

# Accumulation of lead, zinc and cadmium in plant seeds growing in metalliferous habitats in Bulgaria

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Seeds from some plants were collected from metalliferous region in Bulgaria. In this area the concentrations of lead, cadmium and zinc in soil were 3500 mg kg<sup>-1</sup>, 280 mg kg<sup>-1</sup> and 30 mg kg<sup>-1</sup>, respectively. Plants accumulate heavy metal ions in their seeds selectively. It appears that peanut and corn seeds accumulate mainly Pb ( $5\cdot2-9\cdot6$  mg kg<sup>-1</sup>), pea seeds accumulate mainly Cd ( $1\cdot0-1\cdot2$  mg kg<sup>-1</sup>), and wheat seeds accumulate mainly Zn ( $59\cdot4-73\cdot2$  mg kg<sup>-1</sup>). This shows that the accumulation of heavy metals in the seeds of plants from some polluted regions may reach dangerous levels, especially if they are used as forage or food.

# **INTRODUCTION**

Soil pollution, caused by lead and other heavy metals, is an important problem in Bulgaria, as well as in some other industrialised countries. The plants growing in metalliferous habitats probably have the ability to inactivate the heavy metal via the binding of excess metal ions and/or by changing the chemical composition and physical organisation of their cell membranes. There are numerous investigations (see the reviews by Baker (1987) and Ernst *et al.* (1992)) on the influence of heavy metals on plant metabolism, but data for heavy metal accumulation in the reproductive organs of plants are very limited (Ernst, 1982; Searcy & Mulcahy, 1985*a*,*b*).

In this report we present some data on the accumulation of heavy metals in seeds of certain plants, growing in polluted areas around a metal smelter in Bulgaria.

# MATERIALS AND METHODS

#### Seed material

The seeds investigated were collected near the villages Kuklen and Dolni Voden in southern Bulgaria, a region showing very high contamination with heavy metals. The control samples of seeds were from ecologically pure regions, also in southern Bulgaria.

#### Lead analysis

A sample of the seeds was dried to constant weight at 65°C and metal concentrations were determined by atomic absorbtion spectrophotometry (Hsu & Locke, 1983), after digestion of the samples with concentrated nitric acid.

#### **Extraction of lipids**

The seeds were ground. The extraction of the lipids with a chloroform/methanol (2:1) mixture was performed according to Christie (1973).

#### **RESULTS AND DISCUSSION**

Table 1 lists the concentrations of lead, cadmium and zinc in seeds of some plants from a region showing high contamination with heavy metals (near the villages of Kuklen and Dolni Voden in southern Bulgaria). In this area, the concentrations of lead, cadmium and zinc in soil were 3500 mg kg<sup>-1</sup>, 280 mg kg<sup>-1</sup> and 30 mg kg<sup>-1</sup>, respectively. The heavy metal contents in these seeds were compared with those from control seeds from the same plants, collected from ecologically pure regions in southern Bulgaria.

It is evident that the heavy metal content in some seeds is very high.

In metal-tolerant plants, growing in metal-enriched nutrient solutions, metals are not excluded from the

