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Seasonal variations of cadmium and zinc in *Arrhenatherum elatius*, a perennial grass species from highly contaminated soils

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Cd and Zn bioaccumulation varies seasonally in a perennial grass.

Abstract

There is interest in studying bioaccumulation in plants because they form the base of the food chain as well as their potential use in phytoextraction. From this viewpoint, our study deals with the seasonal variation, from January to July, of Cd and Zn bioaccumulation in three metallicolous populations of *Arrhenatherum elatius*, a perennial grass with a high biomass production. In heavily polluted soils, while Zn bioaccumulation is weak, *A. elatius* accumulates more Cd than reported gramineous plants, with concentration of up to 100 $\mu g\ g^{-1}$. Our results also showed seasonal variations of bioaccumulation, underlying the necessity for in situ studies to specify the date of sampling and also the phenology of the collected plant sample. In our experimental conditions, accumulation is lower in June, leading us to the hypothesis of restriction in heavy metals translocation from roots to aerial parts during seed production.

Keywords: *Arrhenatherum elatius*; Bioaccumulation; Heavy metals; Seasonal variation; Uptake

1. Introduction

Parts of Northern France were contaminated with heavy metals during the last century (François et al., 2004; Davranche et al., 2003; Sterckeman et al., 2000). This has caused significant accumulation of potentially toxic elements in the topsoils around industrial sites (Aruoja et al., 2004). Therefore, through uptake by plants, higher trophic levels in terrestrial ecosystems become exposed to trace metals from the contaminated soils. Heavy metal uptake and accumulation by higher 'plants depends on (1) the nature and the speciation of the metals, (2) soil factors and (3) the characteristics of the plant itself (see Bargagli, 1998; Brooks, 1998). Metal accumulation may depend on to seasonal variation. Some publications have reported the highest foliar levels during spring and the lowest during winter (Martin and Couphtrey, 1982; Wilkins, 1978), whereas others have indicated the highest metal contents (Cd, Cu, Ni, Pb, Sn, Zn) during autumn and relatively low levels during spring (Brekken and Steinnes, 2004;

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Kim and Fergusson, 1994). However, this point remains open and little studied, in spite of the fact that Djingova and Kuleff (1994) established that the developmental stage was the most explanatory factor of heavy metal accumulation in shoots. According to Tyler et al. (1984), this factor could be of great interest to give information about the exposure level. Kabata-Pendias et al. (1993) were able to show that it could help to explain both the translocation kinetics and the localisation of metal within the plants. This factor is also a prerequisite for the use of plants in phytoremediation defined as the process of utilising plants to absorb, accumulate, detoxify and/or render harmless, contaminants in the growth substrate through physical, chemical or biological process (Cunningham et al., 1995; Saxena et al., 1999).

Arrhenatherum elatius, a perennial grass, was selected as a study model for its ability to tolerate high concentrations of heavy metals in shoots. In Northern France, this species grows equally well in unpolluted sites (fallow land, meadow) and in polluted sites such as metalliferous areas near smelters and colliery slag heaps (Deram et al., 2000; Ducousso et al., 1990; Petit, 1980). Potentially, *A. elatius* could also be exploited for its ability to remove heavy metals from soil. It has been experimentally demonstrated that it could be effective for phytoremediation operations when growing either in cobalt/copper/nickel ore either in base-metal tailings rich in lead, with some sort of amendment (Deram et al., 2000). *Arrhenatherum elatius* could also be exploited to remove Cd in the vicinity of smelters in Northern France (Deram, 2003). In this area, the Cd concentration in soils exceeds the maximum guideline values recommended for risk assessment (M.A.T.E., 2000). In this paper, we report Cd and Zn concentrations at different stages of the growing season in *A. elatius* and soil.

Consequently, the aims of this study are (1) to assess, in situ, *A. elatius*'s Cd accumulation strategy during a growth cycle, (2) to study parameters influencing accumulation such as the development stage, pH or Zn concentration in soils, and (3) to optimise the sampling strategy for a better accuracy of analytical results.

