UNIVERSITEIT
GENT

FACULTEIT LANDBOUWKUNDIGE EN
TOEGEPASTE BIOLOGISCHE WETENSCHAPPEN

Academiejaar 2000-2001

SPATIAL VARIABILITY OF CADMIUM IN THE CROWN OF *SALIX FRAGILIS* L. AND ITS IMPLICATIONS FOR LEAF SAMPLING

RUIMTELIJKE VARIABILITEIT VAN CADMIUM IN DE KROON VAN *SALIX FRAGILIS* L. EN DE IMPLICATIES VOOR BLADBEMONSTERING

door

ir. SEBASTIAAN LUYSSAERT

Thesis submitted in fulfilment of the requirements for the degree of
Doctor (Ph.D.) in Applied Biological Sciences
Section Land- and Forest Management

Proefschrift voorgedragen tot het bekomen van de graad van
Doctor in de Toegepaste Biologische Wetenschappen
Optie Land- en Bosbeheer

op gezag van
Rector: **Prof. dr. ir. J. WILLEMS**

Decaan:

Promotor:
Prof. dr. ir. N. LUST

Prof. dr. ir. O. VAN CLEEMPUT **Prof. dr. ir. M. VAN MEIRVENNE**

Table of contents

1 Introduction

Sampling and analysing leaves is described by UN/ECE-EC (1998) and Duquesnay et al. (2000) as an essential tool for monitoring the health state of forests because the nutritional state of a tree is often indicative for processes at the ecosystem level. Where inadequate nutrient supply may be the cause of low tree vitality. High concentrations of certain elements in the leaves may be the effect of intoxication or of high immission levels. Except immission, unfavourable chemical conditions in the rooting zone of the soil may also lead to imbalances in nutrient supply and as a consequence to an imbalanced nutrition of trees (UN/ECE-EC, 1998).

Because the use of plant analysis in forestry stems from the aid of foliar analysis as a diagnostic tool in plantation fertilising, in most research stress was laid on nutrients. Forest fertilisation is generally seen as a mean to increase growth but Leaf (1973) points up other goals, such as biotic and abiotic disease control, insect control, increased seed production in seed orchards, foliage colour and retention in Christmas trees, increased sugar yield, increased resin yields, improved recreational site development, range and wildlife browse values, and alteration of wood properties. So in forestry, plant analysis was optimised to diagnose the need for fertilisation in achieving these goals. Subsequently, from the early thirties (Mitchell, 1934, 1936, 1939) until the late eighties (Erdmann et al., 1988; Lemoine et al., 1990; McLennan, 1990), with the emphasis in the sixties and seventies, research activities focussed on the distribution of N, P, K in tree crowns and their sampling location. Variability of nutrients within a tree, a season and between seasons was studied but the early workers could not test the representativity of the data set because of the lack of powerful computers and adequate statistical tools. Only one researcher was found to sample more than 4 heights within the crown (White, 1954). All others, even in the late seventies and eighties based their conclusions on sampling three to four heights, often with only one sample per height level. Based on these research findings it was concluded that most elements showed a modest variation coefficient. Marshall & Jahraus (1987) give variation coefficients of 10 to 17 % for N, P, K and 40 to 75 % for Fe, Zn and Cu. Many other workers confirm these ranges. Based on these limited data sets the amount of (sub)samples needed to take a representative sample was calculated and a general sampling location for nutrients was recommended by Leaf

(1973) and van den Driessche (1974). Leaf samples should be taken from the upper third of the crown from undamaged leaves grown in full light conditions. Because the lack of methodological publications since the early nineties it seemed that the research community considered this topic sufficiently studied.

