 2000).

4.4.2.2. The enhancing effect of the applied Nile compost:

Data presented in Tables (16-23) show that the application of Nile compost at a rate of 2% to the heavy metal-contaminated soils could significantly increase concentrations of the studied heavy metals in roots, stems and leaves of geranium plants as compared with the control plants.

Maximum concentrations of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb in roots were 3798, 2004, 734, 721, 100, 343 and 422 mg kg^{-1}, respectively. The corresponding maximum concentrations in stems were 2503, 1293, 861, 479, 201, 92, 356 and 241 mg kg^{-1}, respectively, whereas those in leaves were 2676, 1378, 1290, 447, 217, 69, 463 and 332 mg kg^{-1}, respectively. The enhancing effect of the applied organic compost on metal ion concentration in geranium plants may be attributed to the increase in the bio-availability of these metal ions in soil which may result from the reduction in soil pH by the organic acids formed upon its decomposition. Also, CO_2 gas exhaled by the decomposers would dissolve in water forming H_2CO_3 that contributed in reducing soil pH. Further more, organic matter is well known by its importance in transportation and accumulation of metallic ions present in soils as chelates of various stability and supplying these ions to plant roots (Kitagishi and Yamane, 1981). Also, the organic compost itself is a source of heavy metals (see Table, 4). On the other hand, addition of the organic compost might improved physical, chemical and fertility status of the investigated soils and hence change media to be more suitable for plant to take up the metal ions. It is worthy to indicate, however the organic residues, that are used for composting, should have as low concentrations of the heavy metal ions as possible to avoid their hazardous consequent effects on soil and plant grown thereon.

4.4.2.3. The enhancing effect of the applied EDTA:

Data presented in Tables (16-23) illustrate that EDTA applied at a rate of 1 mg kg^{-1} soil could increase significantly concentrations of Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb in roots, stems and leaves of geranium plants grown on the studied heavy metals-polluted soils.

Maximum concentration values of these metal ions in roots were 4091, 2144, 907, 876, 207, 113, 379 and 485 mg kg^{-1}, respectively. The corresponding maximum concentrations in stem were 3117, 1465, 1029, 505, 227, 101, 397 and 314 mg kg^{-1}, respectively and in leaves were 3037, 1492, 1346, 477, 246, 88, 530 and 372 mg kg^{-1} respectively. Enhancement of metal accumulation in plants grown on soil amended with EDTA was reported by Blaylock et al. (1997); Dan et al. (2002); Dube et al. (2004); Cui et al. (2004) and Meers et al. (2005). Blaylock et al. (1997) stated that EDTA is an effective chelate compound that has been found to increase the solubility of heavy metals in soil and their bio-availability to plants. They reported the enhancement of Pb accumulation in Indian mustard from soils amended with EDTA.

Cui et al. (2004) found that EDTA increased average Pb concentrations up to 1708 and 246 mg kg^{-1} of Indian mustard and wheat shoots. They added that shoot Zn concentrations followed similar trends to Pb and averaged 352 and 221 mg kg^{-1}.

Meers et al. (2005) pointed out that EDTA has been found to enhance shoot accumulation of heavy metals by sunflower. However, they stated that the enhancement effects were still considered insufficiently to be consided efficient remediation.

4.4.3. Effect of the different applied amendments on translocation of heavy metals within plant organs:

Data presented in Tables (16-23) reveal that Fe, Mn, Cu, Cd and Pb were found in roots in concentrations higher than their concentration in stems or leaves. These results are in well agreement with those of Dube et al. (2004) whose investigation was conducted on different plant species i.e. wheat, mustard, cabbage and Cauliflower. Salib (2005) in his work on geranium plant went to similar results. Stems seemed to have higher concentrations of these metal ions than those of leaves in geranium plants of the control treatment (which did not receive amendment). However, application of the different used amendments i.e. Sulfur at a rate 0.5%, the Nile compost at a rate of 2% and EDTA at a rate of 1mg kg^{-1} soil enhanced leaves accumulation of Fe, Mn, Cu, Cd and Pb at the expense of stem, however the concentrations of these metal ions remained higher in roots. On the basis of these results, the biological absorption coefficients (BAC) of the heavy metals in geranium plants were evaluated (Table 24).

In general, the biological absorption coefficient (BAC) decreased with the increase in the concentration of heavy metals in soil. The values of the biological absorption coefficient (BAC) were very low for iron and ranged in the control (untreated soils) between 0.035 and 0.06, whereas the values for Mn ranged between 1.88 and 4.18.

The biological absorption coefficient (BAC) values for Zn, Cu, Co and Ni in untreated soils ranged between 2.09- 9.61 for Zn, 2.56-4.19 for Cu, 4.23 - 6.44 for Co and 1.98-3.28 for Ni.

BAC values varied widely for Cd and ranged between 12.91 and 158 while the BAC values for Pb ranged between 2.34 and 15.01, respectively.

Table (24): Biological absorption coefficients (BAC) for heavy metals in geranium plants

Heavy metal	Treatment	Toukh	Abar Abu Saed	Al-Gabal Al-Asfar	Bahr Al-Baqar	Namoul	Shobra Al-Khema
Fe	Control	0.042	0.060	0.043	0.035	0.048	0.041
	Elemental S*	0.075	0.103	0.074	0.062	0.084	0.072
	Compost ©	0.077	0.110	0.079	0.064	0.087	0.074
	EDTA	0.090	0.138	0.093	0.074	0.103	0.089
Mn	Control	2.170	1.880	4.180	2.069	2.582	2.446
	Elemental S*	2.353	2.071	4.591	2.244	2.817	2.664
	Compost ©	2.397	2.125	5.012	2.624	3.036	2.938
	EDTA	2.748	2.507	5.814	3.029	3.577	3.263
Zn	Control	9.605	2.801	2.094	2.402	3.184	9.331
	Elemental S*	10.434	3.071	2.270	2.626	3.703	10.323
	Compost ©	12.159	3.620	0.958	3.128	4.096	12.111
	EDTA	13.304	4.029	2.860	3.346	4.426	13.294
Cu	Control	3.760	3.856	2.103	4.188	3.710	2.555
	Elemental S*	4.998	4.586	2.794	5.298	4.591	3.322
	Compost ©	6.199	5.711	3.172	6.632	6.114	4.178
	EDTA	6.574	6.195	3.355	7.151	6.585	4.455
Cd	Control	129.091	12.913	13.547	157.999	98.764	109.277
	Elemental S*	196.443	16.839	19.356	229.319	148.056	201.509
	Compost ©	299.979	25.787	28.319	363.184	211.049	236.678
	EDTA	369.762	31.439	32.268	472.560	254.048	272.577
Co	Control	4.876	5.347	5.675	4.229	4.615	6.437
	Elemental S*	6.132	6.851	7.222	5.375	5.961	8.048
	Compost ©	6.529	7.402	7.755	5.862	6.395	8.727
	EDTA	7.486	8.468	8.761	6.841	7.439	9.828
Ni	Control	2.818	1.980	2.859	2.314	2.639	3.277
	Elemental S*	3.620	2.440	3.599	2.924	3.327	4.050
	Compost ©	3.860	2.612	4.303	3.482	3.951	4.950
	EDTA	4.967	3.084	4.768	3.850	4.397	5.450
Pb	Control	12.035	12.485	2.344	3.797	15.012	8.412
	Elemental S*	15.497	15.996	3.015	4.836	20.037	10.815
	Compost ©	17.921	18.303	3.571	5.766	23.048	12.293
	EDTA	21.742	22.531	4.111	7.219	27.397	14.907

The biological absorption coefficient (BAC) values showed that geranium plants have very high affinity for Cd, Pb and Zn uptake and accumulation in their leaves. With respect to the total accumulation of Cd, Pb and Zn in shoot of geranium plants calculated in (mg plant^{-1}), results show that geranium plants, grown on untreated soil of Al Gabal Al Asfar, accumulated only 0.3 mg Cd plant^{-1}, 1.602 mg plant^{-1} of Pb and 7.15 mg Zn plant^{-1}.