2. Materials and methods

2.1. Study sites

The study was carried out in the North of France. Three sites were selected on account of their high levels of trace metal elements (Cd, Pb, Zn). They coincide with old slag heaps where residues resulting from the processing of Pb–Zn ores were accumulated. The Auby site (A) is located in the immediate vicinity of the UMICORE plant, which has been processing Zn ore since the end of the 19th century. In the 1970s, the industrial process of Unicore changed. At present, there are no atmospheric emissions from this point source. The Mortagne du Nord site (M) is situated near an old Zn smelter. The activity of this factory ceased in 1963. The Courcelles-les-Lens site (C) is located in the Pb–Zn Metaleurop Nord smelter, which was in operation from 1870 until early 2003. This factory is responsible for an important atmospheric emission of metals, particularly Cd, Pb and Zn, which amounted to 0.8, 18 and 26 t, respectively (data in 2001, DRIRE, 2002). On this site, there is still an enormous slag heap influencing atmospheric emissions, whereas slag deposits have been taken away on the two other sites. Consequently, within a radius of 4–5 km around these two smelters, contents of these metals in the local soils are largely above background values. The highest values of Cd, Pb and Zn may very locally reach 350, 8000 and 40,000 mg kg^{-1} dry weight of soil, respectively (Deram, 2003; Petit, 1980). Such concentrations disturbed the microflora activity and the decomposition of organic litter (Baath, 1989,) particularly in Auby (Grelle, 1998). Table 1 summarises the main physico-chemical soil characteristics of studied sites.

The studied sites are colonised by a particular type of vegetation called 'trace element grassland' which consists of metallophytes such as *Armeria maritima* Willd. subsp. *halleri* (Wallr.) Rothm (present at Auby and Mortagne) and *Arabidopsis halleri* (L.) O'Kane and Al-Shehbaz (present in the three sites).

Arrhenatherum elatius (L.) Beauv. ex J. and C. Presl, known as pseudometallophyte (i.e. not necessarily linked to the presence of metals in the soil), grows on

Table 1
Geometric mean ± standard deviation and median of the chemical and physical parameters of topsoils (0–30 cm) at the study sites

	n	OM	N	C/N	CEC	pH_{water}
		Gmean ± S.D.	Gmean ± S.D.	Gmean ± S.D.	Gmean ± S.D.	Gmean ± S.D.
Mortagne du Nord	7	104.3 ± 46	4.11 ± 0.66	20.53 ± 5.10	12.04 ± 2.41	6.2 ± 0.02
Auby	6	562.9 ± 31.3	9.35 ± 6.91	32.15 ± 15.45	49.70 ± 17.78	6.9 ± 0.1
Courcelles-les-Lens	1	59[b]	1.9[b]	18.05[b]	[a]	8.2[b]

OM, organic matter (g kg^{-1}); N, (Kjeldahl methods); C/N, carbon:nitrogen ratio; CEC, cation exchange capacity ($cmol^{+}$ kg^{-1}).
[a] Missing value.
[b] Data from Duval et al., 1997.

the three sites and develops an important biomass, up to 10 t ha^{-1}. Commonly known as 'tall oat grass' or 'French rye grass', this species use to be a fodder crop, thus determining the sample collection period.

2.2. Sample collection

From January to July 1996, soil-plant columns of *A. elatius* were collected monthly at Auby ($n = 6$), Mortagne du Nord ($n = 6$) and Courcelles-les-Lens ($n = 4$), randomly all over each site, taking into account a distance of at least 30 m between samples. The size of each sample is about 15 × 15 cm, 30 cm deep. Each column was separated into shoots cut 5 cm above the soil level, roots mainly developing in the first 30 cm of the soil and soil samples collected by the initial clearing of the roots. During this step, we check that each *A. elatius* sample corresponds to a single individual.