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Seed and region	Treatment <sup>b</sup>	Metal content <sup><math>a</math></sup> (mg kg <sup>-1</sup> )		
		Pb	Zn	Cd
Pisum sativum L.				
Kuklen	В	$3.84 \pm 0.18$	$55.1 \pm 1.28$	$1.02 \pm 0.17$
Kuklen	Α	$4.41 \pm 0.29$	$56.0 \pm 1.65$	$1.13 \pm 0.23$
Control	В	$0.65 \pm 0.14$	$34.3 \pm 1.60$	$0.45 \pm 0.13$
Control	Α	$0.68 \pm 0.15$	$32.4 \pm 1.01$	$0.49 \pm 0.09$
Zea mays				
Kuklen	В	$5.17 \pm 0.21$	$21.6 \pm 1.15$	$0.19 \pm 0.15$
Kuklen	Α	$5.88 \pm 0.30$	$24.2 \pm 1.26$	$0.24 \pm 0.10$
Dolni Voden	В	$4.60 \pm 0.22$	$22.7 \pm 1.14$	$0.21 \pm 0.14$
Dolni Voden	Α	$5.28 \pm 0.31$	$26.9 \pm 1.98$	$0.19 \pm 0.14$
Control	В	$0.61 \pm 0.39$	$22.4 \pm 1.40$	$0.23 \pm 0.16$
Control	Α	$0.27 \pm 0.11$	$24.2 \pm 1.96$	$0.40 \pm 0.15$
Triticum aestivum				
Kuklen	В	$3.82 \pm 0.25$	$73.2 \pm 2.86$	$0.92 \pm 0.14$
Kuklen	Α	$4.02 \pm 0.25$	$73.5 \pm 2.58$	$0.83 \pm 0.11$
Dolni Voden	В	$2.92 \pm 0.33$	$59.4 \pm 1.98$	$1.93 \pm 0.07$
Dolni Voden	Α	$2.89 \pm 0.29$	$62.5 \pm 1.26$	$1.67 \pm 0.93$
Control	В	$2.54 \pm 0.43$	$19.1 \pm 1.07$	$0.38 \pm 0.08$
Control	Α	$2.62 \pm 0.30$	$24.4 \pm 1.00$	$0.54 \pm 0.11$
Arachis hypogaea L.				
Dolni Voden	В	$5.21 \pm 0.18$	$47.1 \pm 1.23$	$0.45 \pm 0.15$
Dolni Voden	Α	$9.65 \pm 0.18$	$80.0 \pm 1.58$	$1.04 \pm 0.16$
Control I	В	$0.81 \pm 0.22$	$35.8 \pm 1.29$	$0.28 \pm 0.13$
Control I	Α	$2.23 \pm 0.22$	$85.4 \pm 2.03$	$0.58 \pm 0.19$
Control II	В	$0.12 \pm 0.09$	$36.4 \pm 1.29$	$0.29 \pm 0.12$
Control II	Α	$0.42 \pm 0.13$	$70.5 \pm 0.54$	$0.40 \pm 0.07$

Table 1. Accumulation of lead, zinc and cadmium in seeds of some industrial plants from a heavily polluted region in Bulgaria

<sup>a</sup>Mean values for five determinations  $\pm$  SD.

<sup>b</sup>B, Before extraction with CHCl<sub>3</sub>/methanol (2:1, v/v). A, After extraction with CHCl<sub>3</sub>/methanol (2:1, v/v).

reproductive parts (Searcy & Mulcahy, 1985*a,b*), but the metal concentrations in the reproductive parts are lower than in vegetative ones (Ernst, 1982). There are reports that Cd and Zn concentrations are particularly high in the leaves of plants growing around metal smelters, while Cu and Pb are accumulated only in roots (Morishita & Boratynski, 1992).

Our investigations on the lipid changes in leaves, pericarp and seeds of pepper (*Capsicum annuum* L.) after incubation of the plants with lead ions (Stefanov *et al.*, 1995) showed that in the pepper seeds the concentration of lead is twice that in seeds of control plants.

It was found (see Table 1) that plant seeds selectively accumulate different heavy metal ions; for example, peanuts and corn in heavly polluted areas accumulate mainly Pb, pea accumulates mainly Cd, and wheat mainly Zn. This is accordance with the observation that different algal taxa accumulate different types of metals, but no direct correlation could be established between the metal uptake and algal division (Guven *et al.*, 1992).

Various hypotheses have been advanced to explain the mechanisms of plant resistance, but probably the metal tolerance in plants is due to evolution of specific metal-binding proteins similar to metallothioneins (Lolkema *et al.*, 1984). There are reports that about 90% of the heavy metal ions are located in the cell wall fraction of roots (Nishizono *et al.*, 1989; Ernst *et al.*, 1992) or with proteins in water macrophytes (Zolotukhina & Gavrilenko, 1990).

When the total lipids were extracted from the seeds by a chloroform/methanol (2:1) mixture, the lipids were practically free from heavy metals, and the heavy metal content in the extracted seeds (Table 1, treatment A) even increased, which is an indirect indication that the heavy metals are bonded to the non-lipid tissues of the investigated seeds. If lead was associated with the lipids, this means that the extraction procedure releases it from the lipid binding and these lipids can therefore be used in the food industry.

The results show that a part of the heavy metal ions can penetrate into the plant seeds. This can be of importance for their eventual application as forage or food.

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