In the mean time, the field of application of leaf sampling extended from solely nutrients to nutrients and pollutants. From the early eighties till the mid nineties the knowledge of the influence of heavy metals on forest ecosystems increased (Bergkvist et al., 1989; Ross, 1994; Scharenberg & Ebeling, 1996). Stimulated by this work the idea of using leaves as an indicator of environmental pollution established for examples see Landolt et al., 1989; Chang et al., 1992; Dmuchowski & Bytnerowicz, 1995; Helmisaari et al., 1995; Tremolada et al., 1996 among other publications. By that time the interest in leaf sampling methodology attained its lowest level. A few authors e.g. Hall et al. (1975) and Hall (1977), Duncan et al. (1995) and Kim & Fergusson (1994) studied the seasonal variation of Cd, Cu, Pb and Zn seen as pollutants. But no research was found studying the spatial distribution of non essential-elements within a tree crown. Consequently, no conclusions recommending a sampling strategy when using leaf analysis as a pollution indicator were found.

Many authors studying pollution problems in forests and the monitoring program of the United Nations and the European Commission on the assessment and monitoring of air pollution effects on forests (UN/ECE-EC, 1998) have adopted the sampling procedure for nutrients proposed in the early seventies by Leaf (1973) and van den Driessche (1974). This procedure samples fully developed leaves from the upper third of the crown of a dominant or co-dominant tree before the very beginning of senescence (UN/ECE-EC, 1998). But studies can have different objectives i.e. monitoring deficiencies, monitoring toxicity or scientific research. Each objective requires different information and possibly calls for a different sampling strategy. Due to the variation in element concentration in the tree crown there is an opportunity to develop different sampling strategies. Errors made by using an unsuitable sampling strategy definitely compromise all the efforts undertaken to assure quality during the following steps in the laboratory (Markert, 1995; Wagner, 1995).

Upon consideration of the above this study aims to describe the implications for leaf sampling emerging from a description of the spatial variability of the Cd concentration in the crown of *Salix fragilis* L.. The central hypothesis was introduced that the generally used and recommended technique for foliar analysis yields a biased estimate of the average Cd concentration and of the risk that the foliage is polluted. The hypothesis was tested in five steps :

- reviewing the literature to define the generally usedsampling strategy and to estimate the background and elevated Cd concentrations in leaves (chapter 2);
- studying the heterogeneity of single leaves to determine the sample size requirements of a representative elementary sampling unit (chapter 4);
- describing and studying the variability of the Cd concentration in the crown of a *Salix fragilis* based on the representative elementary sampling unit (chapter 5);
- developing a conceptual approach to optimise and verify a sampling procedure based on the spatial description of the Cd concentration in the crown of a willow (chapter 6) and
- testing the representativiness of the Cd distribution and concentration of the single tree within the stand as a justification for the use of a single tree in a methodological study (chapter 7).

2 Review of leaf sampling and Cd concentrations in leaves

2.1 Abstract

Leafs are sensitive biomonitors in forest health and pollution studies : the effect of pollution on the composition of the leaves is the reason for existence of leaf sampling as a diagnostic tool in pollution studies. Less used but also suitable as a biomonitor are the other tree organs : bark, bud, root and wood. Despite studies have different objectives, no differences were found between the common leaf sampling strategies to study the effects from soil pollution, the effects from atmospheric pollution or the effects of forest treatment. Irrespective of the aims of the study dominant or co-dominant trees are sampled in late summer but before leaf yellowing and senescence. Leaves are taken from a single height location, generally the upper third of the crown. Samples consist of leaves from an unspecified number of aspects from the sampling height. Within a stand leaf samples are taken from at least five trees. Among the problems that remain for using leaf sampling in pollution-effect studies prevails the lack of knowledge about the relevant sampling height to account for the vertical variation in element concentration. Due to the vertical element distribution the sampling height can be used to account for the objectives of the study. It was concluded that the conventional sampling strategy to describe the vertical variation i.e. sampling the lower, middle and upper crown, does not satisfy. The background and elevated Cd concentrations were, despite the difference in sampling procedure and tree species, surprisingly consistent : background concentrations are below 1.12 mg Cd.kg^{-1} dry weight, elevated concentration range between 1.12 and 60.0 mg Cd.kg^{-1} dry weight.