2.3. Sample analysis

2.3.1. Soil analysis

Soil samples were analysed in the Laboratory for Genetics and Evolution of Vegetal Populations (G.E.P.V.) at the University of Sciences and Technologies of Lille (U.S.T.L.), according to the techniques of the French Association for Normalisation (AFNOR, 1994). All of them were oven dried (40 °C) to constant weight and sieved through a 2-mm sieve as recommended by the NF X 31-101 (1992) norm. The different samples were treated in accordance with the standard method (NF X 31-151, 1993), that is, digested with a mixture of concentrated nitric and hydrochloric acids in a ratio of 1:3 (v/v) at 130 °C for 2 h. In addition to this analysis, the ammonium acetate/EDTA extractable fractions were also estimated (NF X 31-120, 1992). The concentrations of Cd and Zn were determined by flame atomic absorption spectrometry (Varian Spectr. AA-10).

The pH values (suspension in water) were measured according the standard method NF X 31-103 (1988).

2.3.2. Shoot samples

The growth stage of the aerial parts was noticed in the field according to the Freekes scale (Keller and Baggiolini, 1954; Ducousso, 1985). A simplified six-step scale takes place as follows: (1) tillering, (2) montaison, (3) epiaison, (4) flowering, (5) seed production and (6) fall of seed. Prior to extraction, *A. elatius* shoots were successively washed with tap water and distilled water and then oven dried to constant weight (40 °C for 48 h). The biomass was then assessed as the mean weight of an individual above ground part. The samples were digested with a hot mixture of $H_2SO_4/HNO_3/H_2O_2$ in a ratio of 1:3:3 (v/v/v) at 130 °C for 2 h (Hoenig's methods: Hoenig and Vanderstappen, 1978; Hoenig, 1981). Concentrations of Cd and Zn were determined by flame atomic absorption spectrometry (Varian Spectr. AA-10). Metal contents were given on a sample dried at 40 °C.

2.3.3. Root samples

Root samples were washed with tap water then distilled water, and then meticulously cleaned of adhering material and oven dried at 40 °C for 48 h. The root samples were submitted to chemical analysis with the same protocol as used for the shoot samples. Metal contents were given on a sample dried at 40 °C.

2.4. Data processing

A Principal Components Analysis (PCA) was carried out on the data set of both physico-chemical soil factors including heavy metal contents and Cd–Zn levels in *A. elatius* in order to elucidate possible relationships between soil characteristics and the metal accumulation ability of *A. elatius*. (Software STATISTICA, version 5.1, 1997).

3. Results

3.1. Seasonal variation of the biomass of A. elatius

The mean weight of an individual shoot were assessed for the three populations. All samples included, biomass measures were 9.6 ± 4.2 g at Mortagne du Nord, 8.8 ± 4.9 g at Courcelles-les-Lens and 8.6 ± 2.3 g at Auby.

As expected, biomass grew from spring until summer (Fig. 1). Summer corresponds to the seed maturation period. Consequently, the biomass did not grow any more, especially in the Auby population, which seemed to be phenologically advanced.

3.2. Zinc and cadmium concentrations in soils

Total and NH_4OAc–EDTA extractable Zn and Cd concentrations in soils are summarized in Tables 2 and 3.

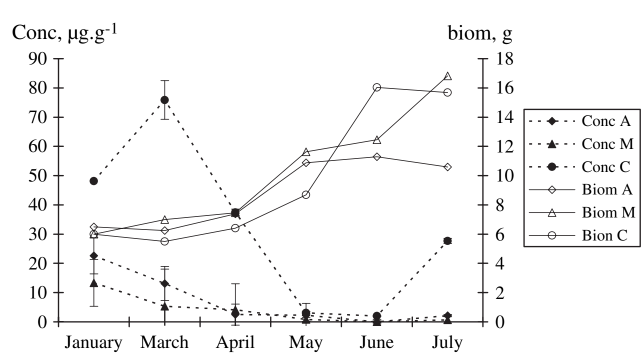

Fig. 1. Biomass (biom) and cadmium concentration in shoots of *Arrhenatherum elatius* (μg g^{-1}) versus months, for Auby (A), Courcelles-les-Lens (C) and Mortagne du Nord (M) sites.