Application of elemental sulfur or organic matter to soils increased the biological accumulation coefficient (BAC) of heavy metals in soil and the effect of organic matter application exceeds the effect of the elemental sulfur application. Geranium plants, grown in Al Gabal Al-Asfar soil amended with Nile compost at a rate of 2%, accumulated only 0.59 mg Cd plant^{-1}, 2.317 mg plant^{-1} of Pb and 3.11 mg Zn plant^{-1}. The depressive effect of Nile compost on decreasing BAC of Zn in Al Gabal Al Asfar soil could be ascribe to the formation of Zn organic compound complex which reduced its mobility and bioavailability and hence its uptake by plants

These results indicate that geranium plants have demonstrated successful Cd phytoextraction while the phytoextraction of the other heavy metals needs long time-periods to make the decontamination more acceptable, economically and environmentally.

Treating soils with 1 mg kg^{-1} EDTA Na_2 had the highest effect on enhancing heavy metal uptake and accumulation in shoots and increased the biological accumulation coefficient (BAC) values by about 220, 136, 138, 170, 155, 260, 166 and

183 % for Fe, Mn, Zn, Cu, Co, Cd, Ni and Pb respectively. On the other hand, significant reductions in geranium dry-weights were accompanied by EDTA treatment, and therefore the extractable amounts of heavy metals from soil by geranium shoots decreased. These results revealed that the use of crops in long-term bioremediation could have more economic values during the phytoextraction process if the concentration of the contaminants in their biomass is below critical levels for livestock consumption.

4.5. Evaluation of using maize in phytoremediation of As-contaminated soil.

Extensive use of synthetic organic pestcides is associated with serious environmental problems for soil, water and air. The arsenical herbcides were and are still used at a wide scale in several countries all over the world. These arsenical herbcides, when applied to soil, rapidy decompose by many micro-organisms found in soil and As is released. Hence, the residual compounds of these arsenical herbcides are more toxic to plant, animal and man than the organic herbicide itself. This part of study was conducted, therefore, to remove or reduce As concentration in soil which might be contaminated with As due to application of arsenical herbcides, irrigation with water contaminated with As or evevn the upward movement of groundwater contaminated with As.

Maize plants were suggested for the phytoextraction of As from Scotch soil artificially contaminated with As. Maize seeds were grown first on organic compost (Table, 6) then transplanted into plastic pots packed with 1 kg As – contaminated soil (one plant/pot) and received nutrient solution (Table, 7).

4.5.1 Maize growth, As accumulation in shoots and grains as influnced by contamination of soil and prolonged sub irrigation used deionized water in As contaminated water.

4.5.1.1. Effect of soil contamination with As on its accumulation in shoots and grains during the different growth stages

Shoots dry weights increased insignificantly with the increase in the level of soil As during V 13 and anthesis stages while a negative significant effect was found after seed maturity (Table 25, Fig. 1). Shoots dry-weights increased during V13 and anthesis for plants grown in soil contaminated with 25 mg As kg^{-1} by 120.54 and 117.79 %, respectively (compared to the control plants); while the increases in shoots dry weight were only 115.29 and 113.27 %, respectively for plants grown in soils contaminated with 50 mg As kg^{-1}. These results indicate that lower application of As stimulated plant growth whoever, increasing the rate of applied As above 25 mg kg^{-1} decreased increment percentage of plant shoots when the its uptake was low and some physiological disorders were accompanied by the increase in As uptake and resulted in reducing the plant growth. The stimulation effect was also observed by (Carborell – Barrachina et al., 1998) who found that applications of arsenate at the concentrations of 0.2 and 0.8 mg L^{-1} (hydroponics culture)

Table (25): Shoot dry weight and As concentration in shoots as affected by soil As

	V 13	Anthesis	Maturity	Mean
Shoots dry weight (g)				
Uncontaminateds soil (control)	3.15	3.35	5.81	4.10
Soil contaminated with 25 mg As kg^{-1}	3.79	3.95	4.46	4.07
Soil contaminated with 50 mg As kg^{-1}	3.63	3.80	3.89	3.77`
Mean	3.52	3.70	4.72	
Shoot As concentration ($\mu g\ g^{-1}$)				
Uncontaminateds soil (control)	0.16	0.23	0.52	0.30
Soil contaminated with 25 mg As kg^{-1}	0.52	2.79	5.41	2.91
Soil contaminated with 50 mg As kg^{-1}	0.73	3.16	7.63	3.84
Mean	0.47	2.06	4.52	

LSD

	Shoot dry weight (g)	As in shoot ($\mu g\ g^{-1}$)
Soil As	n.s.	0.399
Gr. stage	0.383	0.399
Soil As X Gr. stage	0.663	0.692

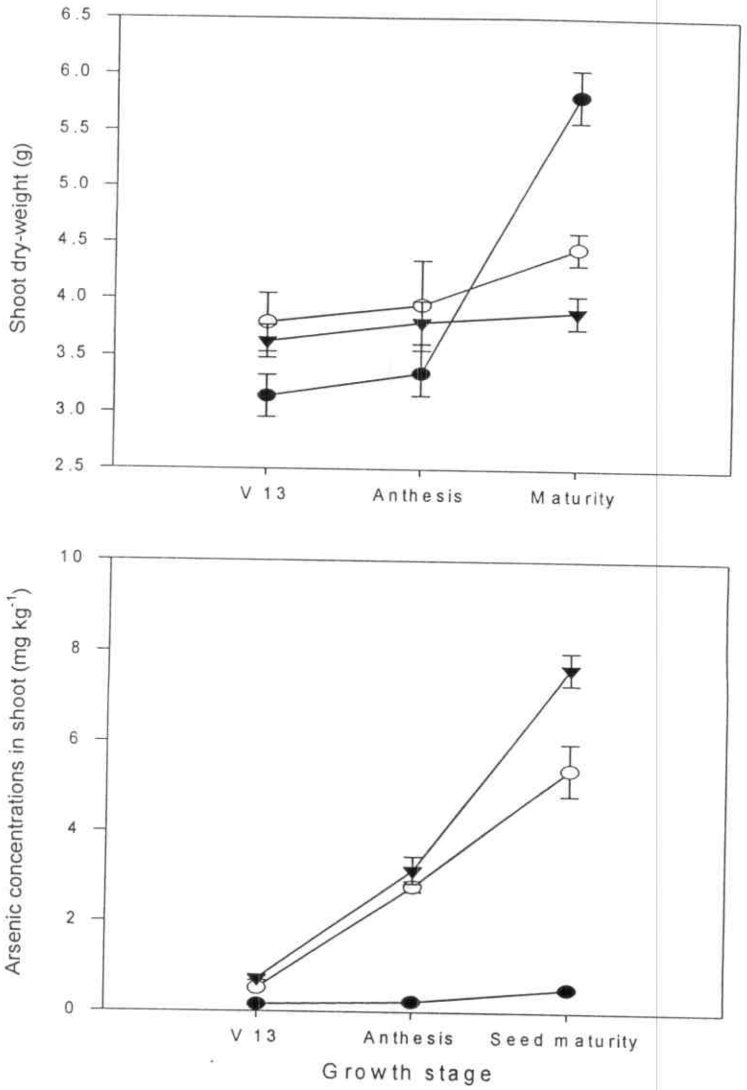

Fig. 1 Shoot dry-weight and As concentrations in maize plants grown in (•) uncontaminated soil, (○) soils contaminated with 25 mg As kg-1 and (▼) soil contaminate with 50 mg As kg^{-1}.

Table (26): Grain As concentration as affected by soil As

Treatment	As conc. ($\mu g\ g^{-1}$)
Uncontaminateds soil (control)	38.90
Soil contaminated with 25 mg As kg^{-1}	284.54
Soil contaminated with 50 mg As kg^{-1}	359.81

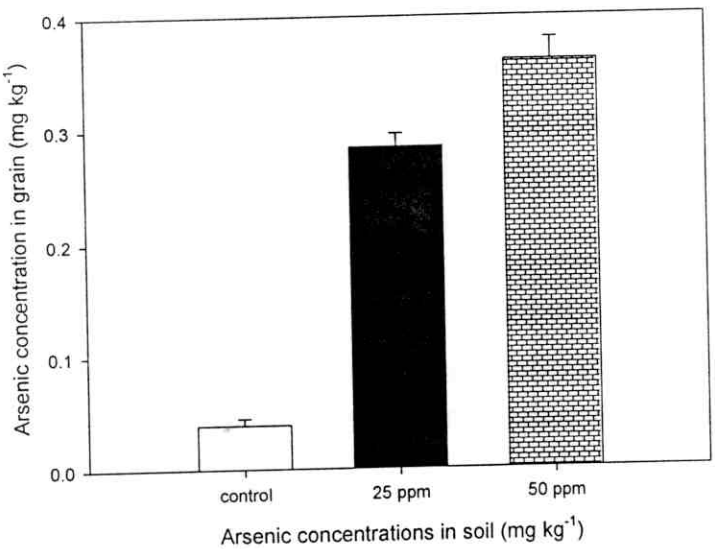

Fig. 2 The concentrations of As in maize grain grown in (open bars) uncontaminated soil, (closed bars) soil contaminated with 25 mg As kg^{-1} and (bricked bars) soil contaminated with 50 mg As kg^{-1} soil.

significantly increased root, shoot and total dry mass production of *Spartina alterniflora* and *Spartina patens* compared to the control plants. On the other hand, the reduction in plant biomass agree with the results obtained by Stoeva et al. (2003) who found that high As contamination decreased plant growth, leaf area and mass bioaccumulation of maize.