2.2 Introduction

The concept of analysing plants as an indication of the available chemical elements was, according to Smith (1962) put forward a century earlier by Weinhold (1862) and Hellriegel (1869). 'If the necessary foundations are first laid by field trials, crop analysis will provide a satisfactory basis for the determination of both the relative and absolute proportions of plant nutrients present and available in soil, and can suitably give the supplementary information needed to evaluate the results of soil analysis'. Generally speaking this hypothesis still appears to be valid. When used in pollution studies, often both leaf and soil analyses are needed and should be treated as complementary information (Ross, 1994; Pilgrim and Hughes, 1994, Kabata-Pendias & Pendias, 1992). Although the idea of using plants as a diagnostic tool in pollution studies is not new (Little & Martin, 1974; Kabata-Pendias & Pendias, 1992) little information is found concerning the in field application of tree sampling in forestry. One field manual in the framework of the 'European Programme on Assessment and Monitoring of Air Pollution Effects on Forests' (UN/ECE-EC, 1998) was found suggesting a sampling strategy, a sampling density, acceptable methods for chemical analysis and data processing for studies concerning heavy metals in tree foliar. In contrast with the lack of methodological information of foliar analyses as a diagnostic tool in pollution studies, is the extended literature of over 100 years of experience with foliar analyses as a diagnostic tool in fertilisation experiments.

Literature dealing with leaf analysis in forest growth and treatment, solution culture and pollution studies was reviewed to define and discuss the generally used sampling strategy for studying the effects of soil pollution on trees. The review focussed on (i) evaluation of leaf, bark, bud, root and wood sampling in plant analyses, (ii) the sampling strategy to account for the spatial and temporal variation in element concentrations in leaves, and (iii) the background and elevated Cd concentrations in deciduous trees.

2.3 Material and methods

2.3.1 Article selection

Mainly, articles published in international peer reviewed journals were used. If a non-reviewed publication was frequently cited in these journals, this publication was considered as a key publication. Key publications were also handled in this literature review. Since the study object is a deciduous tree i.e. *Salix fragilis* L. the review focuses on research concerning deciduous trees. Findings of conifer trees are cited if the reference could be considered as a turning point in research or when similar research is not yet available for deciduous species.

2.3.2 Article handling

Articles were organised in a single table. For the articles given in the rows of the table, the columns of the table contained methodological information and research results. The columns contained the following parameters :

- Author(s);
- year of publication;
- tree species;
- type of research i.e. soil or atmospheric pollution study, forest growth or treatment study and solution culture study;
- analysed plant organ i.e. leaf, bud, bark, wood and roots;
- social position of the sampled tree(s) i.e. dominant and co-dominant, intermediate and suppressed;
- number of heights sampled in the crown i.e. 1, 2, 3, or more;
- bulked sample i.e. whole crown, 4 orientations bulked, unspecified number of locations bulked, samples from different crowns bulked;
- sample preparation i.e. washed or unwashed leaves;
- sampling time i.e. spring, summer, autumn or whole season;
- sampling years i.e. one or more;
- analysed elements i.e. N, K, P, K, Mg, Cd, Cu, Pb and Zn;
- element distribution pattern i.e. I, II, III, IV and V (see Table 2.3);

- seasonal variation pattern i.e. I, II, III, IV, V, VI and VII (see Table 2.4 and Table 2.1);
- number of samples needed to estimate the element concentration;
- precision of the estimation i.e. 5% or 10%;
- background Cd concentration and
- elevated Cd concentration.

If for example an article discussed the differences in results between washed and unwashed leaves, two rows were entered in the table. One for the results of the washed leaves and one for the results of the unwashed leaves. The same methodology was used for different tree species, different social classes, etc. This way 220 citations concerning plant analysis in studies with a different research background were analysed (Table 2.1).