Table 2
Total and mobile zinc concentrations in soils (geometric means ± standard deviation and median, μg g^{-1})

	n	Total zinc (μg g^{-1})		NH_4OAc–EDTA extractable zinc (μg g^{-1})		NH_4OAc–EDTA extractable fraction (%)
		Gmean	Median	Gmean	Median	
Mortagne du Nord	36	7744 ± 9666	3630	2581 ± 1956	2320	64
Auby	36	19928 ± 275	16850	5969 ± 4083	2957	17.55
Courcelles-les-Lens	24	5127 ± 5321	3933	1006 ± 790	824	15.49

For zinc, VDSS = 4500 μg $g^{-1}$and $VCI_{sensitive\ use}$ = 9000 μg g^{-1}.

For comparison, VDSS (French soil concentration values above which soil is considered a pollution source) and VCI (French soil concentration values above which pollution impacts are observable, M.A.T.E., 2002) are presented.

Total Zn and Cd concentrations varied significantly between sites ($p < 0.001$). The highest levels were observed at the Auby site with averages of 164 mg kg^{-1} Cd and 19,928 mg kg^{-1} Zn. A pronounced intra-site variation was observed, i.e. the highest range of Cd concentrations was noted at the Auby site (individual values ranging from 6 to 391 mg kg^{-1}).

The NH_4OAc extractable Zn and Cd concentrations varied between sites ($p < 0.001$) and sampling times ($p < 0.001$). For the three study sites there was a clear concentration increase during spring with highest values in May (Fig. 2).

The percentage of the extractable Cd levels versus total Cd concentrations (median values) varied significantly between sites ($p < 0.001$), the highest values being observed at Mortagne (91.7% for Cd and 64% for Zn).

3.3. Zinc and cadmium concentrations in shoots

Zinc and cadmium concentrations in shoots are summarized in Table 4. Regarding Zn, there was no significant difference between populations ($p < 0.404$). Considering Cd, Courcelles-les-Lens was the population presenting the highest levels ($p < 0.001$), even if this population did not grow on the most polluted site (Table 3). As for soils, an extremely wide range of concentrations was observed (up to 400% at the Auby site). Whatever the population, significant seasonal variations of Zn and Cd concentrations in shoots were observed ($p < 0.001$). Mortagne du Nord, Auby and Courcelles-les-Lens populations varied in the same way. Shoot concentrations were high at the end of winter, decreasing until late spring and finally on the increase again in July. Cadmium variations are presented in Fig. 1, with zinc following the same curve.

3.4. Zinc and cadmium concentrations in roots

The Zn and Cd concentrations in roots are presented in Table 4. Cadmium concentrations varied significantly between sites ($p < 0.001$); however, contrary to concentrations in shoots, no significant variation was observed between sampling times ($p < 0.738$). The Courcelles-les-Lens population presented the highest Cd level: 80.7 ± 22.9 mg kg^{-1} as compared to Auby (38.3 ± 13.6 mg kg^{-1}) and Mortagne du Nord (24.6 ± 15 mg kg^{-1}).

Zinc concentrations varied significantly between sites ($p < 0.005$). Mean concentrations were 3378 ± 957 mg kg^{-1} at Mortagne du Nord, 3723 ± 1033 mg kg^{-1} at Auby, with the lowest concentration (2790 ± 782 mg kg^{-1}) at Courcelles-les-Lens. Contrary to Cd, a significant variation was observed between sampling time due to a statistical difference between root concentrations in June and May at Courcelles-les-Lens ($p < 0.009$).

3.5. Translocation and bioaccumulation factors

For both Zn and Cd, translocation factor or shoot:root ratio were always inferior to 1 in our study. The highest values were noted in March at Courcelles-les-Lens. At all three sites, they decreased to very low in early summer (Table 5).

