OnLine Journal of Biological Sciences 1 (8): 689-693, 2001

The Effect of Industrial Effluents and Vesicular-Arbuscular Mycorrhizae on Nutrient Distribution and Concentration of Wheat and Bean Plants

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Abstract : Several interelemental relationship have been examined in pot-cultivated wheat (*Triticum aestivum*, Giza 164) and *faba* bean (*Vicia faba*, Giza 461) growing naturally and irrigated by industrial effluents enriched with zinc. Zinc concentrations in this industrial waste water were about 5.5 mg L^{-1} (0.84 x 10^{-4} m mol mL^{-1}). In response to industrial effluents, changes in ion distribution, concentration and pigment contents were found in wheat and *faba* bean plants. Industrial effluents induced an increase of pigment contents in wheat and decrease in *faba* bean . In all plant organs of wheat and *faba* bean, Zn concentration increased continuously with irrigation by industrial effluents. The increase in Zn concentration in wheat plants was found to be in the order root > leaf > stem. While, Zn concentrations in *faba* bean plants was in the order root > stem > leaf. There was also an increase of K and Na except for K in stems and roots of wheat plants. Moreover, Ca decreased except in stem and root in *faba* bean plants. Accumulation of Mg in wheat root was increased where thetransport to leaves and stems was decreased. This situation was different in *faba* bean. The internal Feconcentration increased in all plant organs except in leaves of wheat, also its content decreased with time except in leaves o f *faba* bean. When the soil was inoculated with vesicular arbuscular mycorrhizae, changes of ion concentration occurred, where both plants reduced their Zn concentrations but in wheat more than *faba* bean. This could be used for phytoextraction of Zn from soil contaminated by industrial effluents,

Key words: *Triticam aestivum*, *Vicia faba*, vesicular-arbuscular mycorrhizae pigments, elements

Introduction

Inorganic pollutants such as industrial water effluents frequently lead to pollution in fresh water environments such as the River Nile (Abdel-Hamid *et al.*, 1993). Irrigation with contaminated water can lead to accumulate heavy metals such as Zn in the crop (Abbas, 1992). The contamination of soils and plants by heavy metals has been reviewed (Vardaka *et al.*, 1997 and Wheeler and Power, 1995).

Although Zn is an essential element for plant growth, there is also a possibility of Zn pollution. Most plant species are tolerant to excessive Zn concentrations in the soil (Wheeler and Power, 1995). Zinc-deficient plants of various species exhibit great permeability of the plasma membrane resulting in significant leakage of organic and mineral components from root cells (Welch and Norvell, 1993). Zinc may play a role in regulation of transmembrane ion fluxes by preventing oxidation of sulphydryl groups to disulphides in proteins involved in ion-channel gating in the plasma membrane of root cells (Welch, 1995). There is a much evidence that, Zn induced chlorosis in some species such as bean may arise from Zn inhibition of Fe translocation from root to shoot (Agarwala *et al.*, 1977). This problem can be alleviated by an increased supply of Fe.

In soil contaminated with high levels of heavy metals, vesicular-arbuscular mycorrhizae were found to be resistant to these extreme conditions (Weissenhorn *et al.*, 1994). Mycorrhizae can enhance metal uptake, for instance Zn, from metal-deficient soils (Abdel-Fattah and Rabie, 1995). But when these metals are present in the soil at high levels, a decrease in their uptake has been described (Weissenhorn, 1994).

The aim of this study is to investigate the influence of industrial effluents on mineral composition in different plant organs of wheat and *faba*bean as well as the role of vesicular-arbuscular mycorrhizae in changing this distribution. The possibility of phytoextraction of zinc from soils contaminated by industrial effluents was also considered.

Materials and Methods

Seeds of *wheat* (*Triticum aestivum*, Giza 164) and *faba* bean (*Vicia faba*, Giza 461) were surface-sterilized with 1% sodium hypochloride for 5 minutes, then washed with distilled water. Twenty wheat and l0 bean seeds were sown into individual pots each containing 7kg of sieved loam soil (clay: sand, 2:1) collected from cultivated soil in Mansoura. Soil chemical and physical analyses of the experimental soil are presented in Table 1. The above mentioned analyses were perfonned according the standard method of Piper (1950) and Jackson (1958).

Table 1: Chemical and physical data for experimental soils.

