

## **Impact of Cadmium on Field Grown Maize in Three Consecutive Growing Seasons**

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The effect of cadmium (Cd) of atmospheric origin was investigated on certain terms in the heat balance and on the dry matter production of maize grown in the field at two water supply levels (rainfed and irrigated) in Hungary between 2010 and 2012. In contrast to previous experiments, the plants were exposed to pollution every week throughout the vegetation period. Among the plant parameters, a record was made of plant height, leaf area index and the length of the growing period. Water utilisation was estimated on the basis of water use efficiency, and efficiency from global radiation. Polluted maize used more water for the incorporation of unit dry matter, and was unable to absorb as much solar radiation as the unpolluted control plants. Irrigation moderated the negative effect of Cd on these parameters, suggesting that it could be of primary importance in overcoming the effects of Cd pollution of atmospheric origin.

**Keywords:** albedo, cadmium, radiation efficiency, water use efficiency

### **Introduction**

When heavy metal particles are emitted into the atmosphere they form an aerosol, which may be transported by the wind to great distances (Eshleman et al. 1971) and then deposited on plant surfaces (Nagajyoti et al. 2010). Cd endangers human health, and uptake by plants is the first link in the nutritional chain. Plants accumulate it both in the roots and shoots, resulting in toxic symptoms such as growth reduction or cell death (Prasad 1995). According to Wójcik and Tukiendorf (2005), the Cd content in the roots and shoots of young maize plants increases in almost direct proportion with the rise in the Cd concentration of the growth medium.

Many earlier papers discussed the uptake (Wójcik and Tukiendorf 2005) and accumulation (Carpena et al. 2003) of Cd, and the mechanism (Schützendübel and Polle 2002) and extent of the damage (Mogopodi et al. 2008), but with few exceptions these authors dealt with Cd of soil origin. In contrast to earlier analyses, the aim of the present study was to investigate the energetic aspects of the effects of long-term (three-year) Cd pollution of atmospheric origin under field conditions, as this can be expected to give a better approximation of the true nature of contamination than can be achieved in the laboratory.

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### Materials and Methods

The effect of Cd was examined at two water supply levels in the 2010, 2011 and 2012 growing seasons at the Agrometeorological Research Station in Keszthely, where an automatic QLC 50 weather station operates, complemented by a pyranometer of the CM-3 type. Meteorological data were sampled every 6 seconds, and 10-minute means were recorded. Sperlona, a Swiss maize hybrid with a short vegetation period (FAO 340), was sown as the test plant in mid-April each year, at the 70,000 plants/ha density widely used under Hungarian climatic conditions. Describing the Cd toxicity for crop species, maize was the most sensitive and wheat the least (Wójcik and Tukiendorf 1999).

Harvesting was performed in October in 2010, due to the protracted drying down after maturity, and in early September in the other two years.

The soil was Ramann's brown forest soil with a mean density of  $1.46 \text{ Mg m}^{-3}$  in the upper 1 m and an available water quantity of  $150 \text{ mm m}^{-1}$ . Nutrients (180, 80 and  $120 \text{ mg ha}^{-1}$  N, P and K, respectively) were applied in spring, immediately prior to sowing. Other agronomic measures were those recommended for the area by experts from the local University of Agricultural Sciences. Soil and leaf samples were taken in May and September to analyze the initial and final Cd contamination of the maize stand. Soil samples were collected every 0.2 m to a depth of 0.8 m in three replications. Leaf samples were collected from the upper 4<sup>th</sup> or 5<sup>th</sup> leaf levels in May and from around the cob attachment height in September. The leaf surface was not washed before analysis. Cd levels were measured by inductively coupled plasma-OES spectrometry (Optima™ 2000 DV ICP-OES, Perkin-Elmer). The limit of detection (LOD) for Cd was  $0.01 \text{ } \mu\text{g ml}^{-1}$ .