Table 2.1. Overview of 220 available citations concerning leaf analysis in deciduous trees; arranged by research background and decade of the study

Decade	Background of research				Total
	Soil pollution	Atmospheric pollution (*)	Forest growth and treatment	Solution culture	
1940			2		2
1950			4		4
1960			13		13
1970	9	16	26		51
1980	3	26	24	1	54
1990	33	45	6	7	91
2000		5			5
Total	45	92	75	8	220

(*) When the source of the atmospheric pollution was still present at the time of research the results were classified as atmospheric pollution. Otherwise as soil pollution.

2.3.3 Article processing

The table was used to compile the sub-tables 2.1 to 2.16. All tables except Table 2.3 Table 2.4 Table 2.1 Table 2.14 Table 2.15 and Table 2.16 contains the number of citations. When a number of citations was given in a table or in the text a footnote at the end of the chapter was used to give the full references. References were included in Table 2.3 Table 2.4 Table 2.1 Table 2.14 Table 2.15 and Table 2.16. In these tables no footnotes were used.

2.4 Evaluation of leaf, bark, bud, root and wood sampling in plant analyses

Trees are complex organisms composed of several organs, each organ having its pros and cons as an analytical target for describing the chemical composition of the entire tree. At the start of a study the question is put which organ has to be sampled. The best evaluation of the chemical element status of a tree is made if : (i) the sampled organ shows a maximum difference in analytical data between the wanted and unwanted conditions, (ii) a minimum difference in analytical data between trees grown under the same conditions is found, and (iii) a representative element concentration is found for the aim of the study. Still, no such study suggesting a tree organ to sample, based on the above made considerations, was found. Therefore, the dominance of leaves in tree sampling seems to be explained by convenience rather than scientific deliberation.

2.4.1 Leaf

Tree foliage, in being a sensitive indicator for those elements that directly affect photosynthesis (Smith, 1962), is a suitable organ to sample. Moreover Innes (1993), UN/ECE-EC (1998) and Dusquesnay et al. (2000) describe it as an essential tool for monitoring the health state of forests because the nutritional state of a tree is often indicative of processes at the ecosystem level. In addition, the foliage is a convenient portion of the tree as a whole, easy to sample and easy to allocate to an individual tree. Ferm & Markkola (1985) compared the nutritional variation in leaves, twig and bud and concluded that on the whole, leaf analysis was more practical. The ease of sample collection emerged to be decisive in the acceptance of the technique, as outlined by Leaf (1973) and van den Burg (1985).

Since 1970, 94 citations were found using leaf analysis as an indicator of environmental pollution. Because leaves are the aerial organs of trees, leaves are the obvious choice to sample the effects of atmospheric pollution on trees (62 citations)[1]. But leaf analysis is also frequently used to study the effects of soil pollution on trees (32 citations)[2]. Nevertheless, there are a number of alternatives. At least four other organs have been suggested as potentially useful for diagnostic purpose: bark, bud, roots and wood.

2.4.2 Bark

No references were found using bark analysis to characterise soil pollution. Zinke (1968) and Dell et al. (1987) applied bark analysis to determination the nutrient status of trees. With the rise of pollution studies tree bark was used as a tool to indicate and characterise atmospheric deposition of airborne organic and inorganic pollutants. Bark of deciduous trees (5 citations)[3] and bark of conifer trees (6 citations)[4] were found advantageous as an organ to sample. The advantages are summarised. Bark is inert after formation, shows rather good accumulation properties and it is easy to handle as sampling material. Bark was found to be less sensitive as a biomonitor than mosses and lichens but could nevertheless be used for monitoring atmospheric pollution because it is available almost everywhere and samples can be taken very easily (Lippo et al., 1995). As a disadvantage the missing information of the effects of stemflow on trace element levels in bark is given. Deposited elements may be washed off resulting in a decrease in the bark mineral content, but, on the other hand, stemflow may transport large quantities of elements from extensive surface of leaves and branches down to lower parts of the stem (Wolterbeek & Bode, 1995).