Components	Values
Chemical analysis	
Organic carbon	0.45%
SO_4^{2-}	0.015%
Cl^-	0.078%
HCO_3^-	0.062%
$CaCO_3$	2.60%
K^+	19 mg kg^{-1}
Na^+	193 mg kg^{-1}
Ca^{2+}	240 mg kg^{-1}
pH	7.79
Physical analysis	
E.C.	3.55 m.mhos
Sand	26.2%
Silt	30.8%
Clay	43.0%
Mean porosity	40.4%
Mean W. H. C.	61.0%
Mean moisture content	27.3%

There were 3 groups of pots of each plant species each containing 7 pots as a replicates, The first set was irrigated with natural Nile water containing Zn concentration of 0.91 mg L^{-1} as controls. The plants of the second set were irrigated with polluted water containing about 5.5 mg L^{-1} Zn. This water was collected from an area of industrial discharge from Talkh factory of fertilizers. This polluted water will be used for irrigation of about 21 ha, in the Gammesa region. The third set of plants was irrigated with polluted water after inoculation of the soil with vesicular arbuscular-mycorrhiza (VAM). VAM inoculum was prepared as recommended by Abdel-Fattah (1997).

Irrig ation was carried out according to the usual practice by adding equal amounts of water to maintain the water holding capacity at 60% of the water-holding capacity of the soil. Plants were exposed to normal day length with natural illumination in a greenhouse of Faculty of Science of Mansoura in winter season (November 1997). The photoperiod was approximately 10 h. and the day/night temperature vas about 20/14$\pm$2°C.

Plant sampling was carried out at 3 successive, phenological stages referred to as vegetative (for wheat and bean 32 days after sowing); flowering (for wheat after 83 days and for bean after 56 days) and fruiting (for wheat after 154 days and for bean after 106 days). At sampling time, the plants were collected from each pot and separated into shoots and roots for measurement of pigments in leaves and nutrient elements in leaves, stems and roots.

Data were first subjected to analysis of variance (ANOVA). If ANOVA showed significant ($P < 0.05$) effects, the least significant difference (L.S.D) was used to compare treatments (Snedecor and Cochran,1976).

The plants were harvested at different stages of age (vegetative, flowering and fruiting stages). At each time of harvesting, leaves were collected for chlorophyll analysis. The roots of the harvested plants were washed by tap water to remove soil particles. Then they were rinsed in distilled water and blotted lightly. Thereafter, plants were separated into roots, stems and leaves. Samples were dried at 80°C for 48h.

Estimation of photosynthetic pigments: Chlorophyll a, chlorophyll b and carotenoids represent total pigments calculated as mg kg^{-1} fresh weight were determined in leaves by a spectrophotometric method (Metzner *et al.* 1965).

Nutrient element concentration: For estimation of cations, dry samples were digested in a mixture of concentrated nitric acid and perchloric acid (4:1, v:v) and made up to a fixed volume with deionized distilled water (Chapman and Pratt, I978). Flame-emission spectrophotometry was used for determining K and Na while Ca, Mg, Fe and Zn were measured by atomic absorption spectrophotometry.

Results and Discussion

Changes in pigments: The irrigation of wheat plant with industrial effluents either alone or in combination with VAM significantly increased the total photosynthetic pigments throughout the vegetative and flowering stages but declined again at fruiting stages (Fig. 1).

The increase in pigments may be attributed to increased biosynthesis of pigments and/or decreased of their degradation. Here, increase in Zn concentration in soil irrigated with industrial effluents led to an increase of Zn concentration in leaves (Fig. 4). It must be noted that the Fe content of leaves decreased, and the chlorophyll increased concomitantly with increased Ca in the leaves. The Ca ions may play a protective role. This may arise from complex interaction of Ca with Zn and Fe which have similar ionic indices (Baker, 1978). Abbas and Shukry (1993) found that Zn at low concentration (less than 200 mol L^{-1}) increase the chlorophyll content of maize. Furthermore, Vardaka *et al.* (1997) found that chlorophyll increased, where there is a