A motorised sprayer (SP 415) was used to apply a Cd concentration of 10  $\mu\text{M}$  at weekly intervals only for plants avoiding soil pollution. This rate was chosen to simulate moderate – atmospheric origin – Cd pollution for maize and was determined after a consideration of the wide range of values given in the literature (Rüegsegger and Brunold, 1992; Wójcik and Tukiendorf 2005). Cd pollution was repeated eleven times, spraying a total of  $61.6 \text{ } 10^{-2} \text{ } \mu\text{g Cd m}^{-2}$  and  $550 \text{ ml water m}^{-2}$  per season. This additional water amounted to 0.08–0.2% of the seasonal rainfall sum in the vegetation period.

Evapotranspirometers (ETR) of the Thornthwaite-Matter type with a volume of  $4 \text{ m}^3$  (metal containers  $2 \times 2 \text{ m}$  in area, 1 m in depth) were filled with a monolith from the surrounding field, layered as in the natural state, and arranged in a block design with four replications.

Growth was estimated once a week by measuring the leaf area of five sample plants from each treatment with a LI-300A automatic planimeter (LI-COR, Lincoln, NE). These values were then used to calculate the leaf area index (LAI). At the end of the vegetation season the maize was dried to constant weight at  $65^\circ\text{C}$ , after which the total aboveground biomass (TDM) was determined. The relationship between yield and water utilisation was determined in terms of water use efficiency (WUE), given as the water quantity in litres emitted by the plants in the course of evapotranspiration for the production of 1 kg TDM.

The  $4 \text{ m}^2$  surface area of the evapotranspirometer was not large enough for the determination of canopy radiation parameters, so it was necessary to form plots of at least 0.3 ha

for each treatment in the non-irrigated experiment to provide the measurement conditions required for the CMA-11 albedometer (Kipp & Zonen, Vaisala, Finland). The adjustable posts holding the sensors and data collectors (Logbox SD; Kipp & Zonen, Vaisala) were placed in the middle of these plots. The height of the sensor was adjusted each week as the maize grew, so that the albedometer was always at least 1.5 m above the canopy. Plots measuring 4 m<sup>2</sup>, equal to the size of the ETR, were designated along the diameter of the larger fields used for radiation measurement in five replications per plot. The ETR equipment was set up in a block design within the field, 50 m from the southern edge.

The net radiation ( $R_n$ ) was calculated as the difference between the incoming short wave radiation ( $R_{ns}$ ) and the outgoing long wave radiation ( $R_{nl}$ ):

$$R_n = R_{ns} - R_{nl} \tag{1}$$

The albedo ( $a$ ) and global radiation ( $G$ ) data were required to calculate  $R_{ns}$ :

$$R_{ns} = G(1-a) \tag{2}$$

The value of  $R_{nl}$  was determined using the equation given by Allen et al. (1998):

$$R_{nl} = \sigma [T_{meanK}^4] (0.34 - 0.14\sqrt{e_a}) (1.35 \frac{R_s}{R_{so}} - 0.35), \tag{3}$$

where  $\sigma$  is the Stefan–Boltzmann constant [ $4.903 \times 10^{-9} \text{ MJ K}^{-4} \text{ m}^{-2} \text{ day}^{-1}$ ];  $T_{mean,K}$  is the mean temperature during a 24-h period [K];  $e_a$  is the actual vapour pressure [kPa];  $R_s/R_{so}$  is the relative shortwave radiation (limited to = 1.0);  $R_s$  is measured solar radiation [ $\text{MJ m}^{-2} \text{ day}^{-1}$ ] and  $R_{so}$  is calculated clear-sky radiation.

The energy of the net balance was then divided between the consumers: sensible heat flux ( $H$ ) for warming, latent heat flux ( $LE$ ) for evapotranspiration, ground heat flux ( $G$ ) to ensure the heat supply of the soil, the energy fixed by photosynthesis ( $P$ ) and stored energy ( $S$ ), using the equation:

$$R_n = H + LE + G + P + (\Delta S). \tag{4}$$

As the components of the heat balance were calculated daily, the values of  $P$  and  $S$  were negligible (Allen et al. 1998). The energy required for the phase change of the water evaporated ( $2.45 \text{ MJ m}^{-2}$  energy is required to evaporate 1 mm water) was used for the determination of latent heat ( $LE$ ) (Jones 1983). The Monsi–Saeki equation (Monsi and Saeki 1953) served as the basis for the determination of  $G$ :

$$I_g = I_o e^{-kLAI}, \tag{5}$$

where  $I_g$  and  $I_o$  are the radiation measured at ground level and at the top of the canopy, respectively;  $k$  is the extinction coefficient and  $LAI$  is the leaf area index. Sensible heat ( $H$ ) was expressed as the residue term of Equation (4).