As accumulation in shoots increased more than 10-times between V13 and seed maturity when grown on contaminated soil (Fig. 1). The plants grown on more contaminated soil exhibited higher shoot As contents and the large differences in As accumulation in shoots were developed between anthesis and seed maturity. As concentrations in maize grains also increased significantly with the increase in soil As (Table 25 and Fig. 2).

4.5.1.2. Accumulation of arsenate in shoots during the reproductive stages of maize

Shoot dry weight increased insignificantly during grain filling until the 20^{th} day after silking (DAS). Significant reductions in shoot dry-weight were noticed after that (Table 27 and Fig. 3). Such reductions were more pronounced in plants grown in contaminated soils. On the other hand, As concentration in maize shoot increased insignificantly during grain filling periods until the 20^{th} day after silking (DAS). Significant increase in plant shoot grown in contaminated soil were found between the 20th DAS and seed maturity.

Table (27): ShootS dry-weight (G) and As accumulation in shoot (µg g^{-1}) during grain filling.

	Stages of grain filling						
	0 DAS	5 DAS	10 DAS	15 DAS	20 DAS	Maturity	Mean
	Shoot dry weight (g)						
Control soils	3.57	4.07	4.70	5.28	5.62	5.81	4.84
Contaminated soil	4.10	4.66	5.30	5.21	6.19	3.89	4.89
Mean	3.84	4.37	5.00	5.25	5.91	4.85	
Shoot As concentration (µg g^{-1})							
	0 DAS	10 DAS	10 DAS	15 DAS	20 DAS	Maturity	Mean
Control soils	0.258	0.271	0.336	0.368	0.416	0.520	0.362
Contaminated soils	2.714	2.961	3.087	3.133	3.366	5.408	3.445
Mean	1.486	1.616	1.712	1.751	1.891	2.964	

LSD

	Dry weight (g)	As in shoot (µg g^{-1})
Soil As	n.s.	0.293
Gr. stage	0.516	0.508
Soil As X Gr. stage	0.730	0.719

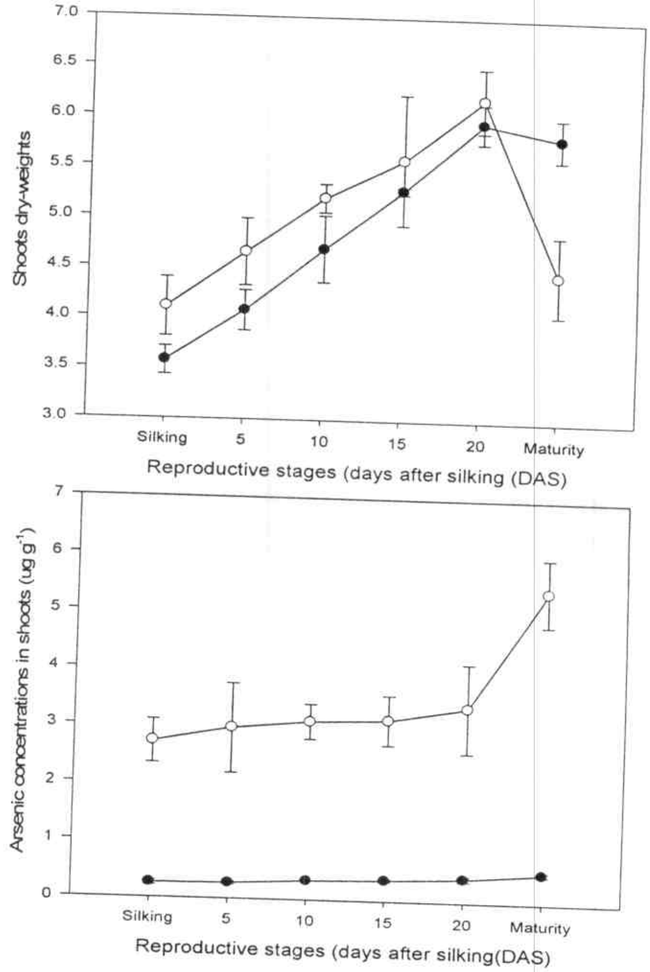

Fig. 3 Changes in the dry weights and As accumulations in plant shoot grown in (•) uncontaminated soils and (○) contaminated soil during grain filling stages

4.5.1.3. Effect of prolonged sub-surface irrigation on As accumulations in shoots and grains

Sub-irrigating the uncontaminated soils for 3-successive days with deionized water had no significant effect on shoot dry weight or As concentrations in either shoots or grains, while prolonged sub irrigating the contaminated soil had significant effects on As concentrations in both shoots and grains with no significant effect on shoots dry weights. Arsenic concentrations decreased significantly in shoots of plants grown in contaminated soils subjected to prolonged sub irrigation with deionised water during V13, anthesis and seed maturity by 28.3, 37.8 and 36.6 %, respectively (Table, 28 and Fig., 4). Significant reductions in As concentrations in grains were found when prolonged sub irrigation took place during anthesis and seed maturity while its effect was insignificant when prolonged sub irrigation took place during V13.

Sub irrigation with As contaminated water for 3 successive days had significant effects on shoot dry weight and As concentrations in shoot and grain. Sub irrigation during V13 and anthesis decreased shoots dry-weights by 34.1 and 20.30%, respectively with no significant effect when took place during seed maturity. On the other hand, As concentration in shoot increased by 14, 37.6 and 1.78 times, respectively when sub irrigation took place during V13, anthesis and maturity. Shoot As concentrations for maize plants sub irrigated with contaminated water for 3 successive days during V13 and anthesis were even higher than the concentrations found in plant shoots grown in soil contaminated with As. This refers to the danger of using contaminated water in irrigation.

Although the increase in As concentrations in maize shoots were significant when sub irrigation took place during anthesis than during V 13 but insignificant differences were found in As concentrations in grain between these two treatments. One possible explanation is that the formed phytochelatins during V13 might have lower molecular weight and formed less stable complexes with As; therefore, during grain filling periods, some of these complexes might be decomposed and transferred to the grains. While during anthesis stage, the formed phytochelatins might have higher molecular weight and formed more stable complexes with As and thus reduced the transfer of As to the grains. Queirolo et al. (2000) found very high As concentrations 1850 $\mu g\ kg^{-1}$ in maize and 860 $\mu g\ kg^{-1}$ in potatoes grown on Andean villages, northern Chile. They related these high values to the concentrations of As in the irrigation water, which are aproximately 50 $\mu g\ L^{-1}$ at Talabre and 150-250 $\mu g\ L^{-1}$ at Socaire.