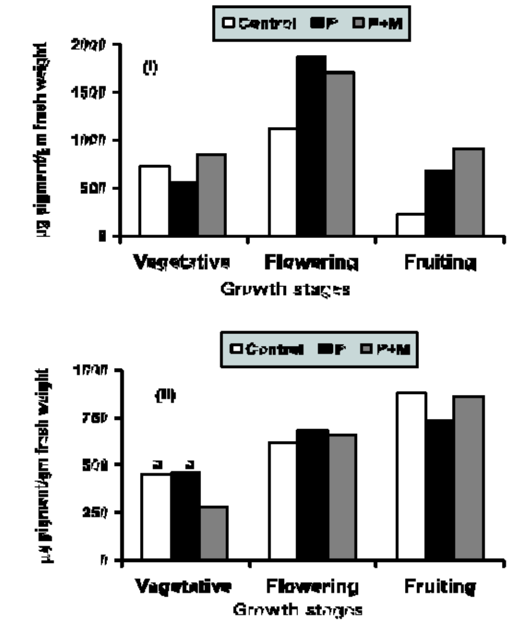

Fig. 1: Effect of irrigation with pure Nile water (Control), industrial effluents (P) and VAM (P+M) on total pigments in leaves of (I) Triticum aestivum and (II) Vicia faba plants throughout the period of growth. The bars with the same letter are not significantly different at p-level =5% (LSD-test)

positive mutual correlation between the Zn, K and Ca concentrations in wheat leaves. The increase in chlorophyll content of leaves irrigated by industrial effluents inoculated by VAM, may be due to the increase in Fe concentrations of leaves. These results are in agreement with those obtained by Bavaresco and Fogher (1996). In *Vicia faba*, the total pigments significantly. decreased in comparison with the control following irrigation by industrial effluents. This was due to the decrease in Fe concentration in leaves (Brooks 1998). This can be alleviated biologically by inoculation of soil with VAM, where the total pigments significantly increased. These results are in agreement with those obtained by Bavaresco and Fogher (1996) who found that, the inoculation with VAM increased Fe and chlorophyll concentrations in leaves of the grapes (*Vitis vinifera*).

Changes in ion nutrient element concentration: Irrigation with industrial effluents increased the Na concentration in all of the wheat and *faba* bean organs (Fig. 2). However, the greatest increase of Na concentration was found in roots and stems whereas the lowest increase was detected in leaves throughout the growth period. Again, the least effect of industrial effluents in combination with VAM on internal Na concentration was found to be in roots and stems of wheat plant.

After irrigation by industrial effluents K and Ca concentrations (Figs. 2 and 3) were low in roots and stems of wheat due to an increase in Na uptake. However, the greatest increase in concentration was found in leaves in relation to control levels. This may be due to the high mobility of K. There was an increase in K and Ca concentration in all organs of *faba* bean. This result was in accordance with those obtained by Amer

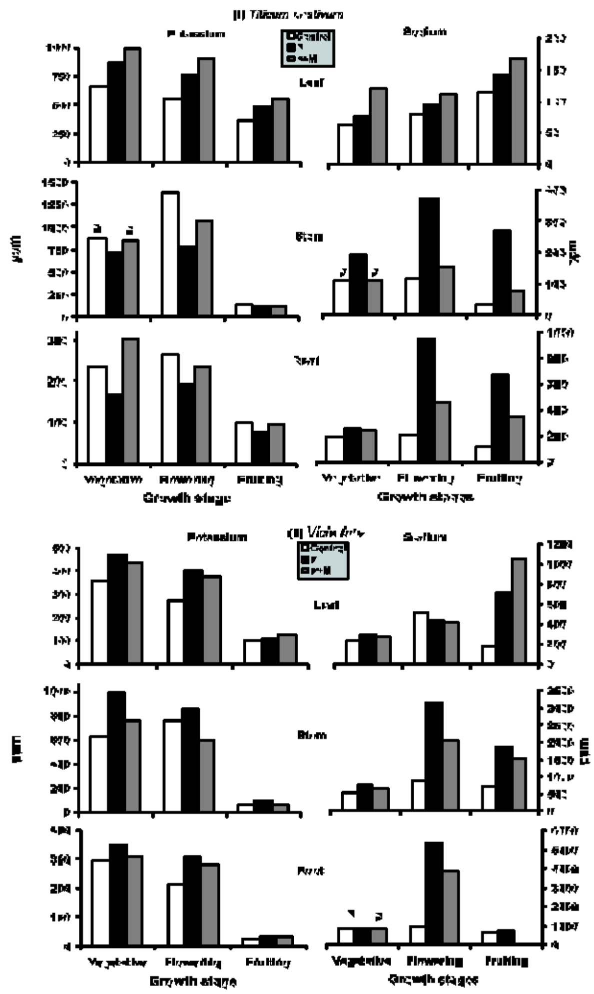

Fig. 2: Effect of irrigation with pure Nile water (Control), industrial effluents (P) and VAM (P+M) on potassium and sodium distribution in different organs of (I) *Triticum aestivum* and (II) *Vicia faba* plants. The bars with same letter are not significantly different at p-level = 5% (LSD-test)

et al. (1993) who stated that, the increase in shoot K was due to Zn addition rather than K application. This result suggests at Zn favours K uptake by *faba* bean plants.

