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Cadmium and Micronutrient Accumulation in Yarrow

By

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With 4 Figures

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Summary

CHIZZOLA R. 2005. Cadmium and micronutrient accumulation in yarrow. – Phyton (Horn, Austria) 45 (2): 159 – 171, 4 figures. – English with German summary.

The accumulation and partitioning of the trace elements Cd, Cu, Mn and Zn was studied in yarrow plants (*Achillea millefolium agg., Asteraceae*) grown in a pot experiment in the green house with and without additions of Cd, Zn or a combination of both metals to the substrate. Two soils were tested and the the plants were harvested four times during one year. As the Cd concentrations in the plants reached up to 10 $mg.kg^{-1}$ when the Cd supply in the soil was 3 $mg.kg^{-1}$, the yarrow can be characterised as a plant accumulating easily this heavy metal.

The Cd accumulation in the plant was little influenced by a simultaneous addition of Zn: only in stems with flowers of the plants grown on a sandy soil a small reduction of the Cd contents could be observed. Zn had no influence on the Cd uptake. By the addition of Zn and Cd at once at the beginning of the experiment enhanced Cd and Zn levels were reached in the plants throughout all harvest times, indicating that Cd present in the soil will remain available to plants for a long time. Furthermore between the 3rd and 4th harvest, during an intensive growth period in early summer, a new mobilisation of Cd but not of Zn, Mn and Cu occurred from both soils, leading to enhanced Cd contents in the plants.

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The Cu and Mn contents in the plants were not influenced by the Cd and Zn additions to the soils.

Zusammenfassung

CHIZZOLA R. 2005. Cadmium- und Zinkaufnahme bei der Schafgarbe. – Phyton (Horn, Austria) 45 (2): 159 – 171, 4 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Aufnahme und Verteilung der Spurenelemente Cd, Cu, Mn und Zn in Schafgarbenpflanzen (*Achillea millefolium agg., Asteraceae*) wurde in einem Gefäßversuch im Glashaus untersucht, bei dem den zwei Versuchsböden Cd, Zn oder eine Kombination beider Metalle zugesetzt wurde. Die Pflanzen konnten vier Mal innerhalb eines Jahres beerntet werden. Da die Pflanzen bis zu 10 $mg.kg^{-1}$ Cd bei einem Zusatz von 3 $mg.kg^{-1}$ im Boden anreicherten, kann die Schafgarbe als Cd-Akkumulatorpflanze angesehen werden.

Die Cd-Aufnahme wurde nur wenig durch eine gleichzeitige Gabe von Zink beeinflußt: Nur in den Blütenstängeln der auf einem sandigen Boden gewachsenen Pflanzen konnte eine geringe Verringerung der Cd-Gehalte festgestellt werden. Aufgrund der einmaligen Zugabe von Cd und Zn zu Versuchsbeginn waren in den Pflanzen über die gesamte Versuchsdauer erhöhte Cd- und Zn-Gehalte zu verzeichenen, was darauf hinweist, dass diese Schwermetalle, einmal in den Boden gelangt, lange Zeit verfügbar bleiben. Außerdem trat zwischen der dritten und vierten Ernte während einer Periode intensiven Wachstums im Frühsommer eine neuerliche Mobilisation von Cd, nicht aber von Zn in beiden Böden auf, was zu erhöhten Cd-Werten in den Pflanzen führte.

Die Cu- und Mn-Gehalte der Pflanzen wurden durch die Cd- und Zn-Gaben zu den Böden nicht beeinflußt.

Introduction

Yarrow, *Achillea millefolium* agg. (*Asteraceae*) is an aggregate of variable (sub)species widespread in Central Europe occuring in various habitats. The plants contain essential oils and sesquiterpenoid bitter substances therefore they might be used as medicinal plants acting spasmolytic and antiphlogistic in the case of unspecific disorders of the gastrointestinal tract (JURENITSCH & al. 1992). On the other hand, yarrow has turned out to be a plant exhibiting sometimes enhanced Cd-levels in the herb (KABELITZ 1998). Contamination situations with heavy metals concern in most cases a simultaneous stress of more than a single element so that they may interact in their bioavailability and uptake by the plant. Zn has some chemical similarities with Cd, and interactions between these two elements in the plants have already been signalised where antagonistic effects prevail (MCLAUGHLIN & SINGH 1999).