Table (28): The effect of sub irrigation for 3 successive days on shoots dry weights and As concentrations in both shoots and grains

Treatment		V 13	Anthesis	Maturity	Total
Shoot dry weight (g/plant)					
Uncontaminated soil	No sub Irr	5.81	5.81	5.81	5.81
	Sub-Irr dstl water	6.42	6.01	5.74	6.06
	Sub-Irr cont. water	4.23	4.79	6.17	5.06
Contaminated soil	No sub Irr.	4.46	4.46	4.46	4.46
	Sub-Irr dstl water	4.99	5.08	4.56	4.88
	Total	5.18	5.23	5.35	
Shoot As concentration ($\mu g\ g^{-1}$)					
Uncontaminated soil	No sub Irr	0.52	0.52	0.52	0.52
	Sub-Irr dstl water	0. 55	0.41	0.76	0.57
	Sub-Irr cont. water	7.28	19.57	0.92	9.26
Contaminated soil	No sub Irr	5.41	5.41	5.41	5.41
	Sub-Irr dstl water	3.879	3.37	3.43	3.56
	Total	**3.53**	**5.86**	**2.21**	
Grain As concentration ($ng\ g^{-1}$)					
Uncontaminated soil	No sub Irr	38.90	38.90	38.90	38.90
	Sub-Irr dstl water	40.60	27.30	54.20	40.70
	Sub-Irr cont. water	193.50	183.60	60.97	146.02
Contaminated soil	No sub Irr	284.54	284.54	284.54	284.54
	Sub-Irr dstl water	260.18	163.87	234.50	219.52
	Total	163.54	139.64	134.62	

LSD

	Shoots dry weights	As in shoots	As in grains
Sub-Irr	0.538	0.86	33.44
Gr. stage	Ns	0.67	Ns
Sub-Irr X Gr. stage	0.923	1.49	57.93

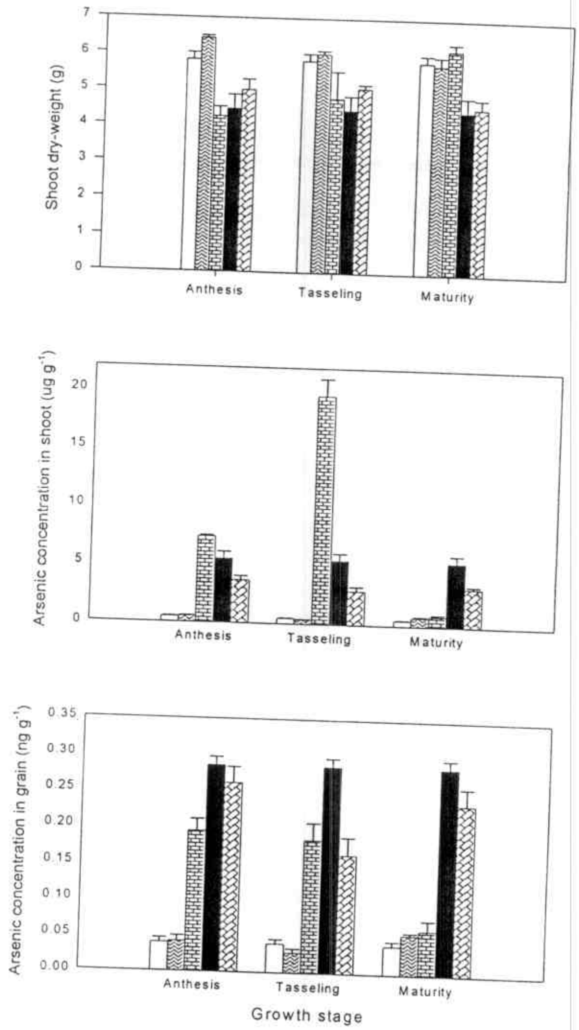

Fig. 4 The effect of prolong sub irrigation on shoot dry-weight, As concentrations in shoots and grains: (open bars) for uncontaminated soils not flooded, (zig zag bars) uncontaminated soils flooded with deionised water, (bricks bars) uncontaminated soils irrigated with contaminated water, (closed bars)contaminated soils not flooded and (bricks diag. bars) contaminated soils flooded with deionised water .

4.5.1.4. Comparing As concentrations in shoots and grains of different varieties as affected by As contamination in soil

Analysis of variance revealed that there were genotypic difference in shoots dry-weights and As concentrations in shoots. As concentrations in shoots of the Egyptian varieties grown in the contaminated soil were about one- half the concentrations found in Alarik variety plants (Table, 29), while shoots dry weight of the Egyptian varieties were more than double shoot dry weight of Alarik variety (Fig. 5) and therefore the dilution effect for As concentrations in maize shoot was found in the Egyptian varieties compared with Alarik variety. Also, analysis of variance indicates that, there were no significant genotypic differences in As concentrations in grain and the concentrations of arsenic in grains were always below the maximum acceptable level (2 mg/kg for As in feedstuffs of animals) proposed by the European Union (European Commission, 2003).

4.5.2.1. Arsenate uptake

The analysis of variance revealed that arsenate influx was concentration dependent with no significant difference in arsenate uptake between the 6 varieties. Also, the effect of phosphate was highly significant on arsenate uptake. The concentration X phosphate interaction term was also significant (Data are shown in table 30).

Influx data was Michaelis-Menten modelled by a least squares fitting procedure to obtain kinetic parameters. For

Table (29): Genotypic differences in shoots dry weights, As concentrations in both shoots and grains in response to soil As.

	Single cross 3084	Single cross 30K8	Alarik	Mean
Shoot dry-weight (g)				
Uncontaminated soil	14.81	14.52	5.81	11.71
Contaminated soil	11.36	12.70	4.46	9.51
Total	13.08	13.61	5.14	
Shoot As concentration (μg g^{-1})				
Uncontaminated soil	0.36	0.22	0.522	0.37
Contaminated soil	2.32	1.86	5.41	3.49
Mean	1.34	1.04	2.97	
Grain As concentration (ng g^{-1})				
Uncontaminated soil	28.08	15.07	38.90	27.11
Contaminated soil	300.52	323.17	284.54	303.48
Mean	164.30	169.12	161.72	

LSD

	Shoots dry weights	As in shoot	As in grain
Variety	1.45	0.65	ns
Soil As	1.19	0.53	22.25
Variety X Soil As	ns	0.92	38.53

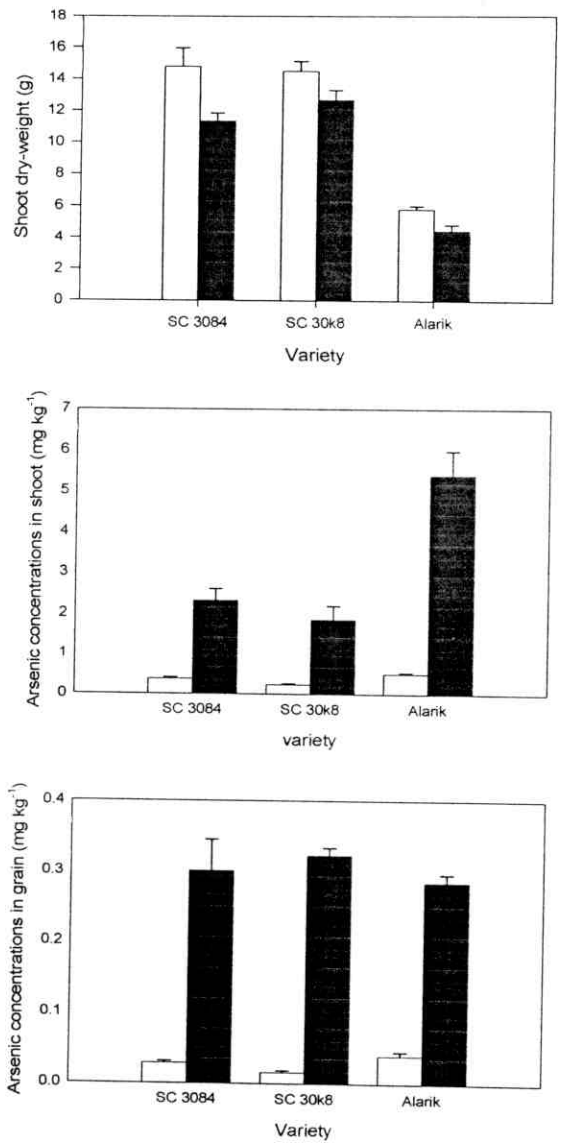

Fig. 5 Difference between shoot dry-weight and As concentration in shoot and grain of different varieties grown in uncontaminated (open bars) and As contaminated soils (closed bars).

phosphate starved plants, arsenate influx in all 6 varieties showed concentration dependence (Fig. 6) and best fitted a four-parameter Michaelis-Menten model corresponding to an additive high and low affinity uptake system (Table 33). Between genotypes the K_m values of the high affinity uptake system ranged 7-fold between 0.010 and 0.076 m*M*. The corresponding values for the low affinity system ranged between 0.71 and 2.29 m*M*. V_{max} values for the high affinity uptake system ranged between 56 and 296 nmol g^{-1} f.wt. h^{-1} and for the low affinity uptake system this range was 432 and 718 nmol g^{-1} f.wt. h^{-1}.