The least effect or non-significant changes were found for Mg in polluted wheat tissues with the exception of roots. Mg concentrations were high relative to the control for all plant parts treated with industrial effluents and VAM. The Mg concentrations in all organs of *faba* bean in comparison with the control were high, except in roots. Because of the requirement of Mg for chlorophyll formation it may be assumed that shortage of Mg will have a negative impact on carbon fixation and thus on the carbohydrate status of plants (Lasley and Garbar, 1978).

Irrigation by industrial effluents in combination with VAM again caused a significant increase in the internal Fe concentration in stems, and a non-significant change in roots (except at vegetative stage) as shown in (Fig. 4). For leaves,

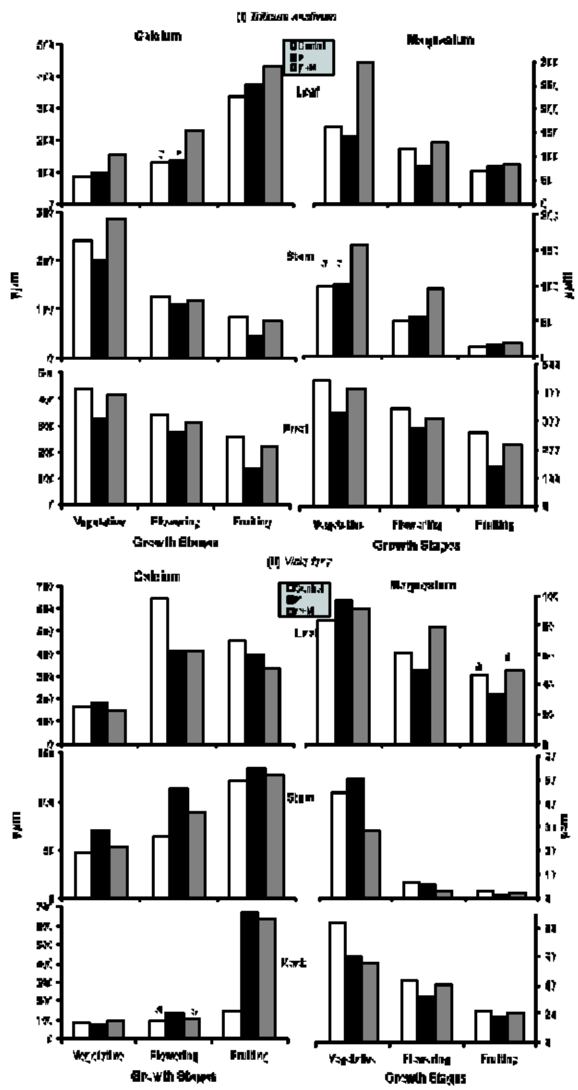

Fig. 3: Effect of irrigation with pure (Nile water (Control), industrial effluents (P) and VAM (P+M) on calcium and magnesium distribution in different organs of (I) *Triticum aestivum* and (II) *Vicia faba* plants. The bars with same letter are not significantly different at p-level = 5% (LSD-test)

there was a significant decrease and increase of Fe in response to irrigation by industrial effluents and in combination with VAM respectively. These results are in agreement with those obtained by Megalah *et al.* (1994), who stated that, increase in Zn level led mostly to a decrease of Fe content in corn plant. The Zn concentration of industrial waste water is about 5.5 mg L^{-1}, thus the irrigation by this water led to an increase in the internal Zn concentration in 11 organs of wheat and faba bean plants (Fig. 4). These results are also in agreement with those obtained by Megalah *et al.* (1994) who studied same results in corn plants.