The aim of this work was to study in yarrow plants the accumulation of Cd and the micronutrients Zn, Cu and Mn in dependence of easily available Cd and Zn added to the soils, the soil type, and the duration of the heavy metal stress. The focus will be on the repartition of these elements in the above ground plant parts and possible interactions between them. As yarrow is a perennial plant, the development of the micronutrient status and Cd stress can be followed over a longer time period.

Material and Methods

Plant Material

The experimental plants were raised from cuttings obtained from a single plant of *Achillea millefolium* L. (*Asteraceae*), variety ProA in order to dispose of genetically homogeneous material.

Pot Experiments

The cuttings were first grown in small containers with a standard peat substrate (ED63). As they reached about 5–10 cm in hight they were transplanted to the experimental pots.

Two different soils were used. Soil I was a sandy topsoil from Groß-Enzersdorf, Lower Austria, soil II a limy and humus garden soil. The content of trace elements under study of both soils is given in table 1. To assure a good porosity of the substrates, the soils were mixed with Granuperl®, an inert, glassy pumice-like material. Cd and Zn were added at the beginning during preparation of the substrates using a $CdCl_2$ solution and a $ZnSO_4$ solution. These additions gave a surplus of 3 $mg.kg^{-1}$ Cd and 200 $mg.kg^{-1}$ Zn in the substrate respectively. By setting an untreated control and providing to the plants both elements alone or in combination four different treatments were realised. Each treatment was replicated four times.

Table 1. Content of trace elements in the uncontaminated soils under study ($mg.kg^{-1}$ dry weight).

	Soil 1 sandy top soil	Soil 2 garden soil
Cd	0.07	0.12
Zn	30.00	58.60
Cu	5.40	9.10
Mn	279.00	387.00

The mixing of soil with Granuperl provoked an increase in soil pH. Therefore in the sandy soil pH-values between 7.3 and 8.0 and in the garden soil between 7.2 and 7.6 were recorded.

As yarrow is a perennial plant with intensive vegetative growth, harvests were done when the plants were sufficiently regrown. By this way four harvests were carried out on 25^{th} october, 12^{th} december, 17^{th} may and 19^{th} july.

Plant Analysis

About 0.3 g of dried powdered plant material was digested with 5 ml conc. HNO_3 suprapur in PTFE beakers in the microwave oven MLS-Ethos 900 (MLS, Leutkirch/Allgäu, Germany). Cd of samples low in Cd was measured by AAS with graphite tube furnance and Zeeman background correction on a Hitachi Z-8100 apparatus (Hitachi Ltd., Tokyo, Japan). Cu, Mn, Zn and samples with higher Cd contents were analysed by flame AAS with an acetylene-air flame with the same system. Analytical quality was checked through analysis of the reference material V-10 hay powder provided by IAEA, Vienna. For statistical analysis of data we used SPSS for Windows version 9.0.

Results

To study the effects of Cd and Zn on trace metal uptake over a longer period, the experimental factors Cd and Zn supply, soil types, and harvest time have been included in the experimental design. Table 2 gives an overview of the influences of these factors and their interactions on trace element accumulation in the plants according to a multifactorial ANOVA.

Table 2. Sources of variance in Cd and micronutrient content in Cd and Zn treated *Achillea millefolium*. According to multifactorial ANOVA with sign. of F.

	Ground leaves				Stems & Flowers			
	Cd	Zn	Cu	Mn	Cd	Zn	Cu	Mn
Main Effects:								
Cd-Addition	***	ns	ns	ns	***	ns	*	ns
Zn-Addition	ns	***	ns	ns	***	***	ns	ns
Soil	***	***	***	***	***	***	***	***
Harvest	***	***	***	***	***	**	**	***
Interactions:								
Soil – Harvest	***	***	***	***	***	ns	**	***
Cd-Addition – Soil	***	ns	ns	ns	***	***	**	ns
Cd-Addition – Harvest	***	ns	ns	ns	***	ns	ns	ns
Cd-Addition Soil – Soil Harvest.	***	ns	ns	ns	***	***	**	ns
Zn-Addition – Soil	*	***	ns	ns	***	***	**	ns
Zn-Addition – Harvest	*	***	ns	ns	**	*	ns	ns
Zn-Addition – Soil – Harvest	ns	***	ns	ns	**	ns	*	ns
Cd-Addition – Zn-Addition	ns	ns	ns	ns	***	ns	ns	*
Cd-Addition – Zn-Addition – Soil	*	ns	ns	ns	***	ns	ns	ns
Cd-Addition – Zn-Addition – Harvest	*	ns	ns	ns	**	ns	ns	ns
Cd-Addition – Zn-Addition – Soil – Harvest	ns	ns	ns	ns	**	ns	ns	ns