When plants were given a phosphate pre-treatment, arsenate influx in all 6 varieties still showed concentration dependent influx (Fig. 6) and the uptake data were well described by a two-parameter Michaelis-Menten model (Table, 33). The calculated values of K_m are high and indicate that the uptake carriers have low affinity for arsenate, ranging between 1.28 and 4.69 m*M*. The V_{max} ranged between 443 and 1007 nmol g^{-1} f.wt. h^{-1}. It has long been established that arsenate acts as phosphate analogue and is transported across the plasma membrane via phosphate co-transport systems (Meharg and Mancair, 1992).

Kraus et al. (1987) found that phosphate is accumulated and preserved in the root tissue during the early stages of maize development until the reproductive stages of the plant commenced. This might explain the reduction in the rate of arsenate influxes in P-sufficient plants as the stored phosphate in the maize roots reduced arsenate influx.

Table (30): Arsenate influx in P-sufficient and P- deficient plants (nmol g^{-1} f.wt. h^{-1}) as affected by As levels and maize varieries.

Conc	+P		+P	-P	+P	-P	+P	-P	+P	-P	+P	-P
	Single cross 10		Single cross 30k8		Single cross 2030		Single cross 3084		Three-way cross 310		Three-way cross 323	
0.000	0	0	0	0	0	0	0	0	0	0	0	0
0.010	2.464	29.734	1.763	41.951	3.199	33.260	4.272	54.980	2.023	42.379	1.448	29.540
0.025	4.480	54.399	3.933	86.320	4.219	83.211	7.075	171.384	2.850	87.161	4.588	52.657
0.050	5.750	144.383	11.985	125.655	14.933	90.436	30.432	208.626	3.984	96.367	8.646	74.527
0.100	19.374	156.368	20.655	161.003	21.975	253.639	55.382	218.335	18.706	165.251	16.963	97.574
0.250	44.912	245.447	57.757	192.675	46.108	292.498	97.107	275.097	42.690	258.756	26.376	156.342
0.500	95.089	304.968	111.559	246.114	83.907	339.092	194.018	377.441	88.136	316.716	73.246	205.858
1.000	283.097	416.095	228.616	418.603	189.489	500.185	294.706	482.951	196.890	458.394	235.589	342.836
2.500	333.635	563.819	282.130	537.436	348.366	580.699	470.324	525.068	315.596	553.384	339.110	450.000

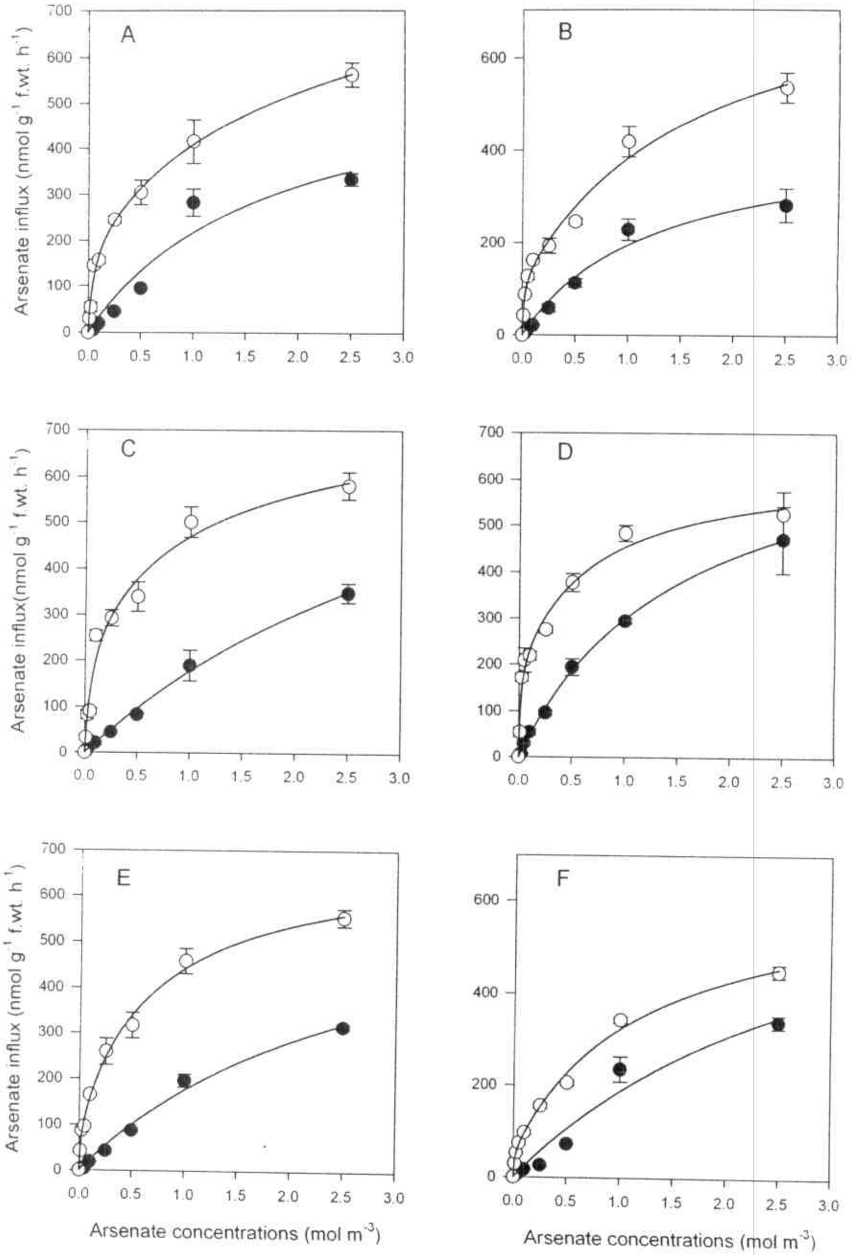

Fig. 6 Arsenate influx nmol g^{-1} f.wt. h^{-1} in excised roots of P-sufficient (•) plants and P-starved (o) plants. A= SC 10, B= SC 30k8, C=SC 2030, D= SC 3084, E=TWC 310, F= TWC 323.

4.5.2.1. Arsenite uptake

Analysis of variance revealed that arsenite influxes were concentration dependent with significant difference between the varieties in arsenite uptake. Also the effect of phosphate was highly significant. The concentration X phosphate X variety interaction term was significant as was the variety X concentration term showing that there were strong genotypic differences in the way that arsenite influx was regulated by arsenite concentration and phosphate (Data shown in Table 31). Arsenite influx in all 6 varieties followed saturation kinetics and that the influxes were well described by a two parameter Michaelis –Menten model (Fig. 7, Table 33). The K_m values for arsenite influx in P-feed plants ranged between 2.1 and 4.5 m*M*. The K_m values increased when the plants were starved for 7 d. The varieties having the lowest K_m values for P-sufficient plants recorded the smallest increases in K_m values for P-deprived plants. The increase in K_m on phosphate starvation ranged from 102-208 %. The V_{max} values in P-feed plants ranged between 1352 and 2128 nmol g^{-1} f.wt. h^{-1}. V_{max} values increased when plants were phosphate starved. This increase ranged from 228 – 114 %.

Table (31): Arsenite influx in P-sufficient and P- deficient plants (nmol g^{-1} f.wt. h^{-1}) as affected by As levels and maize varieties.