Zn concentrations were in the order of root > leaves > stems in wheat and root > stem > leaves in faba bean plants. Neite

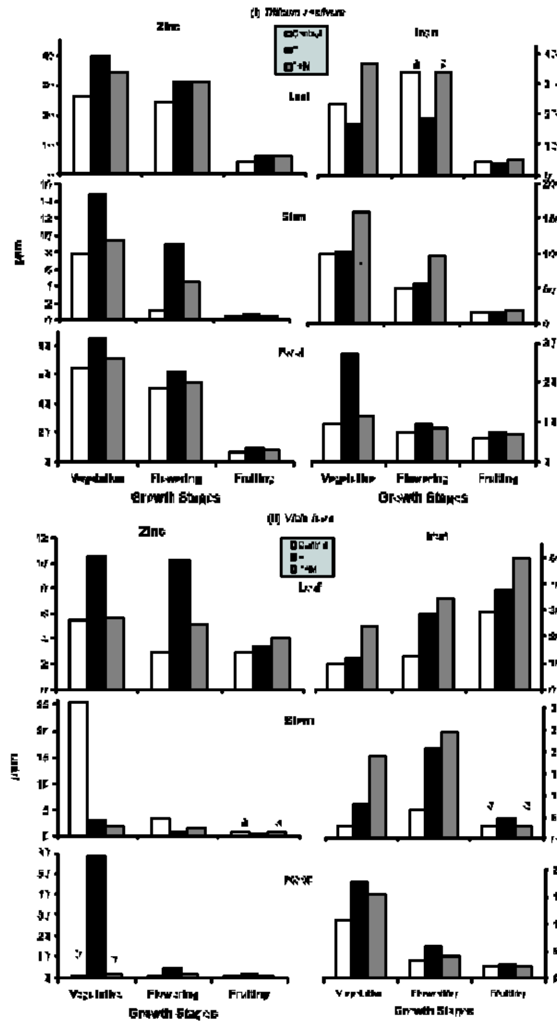

Fig. 4: Effect of irrigation with pure Nile water (Control), industrial effluents (P) and VAM (P+M) on zinc and iron distribution in different organs of (I) *Triticum aestivum* and (II) *Vicia faba* plants. The bars with same letter are not significantly different at p-level = 5% (LSD-test)

et al. (1991) found same results. This may be due to the ability of wheat root to accumulate and translocate Zn to tops from the roots and stems (Luwe, 1995). In contrast, the translocation rate in faba bean was low. These results were confirmed by Wheal and Rengel (1997). Whereas highest levels of macro nutrients can usually be found in the leaves, micro nutrients and potentially phytotoxic metals such as Zn are mainly accumulated in roots. This may be due to some precipitation of these cations in the root (Luwe, 1995).

Metal micro nutrients such as Fe and Zn are thought to be transported predominantly as chelates by chelating organic molecules (Schmidke and Stephan, 1995). It is apparent from Fig. 4, that, the wheat plant can be considered as hyper accumulator of Zn, but faba bean plant less so. These results are in agreement with those of McGrath *et al.* (1993), who reported that 2 populations of *Thlaspi caerulescens*, one took up more Zn than the other.

On the basis of the results obtained, it could be concluded that: wheat plant can accumulatemore Zn than faba bean, i.e. wheat can remove Zn from agricultural soil contaminated by industrial effluents by raising Zn content of grains as recommended by Chaney *et al.* (1997). However the removal rate is not constant, and may vary with the age of the plant. This is the relationship between yields and plant metal concentration in relation to Zn (McGrath, 1998). These are the variables in the efficiency of phytoextraction processes.

Arbuscular mycorrhizal fungi can enhance plant uptake of mineral nutrients over broad pH ranges (Clark and Zeto 1996). VAM fungi increase the water and nutrient uptake by extending the absorptive surface of the roots to explore more soil volume (Ruiz-Lozano and Azcon, 1995).

Two main mechanism have been reported dealing with the effect of mycorrhizae on host metal tolerance as follows: Jones and Hutchinson (1986) suggested that metals may bind to the interfacial matrix found between the hyphae in mycorrhizae or accumulate in their vacuolar bodies (Turnau *et al.*, 1994). This binding would then either, reduce further movement of the metal through the mycorrhizae to host tissue, or removed metabolically by the fungus and sequestered in a harmless form within its hyphae. Either of these possibilities, passive binding or metabolic detoxification by mycobionts should lead to increased amounts of the above-ground parts of resistant plants (Abdel-Fattah and Rabie 1995). Consequently plants inoculated with mycorrhizal fungi retained more Zn in root than shoot (Shetty *et al.*, 1994), confirming earlier reports that mycorrhizal fungi alter the translocation pattern of heavy metals in host plants.

To summarize, the study demonstrates that: wheat can accumulate more Zn than bean and the phytoextraction processes were reduced by the inoculation of soil with vesicular arbuscular mycorrhiza.

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