ns = not significant
* = 0.05–0.01
** = 0.01–0.001
*** = <0.001

Cadmium Accumulation

Plants not supplied with Cd had less than 0.2 mg.kg^{-1} Cd in their above ground organs, whereas with the addition of this heavy metal to the soil up to 10 mg.kg^{-1} could be recorded. At the first harvest Cd reached in ground leaves comparable levels on both soils, later on more Cd was present in the plants from the sandy soil. High Cd contents were at the first harvest in the ground leaves, to the second and third harvest these levels

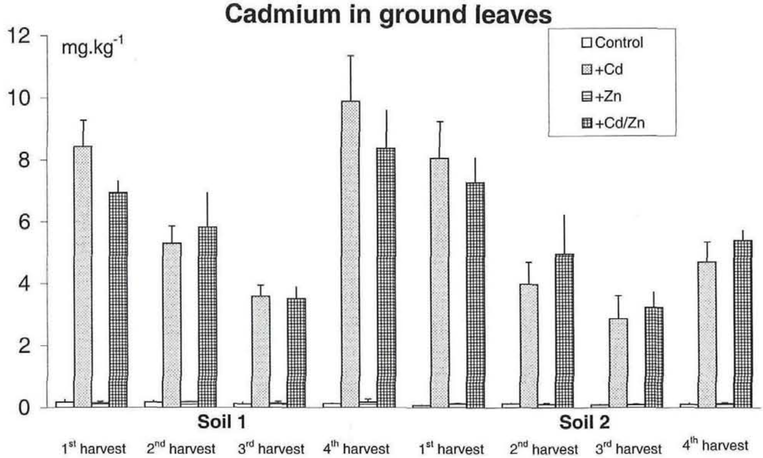

Fig. 1. Time course of the Cd accumulation in the ground leaves of yarrow grown on two different soils treated with Cd, Zn or a combination of both metals. Mean of four replications with standard deviation. Control: no Cd and Zn addition; +Cd: 3 mg.kg^{-1} additional Cd in the soils, +Zn: 200 mg.kg^{-1} additional Zn in the soils, +Cd/Zn: 3 mg.kg^{-1} additional Cd and 200 mg.kg^{-1} additional Zn in the soils.

decreased in these plant parts. However at the last harvest a new increase of the Cd content in all plant on both soils was recordable so that ground leaves and stems with flowers were again high in Cd (Figs. 1, 2).

The combined addition of Cd and Zn gave in most cases approximately the same Cd contents in the plant parts as in plants treated with Cd alone. Only in stems and flowers from plants grown in the sandy soil the simultaneous addition of Zn afforded a slight reduction in the Cd contents.

Zinc Accumulation

Zn accumulation was higher in the ground leaves than in stems and flowers and depended strongly upon Zn addition. The control plants had less than 85 and 70 mg.kg^{-1} Zn at any harvest time on both soils in the ground leaves and stems with flowers respectively. The Zn concentrations

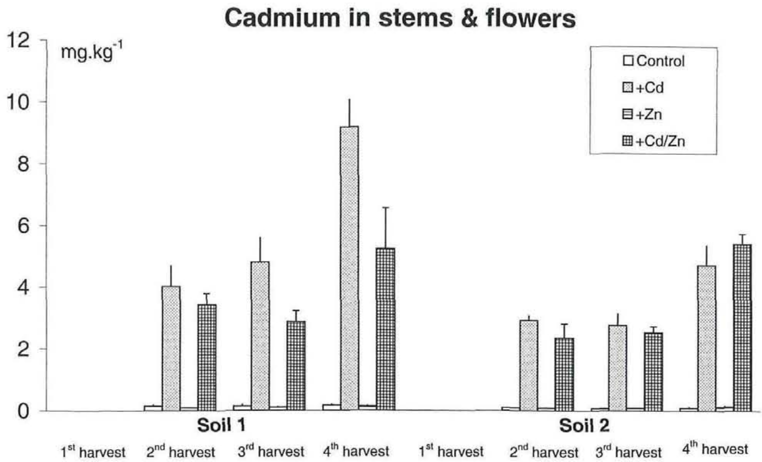

Fig. 2. Time course of the Cd accumulation in the stems with flowers of yarrow grown on two different soils treated with Cd, Zn or a combination of both metals. Further informations see legend fig. 1.

in the plants grown on the garden soil were often slightly higher than in those grown on the sandy soil.