Table 3
Total and mobile cadmium concentrations in soils (geometric means ± standard deviation and median, μg g^{-1})

	n	Total cadmium (μg g^{-1})		NH_4OAc–EDTA extractable cadmium (μg g^{-1})		NH_4OAc–EDTA extractable fraction (%)
		Gmean	Median	Gmean	Median	
Mortagne du Nord	36	36.6 ± 43.8	21.8	33.6 ± 29.4	20	91.70
Auby	36	164.4 ± 155.2	118.6	111.2 ± 86.8	96.2	81.11
Courcelles-les-Lens	24	33.2 ± 17.7	30	33.7 ± 27.7	22	73.33

For cadmium, VDSS = 10 μg $g^{-1}$and $VCI_{sensitive\ use}$ = 20 μg g^{-1}.

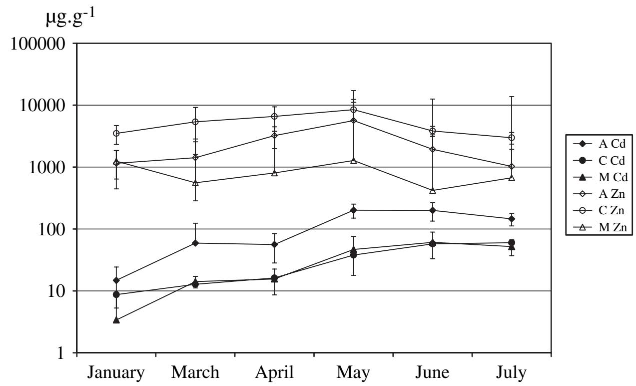

Fig. 2. Cadmium and zinc mobile concentration in soil (geometric means ± standard deviation, μg g^{-1}) versus months, for Auby (A), Courcelles-les-Lens (C) and Mortagne du Nord (M) sites.

The bioaccumulation factors (BAF), defined as a ratio of concentration in shoots to the extractable concentrations in the soils, were used for quantitative expression of accumulation and are summarized in Table 5.

The highest values were observed at Courcelles-les-Lens and the lowest at Auby. There was a significant variation between the sampling times ($p < 0.001$). Concentration ratios were superior to 1 at the beginning of the year, except for Zn ratio at Auby. Those ratios decreased notably until late spring and increased again in July.

4. Discussion

4.1. *Arrhenatherum elatius bioaccumulation*

Data in Table 4 show that the Zn contents in aerial parts of *A. elatius* are weak compared with those of Zn hyperaccumulator plants. The Zn content is about 1220 mg kg^{-1} (Gmean) at Auby (maximum value: 5000 mg kg^{-1}), whereas the typical mean Zn concentrations in hyperaccumulator species are >10,000 mg kg^{-1} (Brooks, 1998).

Table 4 also shows that some *A. elatius* individuals from Courcelles-les-Lens are able to accumulate up to 101.85 μg g^{-1} Cd in shoots. Such high concentrations may allow considering this plant as a Cd hyperaccumulator according to Brooks (1998) (here defined as >100 μg g^{-1} in dry plant material). Until now, only two plant species have been known as Cd hyperaccumulators, i.e. the Brassicaceous *Thlaspi caerulescens* (Brooks, 1987, 1998) and *Arabidopsis halleri* (Bert et al., 2000). However, in the present study, because of non-constant hyperaccumulation of Cd in the three sites, and because the shoot:root ratio is always <1, *A. elatius* would not appear to be a true Cd hyperaccumulator. This is in accordance with the results from other studies demonstrating that monocotyledonous plants take up less Cd than dicotyledonous ones (Matthews and Thornton, 1982). Moreover, it must be emphasized that washing leaves in deionised water clearly removes a very large percentage of the initial metal burden. The superficial deposition removed by washing comprises soluble and insoluble fractions. The more soluble metals, Zn and Cd, are more likely to achieve foliar penetration in solution-form through the guard-cells and cuticle (Little, 1973). This way of contamination is probably low on the sites of Auby and Mortagne du Nord in the absence of industrial activity. It could be different on the Courcelles-les-Lens site due to important atmospheric emissions and fall-out. Therefore, we can assume that this trait has little influence on Zn and Cd concentrations in shoots. Indeed, (1) plant concentrations are weak during summer and (2) the Courcelles-les-Lens population does not have the highest Zn accumulation capacity. As a result, such concentrations in shoots, also observed in the laboratory (Deram, 2003), make it possible to consider *A. elatius* as one of the best gramineous Cd accumulators in comparison with numerous data in the literature (Brekken and Steinnes, 2004; Ebbs and Kochian, 1997).