Conc	+P		+P	-P	+P	-P	+P	-P	+P	-P	+P	-P
	Single cross 10		Single Cross 30k8		Single Cross 2030		Single cross 3084		Three-way cross 310		Three-way cross 323	
0.000	0	0	0	0	0	0	0	0	0	0	0	0
0.010	3.583	2.001	10.252	6.363	7.027	3.831	21.328	11.711	5.892	2.4135	10.332	5.670
0.025	13.056	5.065	24.665	16.602	28.967	16.894	43.212	18.670	18.979	11.123	21.760	15.113
0.050	63.222	13.836	46.462	37.777	50.930	44.855	77.636	62.892	41.465	53.478	35.864	47.018
0.100	79.103	37.885	87.911	68.088	67.485	84.662	100.306	144.149	90.643	97.330	62.009	64.072
0.250	125.705	135.373	121.377	151.505	98.313	118.018	272.118	272.566	156.732	202.439	103.126	144.108
0.500	238.147	263.865	221.327	282.444	231.994	336.694	397.171	515.531	241.309	296.440	178.289	243.946
1.000	349.069	456.707	307.363	492.235	375.579	471.731	590.071	766.866	318.038	625.769	358.762	378.182
2.500	769.349	1025.455	645.744	977.305	670.842	891.489	1101.677	1373.445	717.640	1225.038	673.036	879.068

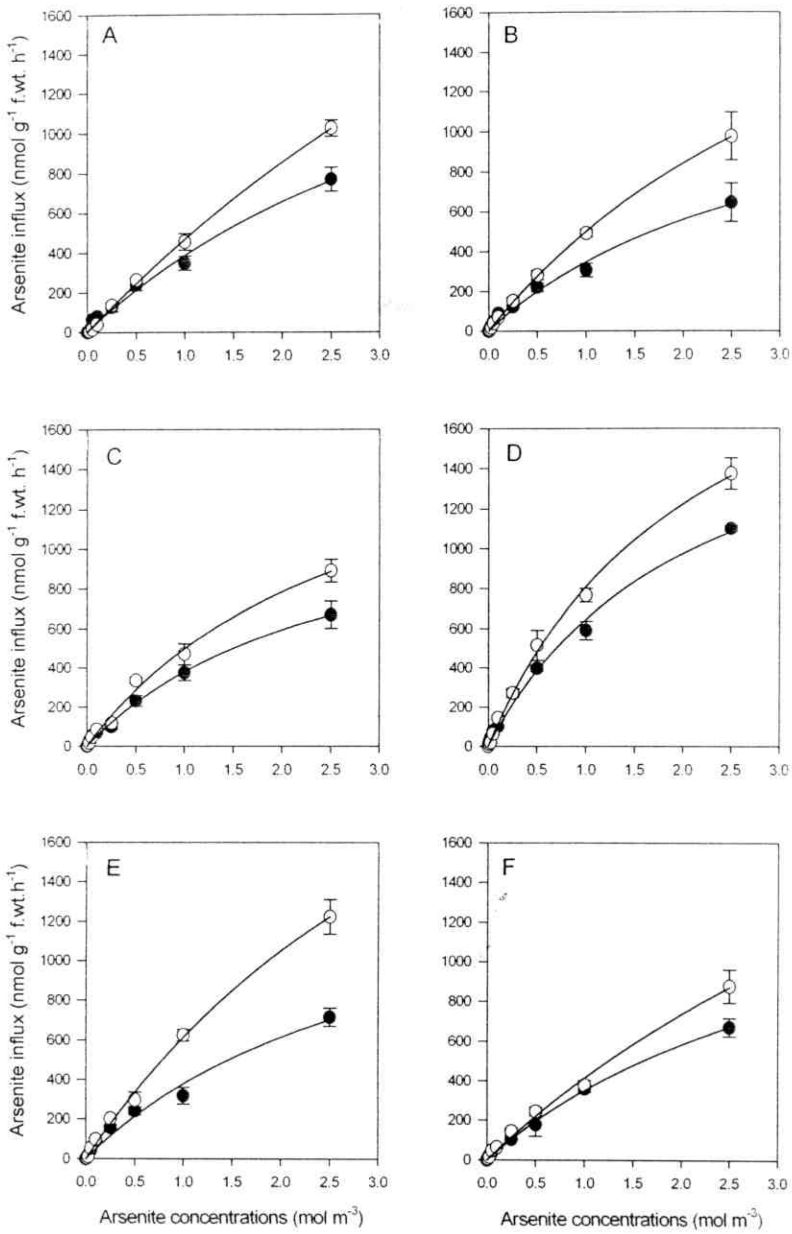

Fig. 7 Arsenite influx nmol g^{-1} f.wt. h^{-1} in excised roots of P-sufficient (•) plants and P-starved (o) plants. A= SC 10, B= SC 30k8, C=SC 2030, D= SC 3084, E=TWC 310, F= TWC 323.

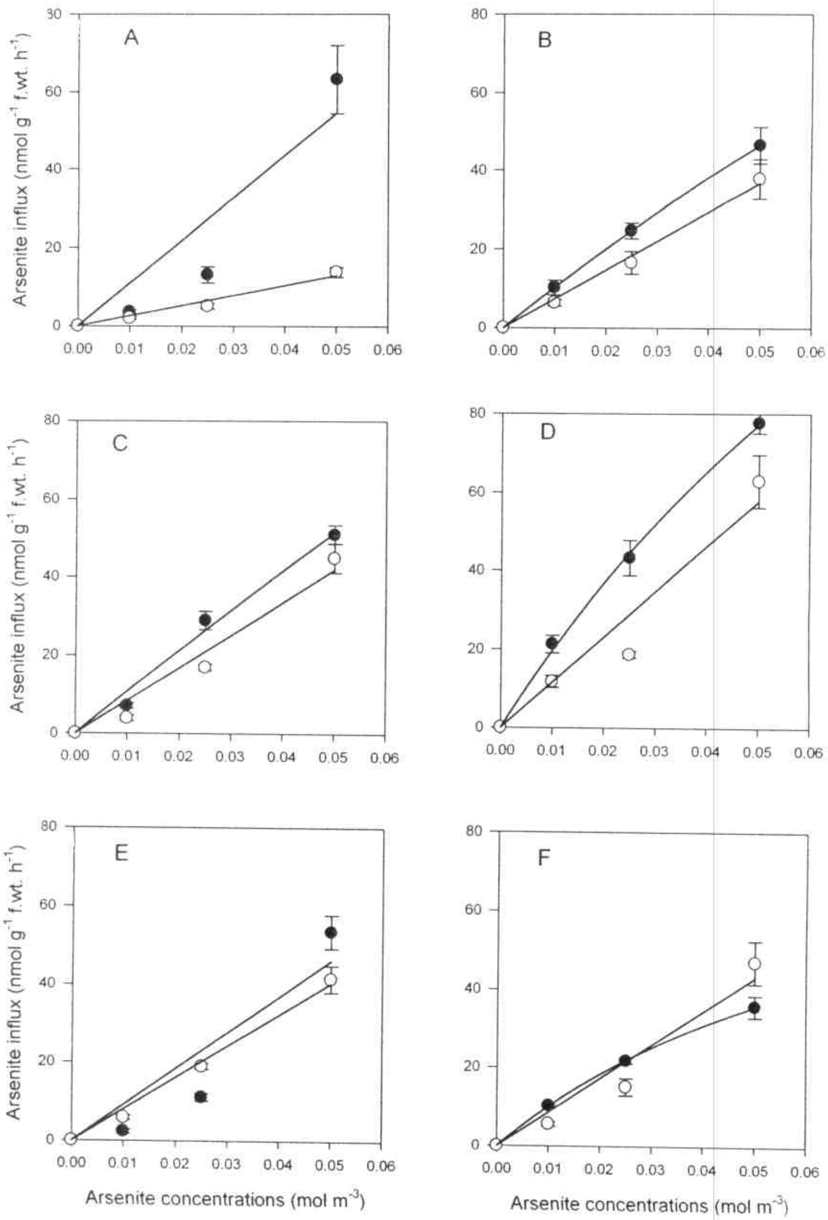

Fig. 8 Arsenite influx nmol g^{-1} f.wt. h^{-1} in excised roots of P-sufficient (•) plants and P-starved (o) plants. A= SC 10, B= SC 30k8, C=SC 2030, D= SC 3084, E=TWC 310, F= TWC 323.

Wang et al. (2002) showed that arsenate and arsenite where taken up by entirely different uptake systems, presenting evidence to suggest that arsenite was taken up by aquaglyceroporins, based upon the detailed findings in yeast by Wysoki et al. (2001) as arsenite is found uncharged $As(OH)_3^o$ at physiological pHs. Arsenite influx was also strongly P dependent, increasing slightly at higher arsenite concentrations in P starved plants (Fig. 8), but at lower arsenite concentrations there were little or no difference in arsenite uptake, and if anything, P starved plants took up more arsenite.