The Zn addition to the soils afforded higher Zn concentrations in the plants throughout the experiment. Finally the Zn levels were around 180 and 220 mg.kg^{-1} in the ground leaves of the plants from the sandy soil and the garden soil respectively. In the stems the Zn values were somewhat lower reaching about 100 and 140 mg.kg^{-1} in the samples from the sandy soil and the garden soil, respectively.

Particularly high Zn levels were recorded in the ground leaves of the garden soil plants at the first harvest when additional Zn was present where values up to 450 mg.kg^{-1} were reached. A simultaneous Cd addition to the soils did not influence the Zn uptake into the plants (Figs. 3, 4).

Manganese and Copper Content

The micronutrients Cu and Mn present in the yarrow plants are presented in table 3 and 4. The levels of these elements were generally higher in plants from the sandy soil than from the garden soil and ground leaves showed higher levels in these elements than stems with the flowers. The highest Mn concentrations were found in the ground leaves at the first harvest and in the case of the sandy soil also at the last harvest where values up to 200 mg.kg^{-1} could be recorded. The lowest Mn concentrations around 20-30 mg.kg^{-1} were found in the stems with flowers from plants grown on the garden soil.

Copper ranged between 3.7 and 15.1 mg.kg^{-1} in the ground leaves and between 2.4 and 12.1 mg.kg^{-1} in the stems and flowers. In some instances

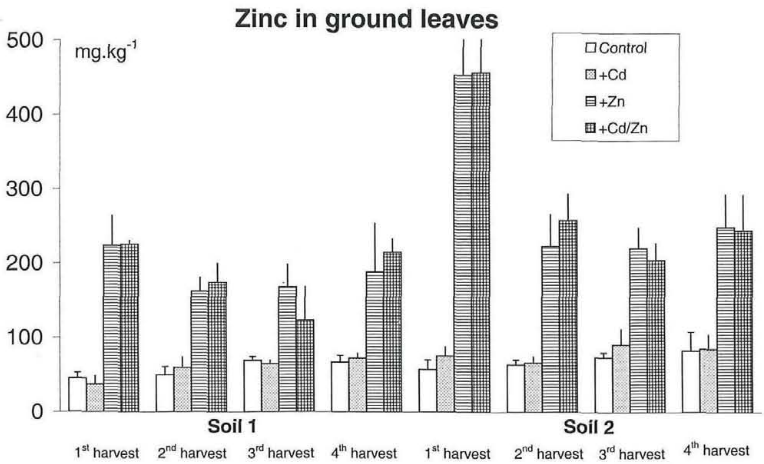

Fig. 3. Time course of the Zn accumulation in the ground leaves of yarrow grown on two different soils treated with Cd, Zn or a combination of both metals. Further informations see legend fig. 1.

the Cu contents varied from one harvest to another, but there was no consistent tendency.

The addition of Cd or Zn to the soil did not influence the Mn or Cu content of the plant parts.

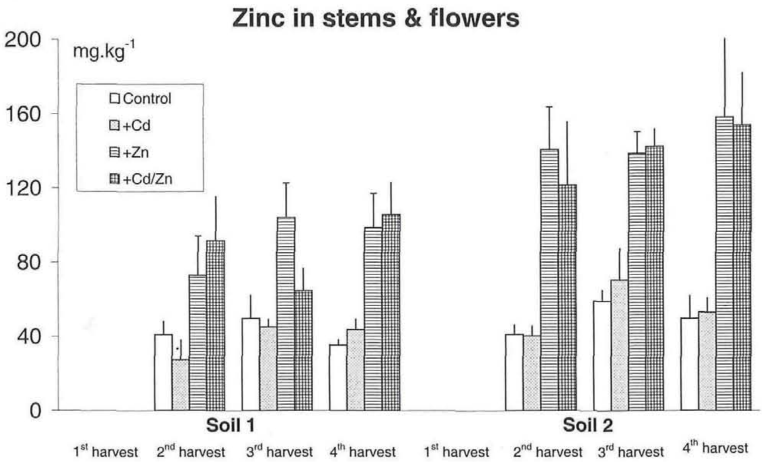

Fig. 4. Time course of the Zn accumulation in the stems with flowers of yarrow grown on two different soils treated with Cd, Zn or a combination of both metals. Further informations see legend fig. 1.