Efficiency ( $\epsilon$ ) was given as the ratio of TDM [ $\text{g}$ ]  $\times 18.18 \text{ kJ g}^{-1}$  (conversion factor) to global radiation,  $G$  [ $\text{kJ}$ ]:

$$e = \frac{TDM \times 18.18}{G} 100 [\%]. \quad (6)$$

The conversion factor 18.18 was the quantity of energy (kJ) required for the production of 1 g dry matter according to Jones (1983).

The effect of the treatments was analyzed with one-way ANOVA, using the Duncan's or Games–Howell simultaneous average comparison tests as a supplement. The basis of the assumption was whether Levene's test on the studied variable gave a difference for the variance. A two-tailed paired *t*-test was applied for the time series analysis. The SPSS GLM Univariate procedure provided analysis of variance for dependent variables (TDM, WUE) by two factors (water supply and contamination level) for three growing seasons. The SPSS program package (SPSS Statistics 17.0; IBM Corporation, New York, US) was used for the analysis.

## Results

### *Weather and maize development*

The weather conditions differed in the three years. In the wet year of 2010 the rainfall sum during the vegetation season was 40% more than the long-term mean, while the air temperature was equal to the long-term mean. By contrast, the summer was extremely dry in 2011 and 2012, with 44% less rainfall in 2011 and 29% less in 2012 than the long-term mean. The two dry years were also hot, with mean temperatures 1.2°C higher than normal in 2011 and 1.6°C higher in 2012. However, in contrast to the weather means for the whole growing season, the values recorded in July, which is considered to be decisive for maize development (tasseling), exhibited no significant difference from the long-term mean (1901–2000) in any of the years.

Cd did not modify maize development as it had no effect on either the length of the phenological phases or on their starting dates. As a result of the abundant rainfall, the drying down of the leaves took a month more in 2010 than in the other years, but of this only one extra week was required for complete maturity (in the control plants, too). The exact opposite was observed in 2012, when drying down took place approx. a month earlier due to the extreme heat in August and the lack of rainfall (monthly sum: 5 mm), which scorched the crop. These changes were also observed in the unpolluted treatment.

Cd tended to reduce the final plant height of maize by around 30–40 cm. Many earlier publications reported similar results, though in these cases the Cd was of soil origin.

In the wet year of 2010 the annual mean LAI of polluted plants was 33.7% lower ( $P < 0.000$ ) in the rainfed plot and 19.7% lower ( $P < 0.000$ ) in the irrigated treatment than that of non-polluted plants (Figs 1 and 2). As the seasonal pattern of change in LAI coming from Cd pollution was the same in the two differently irrigated treatments, results of rainfed crops are presented only. In the hot, dry years the effect of Cd on LAI was somewhat less pronounced in both water supply variants; in 2011 and 2012 the mean annual reduction in the LAI of polluted plants was 10.6% ( $P < 0.01$ ) and 26.9% ( $P < 0.000$ ), respectively, in the rainfed plot and 9.6% ( $P < 0.000$ ) and 10.1% ( $P < 0.008$ ) in the ETR. The

great decline in LAI in 2010 was probably due to the fact that the plentiful rainfall washed the Cd off the plant surface into the soil, from which the maize plants were able to absorb Cd more intensively than from atmospheric deposition.