4.5.2.3. DMA uptake

All terms in the analysis of variance for DMA influx were highly significant indicating that there were very strong genetic variances in DMA influx and that this influx was highly regulated by phosphate. The rate of DMA influx was much lower when compared to arsenate and arsenite (Table 32). DMA influx in all 6 varieties was comprised of linear phase and hyperbolic phase (Fig. 9). Saturable components are well described by a two parameter Michaelis –Menten model. The K_m values for the uptake carriers in P-sufficient and P-deprived plants were low and ranged in P-sufficient plants between 0.009 and 0.022 m*M*. These values increased when plants were phosphate starved for 7 days. There were slight differences in V_{max} values between the varieties, when the plants were phosphate sufficient while the V_{max} values increased and ranged between 0.55 and 1.14 nmol g^{-1} f.wt. h^{-1}. The linear component of DMA influx in P-sufficient plants ranged between 0.17 and 0.3 nmol g^{-1} f.wt. h^{-1}, increasing about 10-fold when plants were

P-starved with values ranged between 2.2 and 4.47 nmol g^{-1} f.wt. h^{-1}. DMA influx decreased dramatically when phosphate was given as a pre-treatment as opposed to phosphate starved plants. Perhaps DMA is being as an arsenate analogue, though the evidence against this is that DMA influx regulation in +P plants was much greater than for that observed for arsenate. The +P treatment tended to decrease influx by 50% for arsenate while this figure was 90% for DMA. Raab et al. (2007) found that DMA was taken up 5-fold less efficiently than arsenate and 2-fold less efficient than monomethyl arsonic acid (MMA) after 24 h of exposure to the roots for a range of plant species. This is generally the pattern found in longer term hydroponic uptake experiments as well for *Spartina spp.* (Carbonell et al. 1998, Carbonell-Barrachina et al. 1998) and rice (Marin et al. 1992). However, no large differences were found in arsenic uptake between arsenic species in turnip (Carbonell-Barrachina et al. 1999) and tomato (Burlo et al. 1999).

Our results showed that arsenate/arsenite/DMA influxes were strongly P dependent. As P is so fundamental to cell function it is perhaps not surprising that P alters regulation of expression of a range of membrane transporters, and potentially altering membrane permeability itself.

Table (32): DMA influx in P-sufficient and P- deficient plants (nmol g^{-1} f.wt. h^{-1}) as affected by As levels and maize varieties.

Conc	+P		+P	-P	+P	-P	+P	-P	+P	-P	+P	-P
	Single cross 10		Single cross 30k8		Single cross 2030		Single cross 3084		Three-way cross 310		Three-way cross 323	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.149	0.185	0.142	0.201	0.179	0.357	0.180	0.284	0.141	0.241	0.173	0.191
0.025	0.247	0.264	0.239	0.414	0.225	0.522	0.259	0.603	0.190	0.409	0.256	0.384
0.050	0.293	0.322	0.307	0.652	0.266	0.868	0.306	0.818	0.274	0.617	0.284	0.580
0.100	0.334	1.129	0.341	0.915	0.333	1.013	0.376	0.894	0.354	0.877	0.302	0.894
0.250	0.369	1.298	0.389	1.212	0.398	1.389	0.439	1.230	0.373	1.668	0.356	1.108
0.500	0.461	1.659	0.463	1.638	0.452	3.021	0.496	3.171	0.463	3.174	0.459	1.707
1.000	0.575	3.442	0.541	2.899	0.780	5.067	0.563	5.460	0.584	4.590	0.609	3.652
2.500	0.947	7.786	0.797	6.207	0.981	10.344	1.640	11.687	0.837	8.429	1.337	7.226

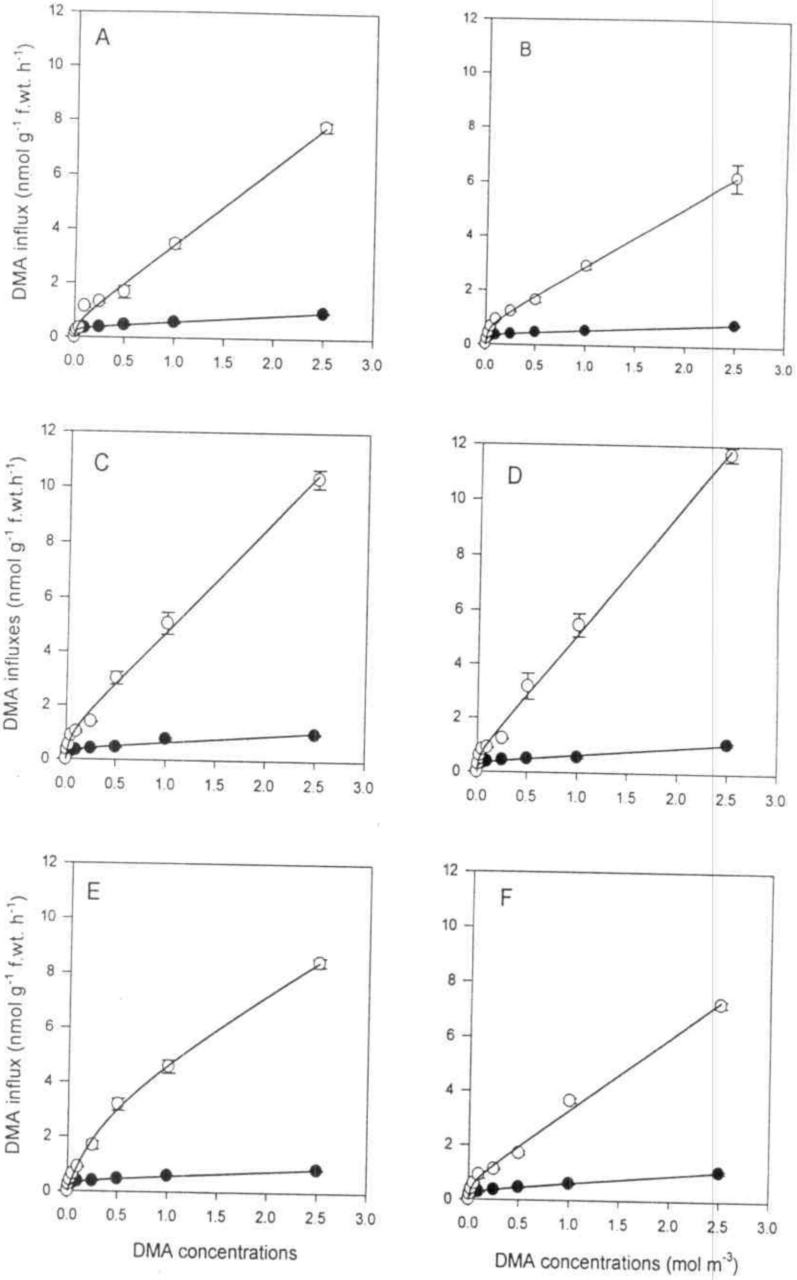

Fig. 9 DMA influx nmol g^{-1} f.wt. h^{-1} in excised roots of P-sufficient (•) plants and P-starved (o) plants. A= SC 10, B= SC 30k8, C=SC 2030, D= SC 3084, E=TWC 310, F= TWC 323.

Table (33): Kinetic parameters of arsenate, arsenite and DMA influxes in P-sufficient and P-deficient plants.