Table 3. Manganese content of yarrow plants treated with Cd and/or Zn. Values in mg.kg^{-1} dry weight. Means of four replications and standard deviation in brackets.

	Treatment	Ground leaves				Stems & flowers			
		Soil 1		Soil 2		Soil 1		Soil 2	
1st harvest	Cont	169	(34.9)	54.7	(12.9)				
	+ Cd	176	(12.7)	78.4	(6.4)				
	+ Zn	133	(10.9)	60.6	(8.3)				
	+ Cd/Zn	163	(11.0)	78.8	(7.4)				
2nd harvest	Cont	137	(31.3)	40.7	(5.1)	63.7	(15.7)	19.1	(1.6)
	+ Cd	118	(12.2)	38.7	(8.8)	44.9	(11.5)	26.1	(8.2)
	+ Zn	95.7	(22.5)	31.9	(3.8)	33.6	(8.7)	17.0	(2.9)
	+ Cd/Zn	133	(31.3)	47.4	(6.9)	58.8	(21.2)	25.8	(9.3)
3rd harvest	Cont	125	(31.1)	39.2	(3.6)	87.7	(29.2)	30.6	(6.7)
	+ Cd	134	(70.3)	33.6	(5.6)	77.8	(20.8)	25.1	(1.0)
	+ Zn	148	(38.0)	31.5	(5.8)	72.5	(8.1)	33.9	(12.0)
	+ Cd/Zn	108	(22.3)	34.6	(5.1)	66.5	(14.8)	29.1	(7.8)
4th harvest	Cont	168	(31.9)	45.9	(26.3)	121	(63.1)	22.0	(11.2)
	+ Cd	162	(82.3)	35.0	(8.0)	91.0	(36.3)	18.9	(3.5)
	+ Zn	199	(18.7)	37.9	(6.8)	84.4	(13.5)	21.5	(4.4)
	+ Cd/Zn	213	(51.1)	34.6	(4.7)	96.9	(5.6)	25.0	(5.6)

Soil 1: sandy top soil, soil 2: garden soil

Table 4. Copper content of yarrow plants treated with Cd and/or Zn. Values in mg.kg^{-1} dry weight. Means of four replications and standard deviation in brackets.

		Ground leaves				Stems & flowers			
		Soil 1		Soil 2		Soil 1		Soil 2	
1st harvest	Cont	9.9	(1.0)	5.0	(1.2)				
	+ Cd	10.1	(1.7)	5.3	(0.3)				
	+ Zn	8.6	(1.6)	4.6	(0.8)				
	+ Cd/Zn	7.5	(1.8)	5.0	(0.6)				
2nd harvest	Cont	15.1	(5.2)	5.3	(1.5)	12.1	(2.8)	4.1	(1.5)
	+ Cd	12.3	(1.2)	4.5	(0.8)	8.8	(2.7)	3.6	(0.4)
	+ Zn	10.6	(0.9)	5.0	(1.2)	7.0	(0.7)	3.7	(0.6)
	+ Cd/Zn	12.9	(3.6)	4.3	(0.9)	8.5	(3.1)	2.9	(0.4)
3rd harvest	Cont	7.6	(0.8)	4.8	(0.8)	6.7	(1.8)	3.8	(0.6)
	+ Cd	6.8	(0.7)	4.3	(0.9)	7.9	(2.2)	3.5	(0.3)
	+ Zn	5.9	(0.7)	4.5	(0.7)	6.0	(1.2)	4.5	(1.0)
	+ Cd/Zn	7.4	(0.5)	3.3	(0.7)	7.9	(0.7)	3.0	(0.2)
4th harvest	Cont	6.5	(0.7)	6.6	(2.2)	4.9	(0.9)	5.2	(1.7)
	+ Cd	8.9	(2.7)	4.2	(1.1)	7.5	(1.6)	2.7	(1.2)
	+ Zn	7.1	(1.1)	4.9	(1.2)	6.1	(0.8)	3.8	(0.9)
	+ Cd/Zn	8.0	(1.2)	3.7	(0.9)	7.3	(1.4)	2.4	(0.7)