The roots provide the highest plant metal concentrations. This is in agreement with works on Cd in many gramineous plants such as *Zea mays* and *Lolium perenne* (Kovacs et al., 1993), *Triticum vulgare* and *Hordeum vulgare* (Bargagli, 1998), or *Triticum aestivum* (Nan et al., 2002).

The bioaccumulation factor bears no relation to the amount of Cd present in the soils from which the plants were collected. Similar results were obtained by Sappin-Didier et al. (2004), who showed that the concentration of Cd in soils is not an explicative factor for wheat grain concentration.

Table 4
Zinc and cadmium concentrations in *A. elatius* shoots and roots (geometric means ± standard deviation, median and range, μg g^{-1} dry weight)

	n		Cadmium			Zinc		
			GMean	Median	Range	GMean	Median	Range
Mortagne du Nord	36	Shoots	5.2 ± 6	2.97	0.01–25	948 ± 677	689	104–2575
		Roots	24.6 ± 15	23	9.6–37.2	3378 ± 957	3454	1903–5400
Auby	36	Shoots	9.4 ± 9.9	3.4	0.01–40	1221 ± 1022	995	125–5000
		Roots	38.3 ± 13.6	35.7	18.8–71	3723 ± 1033	3570	2412–6590
Courcelles-les-Lens	24	Shoots	37.1 ± 31.4	39.5	1.05–102	1171 ± 897	888	139–3149
		Roots	80.7 ± 22.9	72.8	52.5–120	2790 ± 782	2736	1634–4716

Table 5
Zinc and cadmium translocation (TF) and bioaccumulation factors (BAF) for *A. elatius* populations versus months

			January	March	April	May	June	July
Cd	TF	Mortagne du Nord	0.466	0.268	0.266	0.038	0.012	0.044
		Auby	0.665	0.319	0.079	0.055	0.001	0.062
		Courcelles-les-Lens	0.638	0.886	0.39	0.033	0.078	0.34
	BAF	Mortagne du Nord	4.24	0.37	0.27	0.02	0.01	0.03
		Auby	1.52	0.2	0.06	0.01	0.01	0.01
		Courcelles-les-Lens	3.42	5.93	2.3	0.08	0.03	0.46
Zn	TF	Mortagne du Nord	0.48	0.34	0.21	0.12	0.09	0.26
		Auby	0.54	0.5	0.19	0.13	0.07	0.22
		Courcelles-les-Lens	0.73	0.74	0.37	0.08	0.02	0.23
	BAF	Mortagne du Nord	1.19	0.9	0.34	0.07	0.11	0.91
		Auby	0.5	0.42	0.12	0.06	0.05	0.26
		Courcelles-les-Lens	1.37	1.3	1.2	0.21	0.05	0.92

Although originating from metalliferous sites, all three populations take up Cd and Zn differently. For both metals, the highest values of BAF were observed for the populations of the Courcelles-les-Lens site where extractable concentrations in soils were threefold lower than at the Auby site. In other words, the most exposed population is the lowest accumulator. Once more, a cuticle pathway could be considered. Nevertheless, two other parameters are not submitted to atmospheric deposition but directly linked to soil contamination, i.e. the ratio $NH_4OAc-EDTA$ extractable fraction in soil:root concentration and the Translocation Factor (TF or shoot:root ratio). The values measured for Courcelles-les-Lens populations suggest its good accumulation capacity. Consequently, a clear variability of bioaccumulation was observed between sites. In addition to site differences, metal concentrations in plants changed within a single population, as illustrated by a wide range of root and shoot contents.