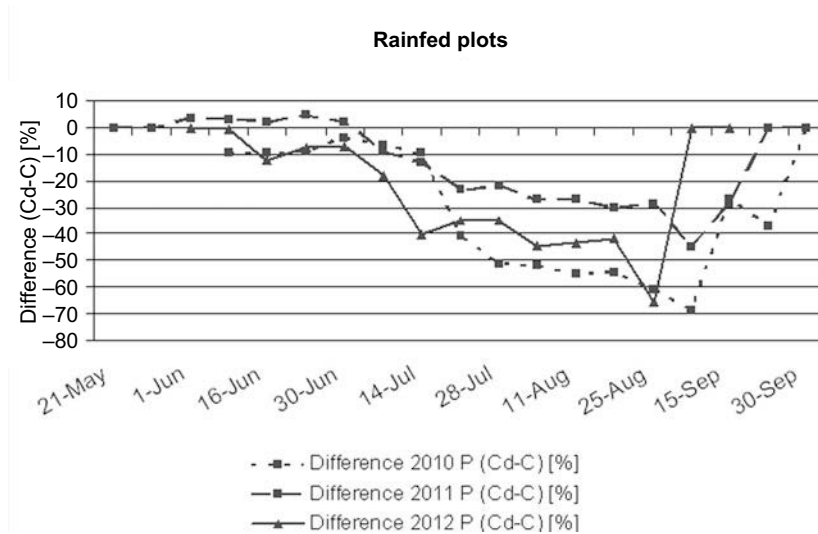


Figure 1. Percentage decline in LAI resulting from Cd pollution in rainfed treatments. The weekly data are the differences in LAI between polluted (Cd) and non-polluted maize (C)

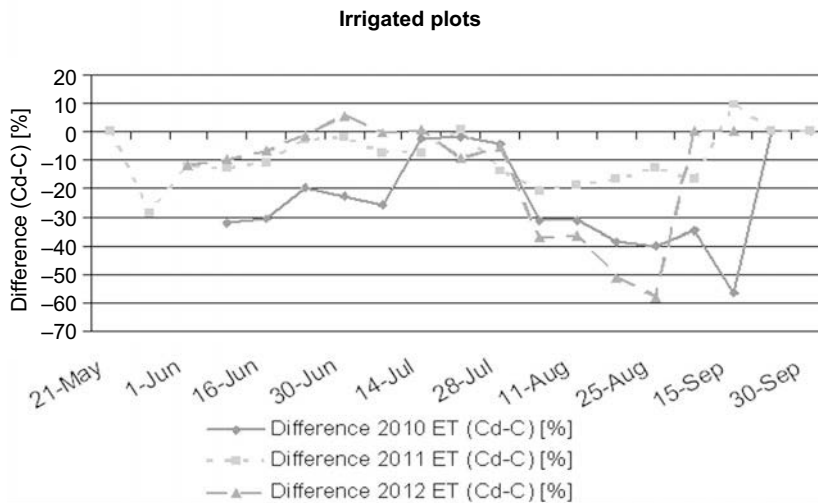


Figure 2. Percentage decline in LAI resulting from Cd pollution in irrigated treatments. The weekly data are the differences in LAI between polluted (Cd) and non-polluted maize (C)

### Changes in evapotranspiration

The maize evapotranspiration was greatly dependent on the year; the warmer the vegetation period, the greater the water loss observed. Consequently, the cumulative evapotranspiration was the lowest in the cool wet year of 2010, compared with which unpolluted maize used 34.5% ( $P < 0.000$ ) more water in 2011 and 19.8% more ( $P < 0.001$ ) in 2012 (Fig. 3). In 2010 and 2012 Cd caused a slight (a few %) but significant drop in the total evapotranspiration. The reason could have been the small LAI of polluted plants. A more pronounced effect of pollution was recorded in 2011, when the water loss was 10.6% less ( $P < 0.000$ ).