Soil 1: sandy top soil, soil 2: garden soil

Soil Type

The soil type is an important factor for the availability of trace elements. In all plant parts and at any harvest time more Cu and Mn were found in plants growing on the sandy soil than in those from the garden soil. For Cd and Zn the differences were less clear. In some cases Cd was better available from the sandy soil. The mobilisation and uptake of Cd at the last harvest was higher for the plants on the sandy soil than on the garden soil. When grown without Zn addition plants from both soils had approximately comparable Zn contents at all harvest times. With the Zn addition plants from the garden soil accumulated more Zn than those from the sandy soil, an effect which was particularly distinct in at the first harvest in the ground leaves and also pronounced in stems and flowers.

Harvest Time

Yarrow is a perennial plant and could be harvested four times after transplanting into the experimental pots. In each case the plants were completely regrown.

The most conspicious elemental changes during the year were the high Cd concentrations with additional Cd present in the soils at the first harvest in the ground leaves. These Cd levels decreased afterwards and finally increased again between 3rd and 4th harvest. In the case of Zn the most noticeable influence of harvest time was in plants from the garden soil, where upon Zn addition to the soil the Zn level in the ground leaves were particularly high at the first harvest and the stabilised approximately at half of the original concentration.

Discussion

Above ground plant material grown on sites not contaminated with heavy metals contain usually less than 0.2 $mg.kg^{-1}$ Cd (ANONYMUS 1992, SCHILCHER 1994), and this concentration was not exceeded in this study in untreated plants. However yarrow has been signalised as a species able to take up easily this heavy metal. Therefore the guide value for Cd in yarrow herb has been set at 0.3 $mg.kg^{-1}$ (ANONYMUS 1992, SCHILCHER 1994). The WHO prescribes that the Cd content of the used medicinal plant preparation must not exceed 0.3 $mg.kg^{-1}$ (WHO 1998). In a survey where 109 yarrow herb samples have been analysed, the 90% percentile value for Cd was 0.49 $mg.kg^{-1}$ (KABETLITZ 1998), indicating that the proposed guide value of 0.3 $mg.kg^{-1}$ is often exceeded. In any case, when Cd was added to the substrate, the yarrow plants took up largely this element. No obvious differences in the growth of differently treated plants could be observed, the toxicity level of Cd was not reached with up to 10 $mg.kg^{-1}$ Cd in the above ground plant parts. In such cases Cd may be bound to metabolically inactive cell compartiments as cell walls (RAMOS & al. 2002).

A similar response could be observed in other Cd accumulation species as St. John's wort and chamomile (Chizzola 1998, Grejtovsky & Pirc 2000, Chizzola & Mitteregger 2005). The presence of Cd in above ground plant parts is strongly dependent to the extent this heavy metal is translocated from the roots to the shoots, a process where great differences have been reported between species and even between varieties of a given species (Florijn & van Beusichem 1993, Schneider & Marquard 1996, Schneider & al. 2002).

High Cd contents in yarrow herb were signalised in plants grown on acidic soils and plant Cd was found to decrease exponentially with increasing soil pH (Radanovic & al. 2001). Zn and Cd in plants decreased when the soil pH increased (Singh & al. 1995). As in the present study the pH of the substrate was high, partly an effect of the added Granuperl, the high availability of Cd and Zn was not only due to a pH effect.

Besides the total Cd content of the soil, the soil type plays also an important role in Cd availability for the plants (Puschenreiter & Horak 2000, Lehoczky & al. 1997, Wenzel & al. 1996). In Cd and Zn uptake experiments using comparable soils as in the present study, the influence of the soil type on Cd accumulation was more important in chamomile than in the yarrow plants reported here (Chizzola & Mitteregger 2005).