Hence, our results suggest that an ecotype variation could explain the differences observed in metal uptake. Ducousso et al. (1990) point out that a phenological shift among *A. elatius* populations originating from sites differently contaminated by heavy metals could induce a genetic isolation of populations. Since *A. elatius* is a pseudometallophyte species, we have compared metal concentrations in plants between metallicolous and non-metallicolous populations (Deram et al., submitted). First results show a very different accumulation capacity between and within metallicolous (developing an excluder strategy) and non-metallicolous populations (developing an accumulator strategy). Similar results were observed in metallicolous and non-metallicolous population of *Thlaspi caeruslescens* (Meerts and Van Isacker, 1997).

4.2. Seasonal variations

It is clear from this study that Zn and Cd concentrations in shoots vary between sampling times with a clear concentration reduction during spring. Similar results were also observed in crops (Jastrow and Koeppe, 1980), in pasture herbage (Matthews and Thornton, 1982; Pissaloux-Piquet and Pauly, 2003) and in various grazing plants (Brekken and Steinnes, 2004). The recorded concentration decrease during spring is generally referred to as a dilution effect due to growth increase (whereby changes in the amount of plant biomass bring about corresponding changes in plant metal contents). The dilution effect was first pointed out by Rains (1971) in *Avena sativa* and confirmed in different plant species by other authors (see Brekken and Steinnes, 2004, for review; Jiang et al., 2004). In the present investigation, when taking into account the plant biomass during the sampling times, the recorded values were contrary to the Zn and Cd concentrations in shoots (Fig. 1). There was a clear increase in biomass from spring to summer at the Courcelles-les-Lens and Mortagne sites while at the Auby site, the weaker observed values may have been due at least in part to an early phenology. For all three populations, the plant biomass was weak in winter. The observed seasonal variations of metal concentrations in shoots could be put down to a dilution effect without an increase in translocation. This means that an increase in biomass is not necessarily linked to a bioaccumulation increase, as this could have been suggested for hyper-accumulator species, which generally have a weak biomass (Salt et al., 1995). Besides, contrary to general opinion the bioaccumulation level does not peak when the biomass is at its highest. If we try to estimate the metal quantity in shoot Q (where Q = biomass × shoot concentration), Q is significantly different, considering the sampling time for Zn ($p < 0.001$) and Cd ($p < 0.036$). Whatever the population, Q is the highest in March and the lowest in June, the period of seed production. From the literature it is well known that the accumulation in plant is higher in leaves than stems and seed, the hypothesis being a protection of the offspring (Djingova and Kuleff, 1994).

The dilution effect does not seem to be compensated for by a translocation increase. In fact, during the growth cycle the translocation factor decreases, indicating a restricted transportation of toxic metals from roots to shoot biomass (stems and weeds). Such 'barriers' could explain the low Cd concentrations in shoots during the maturation of seeds. This phenomenon is often referred to as a strategy for heavy metal tolerance (Brekken and Steinnes, 2004; Dahmani-Muller et al., 2000; Ernst, 1990; Kabata-Pendias et al., 1993).

4.3. Bioaccumulation-influencing factors

The extractable concentrations in soil vary greatly during the sampling times. This seems to confirm previous studies that consider the extractable fraction to have the most spatial and temporal variability (Bargagli, 1998).

In order to relate the different physico-chemical parameters of the soils to the bioaccumulation capacity of *A. elatius*, a Principal Component Analysis was applied. Due to their chemical and physical similarities, the co-existence of Zn and Cd in the environment and their possible interactions in biological systems are of particular interest (Bert et al., 2000; Robinson, 1997; Ross, 1994). Thus, Zn soil contents have also been included in the statistical processing.