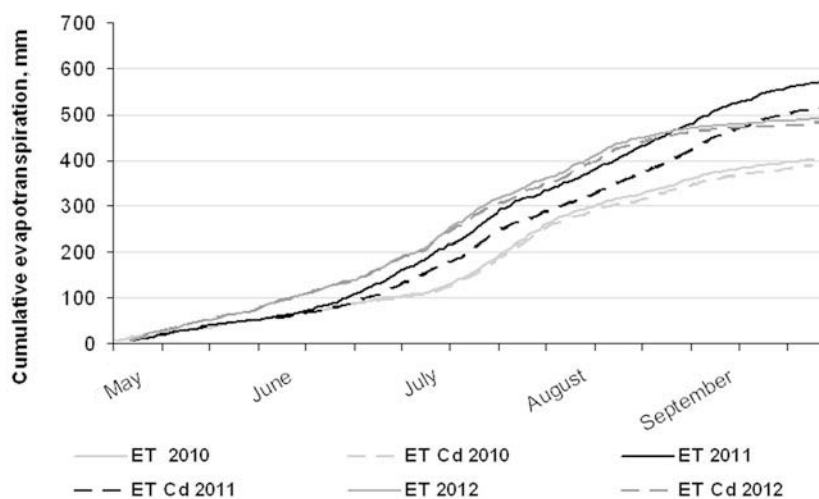


Figure 3. Cumulative evapotranspiration of maize in the 2010–2012 seasons. Water losses in Cd-treated maize and the non-polluted control are designated by broken and continuous lines, respectively

### Effect of pollution on the components of the heat balance of maize

In the present work, the albedo, the most readily measurable radiation property, exhibited the greatest constancy. In 2010 the albedo of the polluted stand was 4.8% higher ( $P < 0.001$ ) than in the control, but only during the vegetative phase. In the generative phase (up to 26 Aug.) the albedo of the treated and untreated stands was much the same, but during the very protracted drying down period a decrease of 6.2% was observed in the polluted stand. In 2011 the albedo of Cd-treated maize was 2.8% ( $P < 0.001$ ) higher than in the control. In this hot, dry year there was little difference between the two stands at the beginning of the vegetation period, while at the end of the season a decrease was again observed in the albedo. However, as drying down proceeded much more quickly in this year, the decrease was only recorded on a few days. In 2012 the annual growth in albedo of the polluted treatment was the highest, 6% ( $P < 0.000$ ). In this year the albedo of the treated

and untreated stands evened out at the end of the vegetation cycle (in the last 1.5 weeks). The less intense soil cover in the Cd-treated plot, due to the lower LAI, may have contributed to the increase in the albedo. Local observations in Keszthely revealed that the soil albedo was around 10–15% higher than that of the plants.

The net balance reflected the smaller radiation absorption of the polluted stand, noted in the discussion of albedo. In the wet year (2010) the decrease was only characteristic of the vegetative phase, and a slight increase was observed during drying down, but this was no longer detectable after full maturity, during the protracted period of drying down caused by the abundant rainfall. In the two dry years the reducing effect of pollution on the radiation absorption of the stand was characteristic of the whole growing season, with values of  $-0.9\%$  ( $P < 0.001$ ) in 2011 and  $-1.2\%$  ( $P < 0.000$ ) in 2012. The low percentage change may be misleading without data on the exact energy content. In 2011 the magnitude of the daily mean energy retained by the polluted maize plants was 118.9 kJ lower in 2011 and 191.1 kJ lower in 2012. This value is of approximately the same order of magnitude as the energy fixed by photosynthesis.

The higher albedo recorded for polluted plants irrespective of the year was indicative of lower energy uptake by the canopy, as confirmed by the plot of the daily changes in the net balance. In the wet year the sensible/latent heat ratio was approximately 50%:50%, and in this case no Cd effect could be detected. In the dry, hot years the lower evapotranspiration of the smaller leaf area of polluted plants led to a reduction in the latent heat of the canopy; this reduction was significant in both years, with values of 7% ( $P < 0.000$ ) in 2011 and 4.7% ( $P < 0.001$ ) in 2012. Due to the smaller leaf area, the polluted canopy was more open and thus warmed up to a greater extent, as confirmed by the higher value of sensible heat, which exhibited an annual mean increase of 10.7% ( $P < 0.05$ ) in 2011 and 7.8% ( $P < 0.000$ ) in 2012.

#### *Total dry matter (TDM) production and water use efficiency (WUE)*

To study the toxicity of heavy metals, the depression in dry matter production of crops is the most frequently used parameter. Changes in TDM revealed a considerable year effect between the wet year of 2010 and the dry year of 2011. In the non-polluted treatment TDM was 5.5% higher ( $P < 0.040$ ) in the ETR treatment (hot weather + supplementary water supplies) in 2011, while the water deficit led to a decrease of 7.4% in the rainfed plot ( $P < 0.025$ ). The negative effect of Cd treatment was observed in the non-irrigated plot, where the loss was doubled (14.6%,  $P < 0.018$ ), but no effect was detected in the ETR treatment.