Zinc levels in plants range usually between 20 and 100 $mg.kg^{-1}$ (Kabata-Pendias & Pendias 1985). With the Zn addition to the soils, up to about 250 $mg.kg^{-1}$ Zn were recorded in the ground leaves and 100–150 $mg.kg^{-1}$ in the stems with the flowers. These levels were lower than the toxicity threshold as no growth depressions could be recorded. By the addition of Zn at once at the beginning, the Zn reserves of the soil were filled up and allowed enhanced Zn values in the plants till the last harvest.

The usual micronutrient range in plant leaves is 20–300 $mg.kg^{-1}$ for Mn and 5–30 $mg.kg^{-1}$ for Cu (Kabata-Pendias & Pendias 1985). The yarrow plants grown on the garden soil may therefore appear poor in Cu.

Cd and Zn were added once at the beginning of the experiment. It was to expect that the availability of these elements should be highest in the first weeks as they need a certain time to be fixed in the soil (Chaney & al. 1999, Pluquet & al. 1990). This might be true for Cd, where in the ground leaves high contents appear at the first harvest and also for Zn in the ground leaves of the plant grown on the garden soil.

The last period between 3^{rd} and 4^{th} harvest in early summer was a season of intensive growth. New root growth might have increased the availability of nutrients and trace elements. In this context it is important to notice that the mobilisation in the soil and the subsequent uptake by the yarrow plants was very marked in the case of Cd on both soil types but not in the case of the micronutrients Zn, Cu and Mn.

In long term experiments, the plant available heavy metal fraction in soils usually decreases with time, but a slight increase in plant availability of heavy metals could be found after the winter time at the beginning of new growth (HORAK & KAMEL 1990). SCHNEIDER & al. 2002 who investigated various varieties of the perennial St. John's wort (*Hypericum perforatum* L.) on four different locations, observed varying Cd contents in the herb during two consecutive years, whether the Cd contents in the plants increased or decreased from one year to the next was dependent upon variety and growing location. In any case, Cd present in the soil remains for a long time available to the plants. In a pot experiment with St. John's worth where Cd was also added at once at the beginning, the Cd content of the herb showed only a slight reduction after two years (CHIZZOLA 1998). In field experiments with perennial plants, herbs from later cuttings show often higher Cd contents than those from earlier ones (PLESCHER & al. 1995).

Although Zn and Cd might be considered as chemically similar elements, in the plant metabolism Zn is a micronutrient, whereas Cd is toxic and occurs ordinary in a very low concentration in the plant. Usually the Zn concentrations exceed more than 100 times the Cd levels. Interactions between Cd and Zn have been signalised for various plants and in most cases Zn reduces the uptake of Cd by roots (MCLAUGHLIN & SINGH 1999, KABATA-PENDIAS & PENDIAS 1985). At the level of the root cell membrane, Cd and Zn show in *Triticum* a competitive interaction, indicating a common transport system (HART & al. 2002). However in sunflower kernels, Zn and Cd are bound to different protein fractions (BETSCHE & al. 2001).

For the Cd accumulation and partitioning in the plant the Zn status seems to be important; a Zn deficiency situation favours the Cd accumulation by the plant (WELCH & NORVELL 1999, GRANT & BAILEY 1997). The Zn levels of the yarrow plants were certainly sufficient since they were within a normal range in the plants not supplied with additional Zn. The observation that additional Zn in the substrate failed to reduce the Cd uptake was also made in young poppy plants (*Papaver somniferum* L.) in short term experiments (CHIZZOLA 1999).

In the present study, the enhanced Cd supply also failed to influence the Zn status of the plants. Whether Cd in the soil may lead to a reduced Zn uptake in the plants depends largely upon the plant species and variety (GREJTOVSKY & PIRC 2000) and its susceptibility to Cd toxicity. For instance in sunflower (*Helianthus annuus* L.), even a supply of 10 mg.kg^{-1} Cd in the substrate did not influence the Zn content in the above ground organs (SIMON 1998).

As yarrow is not only a morphologically and chemically variable species (JURENITSCH & al. 1992, KASTNER & al. 1992) but also shows the tendency to build up higher Cd levels in the herb, it is essential for obtaining a valuable plant drug low in the hazardous Cd to establish field cultures rather than getting the plant raw material from collections in the wild. For this purpose the growing sites with low Cd availability should be chosen carefully and the tillage must afford an optimal supply with mineral and micronutrients.

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