Fig. 3 shows the plot of FCMA ordination. The eigenvalues of axes 1, 2 and 3 were 41.1%, 21.6% and 14.8%, respectively. High scores on Axis 1 corresponded to soil metal concentrations. Significant positive correlations were obtained between Cd and Zn concentrations in soils and between soil pH and Zn levels in plant shoots. As seen from the correlation matrix, the most significant factors influencing Cd accumulation in aerial shoots were Zn levels in shoots ($r = 0.53$) and Cd contents in roots ($r = 0.56$).

These results were completed with a step-by-step regression (without Zn effect), indicating that most explicative parameters were respectively phenology, sampling time, Cd concentration in roots and extractable Cd concentration in soil. Nevertheless, all parameters included in this statistical analysis only constitute a 7.5% explicative model, proving that many more parameters influence Cd bioaccumulation in *A. elatius* shoots such as, for example, Cd speciation (Anderson et al., 2001; Baize, 1997), agronomical properties of soils (François et al., 2004) or soil and plant microflora (Languereau-Leman, 1999; Leyval et al., 1997). In our study, analyses concerning soil parameters are missing. This prevents a full explanation of results, particularly on the extractable fraction in soil. Mainly due to the high buffering power of NH_4OAc–EDTA, variations of heavy metal concentration in soil are not easy to explain from a physico-chemical point of view. That is why we are looking for more biological parameters. This paper presents a study examining seasonal variations of Zn and Cd accumulation, while additional works addressing the influence of fungal colonisation of roots on seasonal variations will be presented elsewhere.

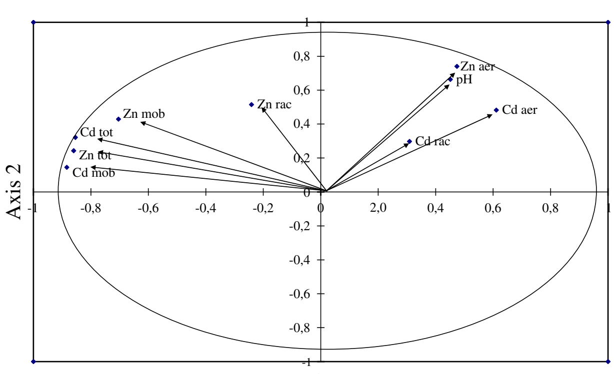

Fig. 3. Factorial map showing axes 1 and 2 in the principal components analysis (PCA). Zn aer and Cd aer, element concentration in aerial part; Zn rac and Cd rac, element concentration in roots; Zn tot and Cd tot, total concentration of element in soil; Zn mob and Cd mob, mobile concentration in soil.

5. Conclusion

It was useful to demonstrate the high Cd accumulation capacity of *A. elatius* since (1) it is a good fodder plant and (2) it is an excellent candidate plant for phytoremediation due to an important biomass (15 t ha^{-1} $year^{-1}$).

Our results have shown seasonal variations of bioaccumulation that imply taking into account the sampling time and repeating analyses during the growth cycle. Without such precautions, the results could provide an inappropriate illustration of the bioaccumulation capacity.

Our results have also demonstrated that the metal extraction does not relate to the biomass level. In other words, the biomass increase is not necessarily linked to the bioaccumulation increase, which is important to bear in mind for phytoremediation technologies. So, with phytoextraction as a goal, the best harvesting month is March. On the other hand, considering plants as a food source for animals, the best harvesting period is summer, as is generally the case.

Furthermore, low shoot Cd concentrations were observed during seed maturation. In field experiments, the phenology seems to be the most discriminant parameter for bioaccumulation capacity. The co-presence of Zn also influences Cd accumulation.

A clear variability of Cd bioaccumulation was observed within metallicolous populations. It is not unlikely that such variability will be observed between metallicolous and non-metallicolous populations.

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