Cultivar	Single cross 10	Single cross 30k8	Single cross 2030	Single cross 3084	Three-way cross 310	Three-way cross 323
Arsenate						
+P						
V_{max}	599	443	1007	755	700	811
K_m	1.75	1.27	4.69	1.52	3.00	3.3
R-Sq	0.9458	0.9796	0.9966	0.9985	0.9916	0.9635
-P						
V^1_{max}	213	121.7	296	204	101	56.4
K^1_m	0.055	0.015	0.076	0.0147	0.019	0.010
V^2_{max}	683	718	475	432	587	596
K^2_m	2.29	1.72	1.45	0.71	0.71	1.23
R-Sq	0.995	0.988	0.978	0.982	0.995	0.995
Arsenite						
+P						
V_{max}	2128	1398	1352	1973	1651	1733
K_m	4.48	3	2.55	2.05	3.36	3.93
R-Sq	0.990	0.985	0.996	0.992	0.989	0.997
-P						
V_{max}	4843	2631	1843	2250	3635	3299
K_m	9.32	4.20	2.69	2.09	4.91	6.95
R-Sq	0.999	1	0.993	0.998	0.998	0.994
DMA						
+P						
V_{max}	0.34	0.38	0.39	0.35	0.39	0.31
K_m	0.011	0.016	0.020	0.009	0.022	0.020
C	0.24	0.17	0.25	0.23	0.18	0.30
R-Sq	0.998	0.999	0.962	0.990	0.995	0.999
-P						
V_{max}	0.55	0.70	1	0.66	1.14	0.64
Km	0.026	0.020	0.037	0.015	0.069	0.024
C	2.90	2.20	3.79	4.47	2.92	2.67
R-Sq	0.994	0.999	0.996	0.996	0.998	0.995

4.5.3. Arsenate, arsenite and DMA toxicity in maize

Short term plant tolerance tests for arsenate, arsenite and DMA were made by measuring maximum root growth (MRG) obtained after one day from growing maize seedlings in media containing concentrations ranging between 0 – 10 mg / L^{-1} (Table 34, Fig. 10). Tolerance indexes for all varieties were calculated following De Koe and Jaques (1993) (Table 35, Fig. 10). Maximum root growth (MRG) was concentration dependent in arsenate, arsenite and DMA toxicity tests. The variety term was only significant for arsenate. Only 2 varieties exceeded 100 % tolerance indexes for arsenate at 0.5 mg As^{5+} L^{-1}. For arsenite all varieties exceed the 100 % tolerance index at 0.5 mg As^{3+} L^{-1}. Moreover, 3 varieties exceed the 100% tolerance index at 1 mgAs^{3+} L^{-1}. In case of DMA all the varieties exceeds 100% tolerance index for all the used concentrations. Thus, the relative toxicity of arsenic species is As(V) > As (III) >> DMA. As arsenate and arsenite concentrations rose there was a sharp decrease in root growth, indicating the high toxicity of these species.

Genetic variation to arsenic toxicity, observed here for arsenite and DMA in maize was also found in field grown rice (Rahman et al. 2007). Laboratory experiments on laboratory grown rice, the most widely studied species in this respect, found genetic variation in response to both arsenate and arsenite (Abedin and Meharg 2002; Dasgupta et al. 2004 and Geng et al. 2006). Rice also showed genetic variation in arsenate and arsenite short term high affinity uptake kinetics (Abedin et al., 2002).

Table (34): Maximum root growth (cm) of the different maize varieties grown under different arsenate/arsenite/DMA concentrations.

Conc. ($mg\ L^{-1}$)	Single cross 10	Single cross 30k8	Single cross 2030	Single cross 3084	Three-way cross 310	Three-way cross 323
			Arsenate			
0.00	2.90	3.83	2.47	2.27	3.67	2.47
0.50	2.40	3.57	2.70	1.07	3.40	2.67
1.00	2.07	1.73	2.03	0.73	1.87	2.00
2.50	1.17	1.27	1.73	0.30	0.87	1.17
5.00	0.90	1.13	0.93	0.33	0.87	1.00
10.00	0.73	0.80	0.33	0.07	0.40	0.33
			Arsenite			
0.00	1.87	2.17	2.17	2.10	2.23	2.33
0.50	2.37	2.03	2.33	2.60	3.10	2.47
1.00	3.30	1.87	2.07	2.47	3.65	2.60
2.50	1.63	1.17	1.57	1.70	2.10	1.73
5.00	1.00	0.63	0.97	0.63	1.47	0.85
10.00	0.33	0.35	0.32	0.20	0.80	0.50
			DMA			
0.00	2.50	2.03	2.13	2.93	1.70	2.43
0.50	2.70	2.67	2.47	3.00	2.17	2.47
1.00	3.08	2.83	3.20	3.20	2.98	2.73
2.50	2.73	3.27	4.08	3.70	2.67	3.43
5.00	2.68	2.70	3.57	3.68	2.53	3.43
10.00	2.63	2.62	2.32	3.00	2.33	3.37

Table (35): **Tolerance index (TI %) of the different maize varieties grown under different arsenate/arsenite/DMA concentrations.**

Conc. (mg L^{-1})	Single cross 10	Single cross 30k8	Single cross 2030	Single cross 3084	Three-way cross 310	Three-way cross 323
	Arsenate					
0.00	100.00	100.00	100.00	100.00	100.00	100.00
0.50	82.76	93.04	109.46	47.06	92.73	108.11
1.00	71.26	45.22	82.43	32.35	50.91	81.08
2.50	40.23	33.04	70.27	13.24	23.64	47.30
5.00	31.03	29.57	37.84	14.71	23.64	40.54
10.00	27.59	20.87	13.51	2.94	10.91	13.51
	Arsenite					
0.00	100.00	100.00	100.00	100.00	100.00	100.00
0.50	126.79	92.97	81.15	123.81	138.81	105.71
1.00	176.79	86.15	74.06	117.46	163.43	111.43
2.50	87.50	53.85	55.00	80.95	94.03	74.29
5.00	53.57	29.23	34.48	30.16	65.67	36.43
10.00	18.75	16.15	11.65	9.52	35.82	21.43
	DMA					
0.00	100.00	100.00	100.00	100.00	100.00	100.00
0.50	108.00	131.15	115.63	102.27	127.45	101.37
1.00	123.33	139.34	150.00	109.09	175.49	112.33
2.50	109.33	160.66	191.41	126.14	156.86	141.10
5.00	107.33	132.79	167.19	125.57	149.02	141.10
10.00	105.33	128.69	108.59	102.27	137.25	138.36

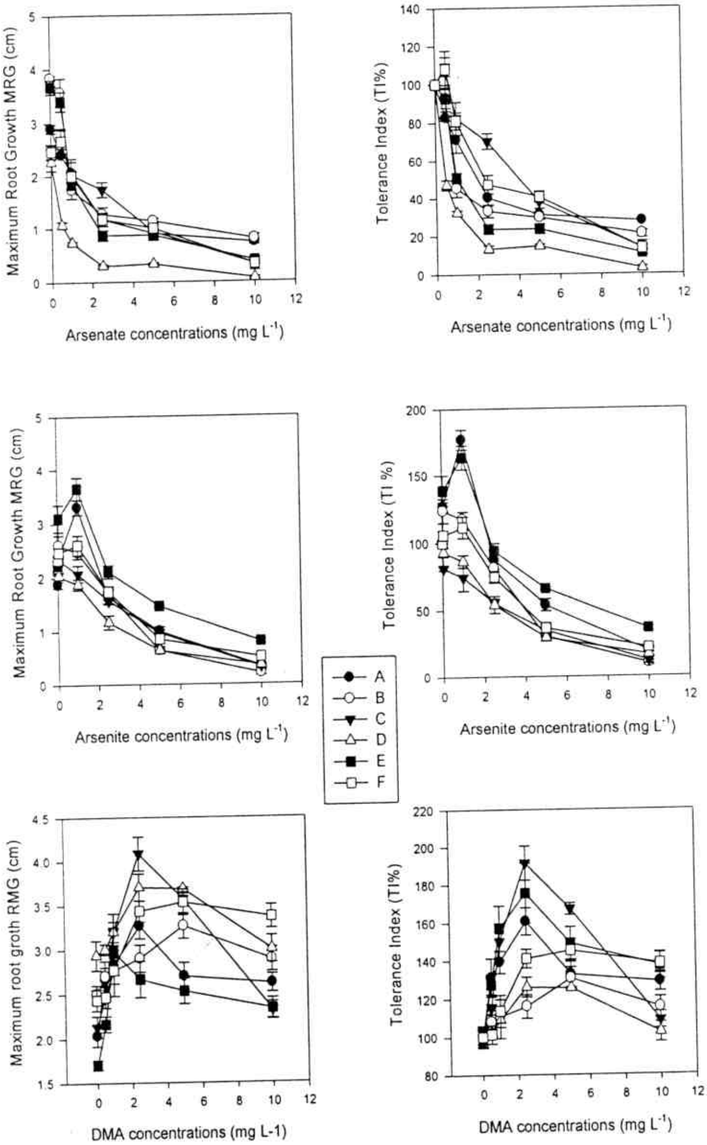

Fig. 10 Maximum root growth and tolerance index (TI %) of the different maize varieties grown under different arsenate/arsenite/DMA concentrations. A= SC 10, B= SC 30k8, C=SC 2030, D= SC 3084, E=TWC 310, F= TWC 323.