In the wet year of 2010 irrigation did not result in any change in TDM. By contrast, a reduction of 28.6% ( $P < 0.000$ ) was recorded in the yield of unpolluted maize in 2011 and 31.1% ( $P < 0.002$ ) in 2012. Irrigation had a positive effect on the TDM of the Cd-polluted stand, as the yield of the irrigated plants was 39.7% ( $P < 0.000$ ) higher in 2011 and 40% ( $P < 0.000$ ) higher in 2012.

The WUE of non-polluted plants was only significantly reduced by irrigation in 2011 (29.6%;  $P < 0.000$ ), when the plants in the rainfed plot used less water for unit dry matter production than those in the irrigated treatment. In the case of Cd pollution, WUE tended

to decrease in response to irrigation, but the change was not significant. The effect of Cd pollution on the two studied parameters investigated in the present work proved to be less pronounced in the ETR treatment than on the non-irrigated plots. The decrease in the TDM of irrigated plants was 9.1 (ns), 18.2 ( $P < 0.002$ ) and 14.1% ( $P < 0.005$ ) in 2010, 2011 and 2012, respectively, while on the rainfed plots the reductions were greater, with values of 24% ( $P < 0.000$ ), 29.5% ( $P < 0.000$ ) and 22.2% ( $P < 0.038$ ). The differences induced by Cd in the WUE values, on the other hand, were greater for the non-irrigated plots, showing increases of 22.3% ( $P < 0.001$ ), 29.6% ( $P < 0.003$ ) and 22.3% ( $P < 0.022$ ) in 2010, 2011 and 2012, respectively, and indicating that the quantity of water consumed for the production of unit dry matter was this much higher on the Cd-polluted plots. The change in WUE caused by pollution in the ETR treatment was only significant in 2012.

Tests on the interaction effect for TDM (a) and WUE (b) showed highly significant impacts of watering level and Cd pollution when the data were combined across years. For TDM the results showed significant main effect for either the water level ( $P < 0.000$ ) or pollution ( $P < 0.000$ ). There was also a significant main effect for the season ( $P < 0.024$ ). The ANOVA table demonstrated the year by water confound effect significant ( $P < 0.001$ ). In the case of WUE the interaction impacts (year, water, and pollution) were even more pronounced. The interaction of year  $\times$  water ( $P < 0.000$ ) and water  $\times$  pollution ( $P < 0.000$ ) revealed highly significant.

#### *Efficiency ( $\epsilon$ ) for solar radiation*

Between 2010 and 2012 the mean annual efficiency (for solar radiation) of non-irrigated maize was 0.92% without pollution. This value deteriorated in the case of Cd pollution, leading to an average efficiency of 0.72% over the three years. The efficiency of irrigated plants was better than that of rainfed maize, with values of 1.16% without pollution and 1.01% for polluted plants, averaged over the three years.

Irrespective of the year, supplementary water supplies improved the radiation utilisation of maize. In the non-polluted treatments the mean annual radiation absorption of ETR-grown plants was 11.5% ( $P < 0.047$ ) better in 2010, 28% ( $P < 0.000$ ) better in 2011 and 31.9% ( $P < 0.002$ ) better in 2012. Cd pollution reduced the  $\epsilon$  values in all the treatments. In the wet year of 2010 the least reduction in maize efficiency was recorded on the polluted, non-irrigated plot: 23.8% ( $P < 0.019$ ). In the dry, hot years of 2011 and 2012 the radiation utilisation of Cd-treated plants was 40.5% ( $P < 0.000$ ) lower on the non-irrigated control plots.

The efficiency was influenced both by the year and by pollution. In 2010 a less pronounced reduction was observed at both water supply levels; the radiation use of Cd-treated plants was 22% ( $P < 0.000$ ) lower without irrigation and 9.3% (ns) lower in the case of irrigation. The changes were greater in the dry, hot years, with reductions of 30.6% ( $P < 0.000$ ) in 2011 and 34.6% ( $P < 0.038$ ) in 2012 on the rainfed plots and 17.8% ( $P < 0.002$ ) in 2011 and 14.3% ( $P < 0.005$ ) in 2012 for the ETR-grown plants after Cd pollution. Irrigation thus, somewhat improved the radiation absorption of polluted plants.



### *Cd content*

The average Cd content of the upper 0.8 m soil profile of the experimental site in Keszthely was  $46.1 \pm 40.44 \mu\text{g kg}^{-1}$  in spring, with values about 10–20  $\mu\text{g kg}^{-1}$  higher in the upper 0.4 m than in the lower part. Irrespective of contamination, the soil Cd content remained at the same level in September, confirming that the additional Cd in the polluted plots originated from the atmosphere and not from the soil.

The Cd content of young maize leaves (in May) was very similar to the soil pollution level ( $42.82 \pm 12.75 \mu\text{g kg}^{-1}$ ) during the three seasons investigated. At the beginning of each growing season, irrigation had no influence on the Cd content of the leaves. Irrespective of the growing season, the annual increment in leaf Cd content ranged from 456–475 and 671–689  $\mu\text{g kg}^{-1}$  in the non-irrigated and irrigated treatments, respectively.

Despite the differences, no significant year effect was found for the Cd accumulation in irrigated and non-irrigated maize. It is important to note that the leaf Cd contents recorded were in excess of the EU limits suggested for livestock feeding purposes (0.3  $\text{mg kg}^{-1}$ ).

Surprisingly, the Cd level in the kernels never reached that observed in the leaves, remaining below 61.5  $\mu\text{g kg}^{-1}$  throughout the experiment. Small amounts of Cd were translocated into grain despite extended time (whole growing season) of Cd pollution. This grain Cd content was close to the Cd level detected in the soil.

### **Discussion**

The lower LAI recorded for contaminated plants, regardless of the water supplies was in agreement with the findings of Drazkiewicz and Baszynski (2005) in maize. Similarly to our observation, Wójcik and Tukiendorf (2005) found more intense drying of older leaves in Cd treated maize. In hot, dry years the smaller LAI reduced the evapotranspiration of Cd-treated maize leading to a lower ratio of latent heat. The ability of Cd to reduce evapotranspiration was due to decreasing number and diameter of vessels and duct tubes as well as declining membrane permeability for water in Cd treated crops (Barceló and Poschenrieder 1990). At the same time, the ratio of sensible heat (used in warming processes) rose, as the smaller LAI resulted in greater energy penetration through the stand, leading to more intensive warming.

Continuous Cd pollution reduced the values of TDM, WUE and  $\epsilon$ . Negative changes of TDM were described earlier by Ekmekci et al. (2008) and Wójcik and Tukiendorf (1999, 2005). The values of WUE and  $\epsilon$  showed that the treated plants made poorer use of the available water and the solar radiation, respectively. Polluted maize used more water for unit dry matter production, and was able to fix less solar radiation than the non-polluted control plants.

The presence of Cd could not be detected in the grain in any of the years. In the stalks, however, concentrations double the EU limit values for fodder crops were recorded. Kirkham (2006) also found very low Cd concentration in maize grain (0.1  $\text{mg kg}^{-1}$ ) after soil contamination. At the same time, the Cd concentration of maize leaves was

10 mg kg<sup>-1</sup>. No reference was found to damage of maize by Cd coming from the atmosphere only.

Oppositely to earlier laboratory studies, Cd toxicity was investigated under field conditions. However, as long as previous works were mostly dealing with the impact of Cd of soil origin, we focused on the effect of airborne Cd deposited directly on maize. Low concentration atmospheric origin Cd induced a slight decrease in TDM, WUE and  $\epsilon$  of maize. Irrigated crops had improved performance in observed crop characteristics. The negative effect of pollution was less pronounced in the irrigated treatment, suggesting that the damage caused by Cd of atmospheric origin could be moderated by supplementary water supplies.

Our results do not indicate Cd accumulation in grain but they confirm its presence in stalks. Consequently, if the production of maize on contaminated areas is unavoidable, the crop should only be used as grain, as the Cd content of the stalks would be a danger to animal health in the case of constant exposure.

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