2.4.3 Bud

Buds were found to be very useful as diagnostics tool (Marion et al., 1968; Leaf, 1970; McColl, 1980; Ferm & Markkola, 1985) but are not yet used to describe the effects of soil or atmospheric pollution on trees. The small portion of buds to the entire tree, the short period buds are available, and the fact that many buds are needed to collect a sample big enough for chemical analyses makes buds since the beginning a rarely used organ (4 citations)[5] for the chemical analysis of tree compartments.

2.4.4 Root

Roots were identified as the main pathway of soil pollution to enter trees (Trüby, 1995). Physical, chemical and biological processes influence the element availability to roots (Ernst, 1996). Then not well known processes determine the uptake of heavy metals by roots. From the roots metals are distributed all over the tree. Many researchers have noted the marked accumulation of soilborne heavy metals in the root system (Jarvis et al., 1976; Garcia Sánchez et al., 1999) resulting in only small amounts transported to the shoots (Sanità di Toppi & Gabbrielli, 1999). If accumulation occurs in roots, roots instead of leaves, should be analysed to reveal the heavy metal and nutrient status of those elements mainly located in the roots (Sieghardt, 1988; Raitio, 1993). Because roots are the tree organ that interacts with the soil roots are the most obvious organ to sample when studying the effects of soil pollution on trees.

Root analysis was exclusively used in soil pollution studies. In literature 45 citations were found tackling soil pollution. Six of these citations[6] referred to the use root analysis. Practical considerations are limiting the frequent use of roots as a diagnostic tool in tree analyses. A lot of labour is needed to separate the roots from the soil particles to collect a sample large enough to provide the required amount for chemical analysis and moreover, it is difficult to allocate a sample to an individual tree. Brundin et al. (1987) have shown that there are seasonal variations in root concentrations, and that these can sometimes be greater than those in the leaves.

2.4.5 Wood

Because trees are long living organisms, wood was expected to reveal the history of local and regional pollution. Wood samples are obtained from basal parts of tree trunks and samples of different annual growth are analysed for their element concentrations. These analytical data are then plotted on a time axis of growth ring age. The observed changes in element concentrations in the wood have been used to infer variations in environmental pollution levels in the past. Since Ward et al. (1974)

and Lepp (1975) suggested the concept, 22 citations applying wood analysis in deciduous trees were found. Six citations[7] deal with soil pollution, sixteen citations[8] with atmospheric pollution. On a regional scale the effects of urbanisation, industrialisation and escalation of the use of leaded petrol were reflected by the metal concentrations in wood (Stewart et al. 1991; Latimer et al., 1996; Jonsson et al., 1997). On a local scale the chemical composition of wood was of use to reconstruct the effects of drainage and the operational characteristics of nearby industrial plants (Stewart et al. 1991; Eklund, 1995; Watmough and Hutchinson, 1996).

The validity of the results has never been shown beyond doubt (Hagemeyer et al., 1992). The distribution pattern of Zn, Cd, Ni and Pb within the xylem of *Fagus sylvatica* L. is, due to radial transport, not stable (Hagemeyer et al., 1992; Hagemeyer et al., 1994). Hagemeyer & Schäfer (1995) found unstable metal concentrations during the annual growth cycle in *Fagus sylvatica* L.. It was concluded that dendroanalysis, in at least *Fagus sylvatica* L., *Picea abies* (L.) Karst, *Picea rubens* Sarg. and *Quercus robur* L. was not a reliable source of information to study former pollution (Hagemeyer et al., 1992; Zayed et al., 1992; Hagemeyer, 1995; Hagemeyer & Lohrie, 1995; Hagemeyer & Schäfer, 1995). However Watmough (1999) and Nabais et al. (1999) believe that if careful sampling strategies are used and suitable tree species chosen, the chemical analysis of tree-rings can provide information concerning historical changes in soil and atmospheric trace metal levels.

2.4.6 Conclusion

The reliability of wood as tissue to study former pollution was not shown beyond doubt. No fundamental reasons could be found to consider bark, bud and root analysis as less suitable in pollution studies. The opposite seems to be true. Fundamental reasons i.e. the fact that roots interact with the soil, were found to favour root analysis in soil pollution studies. When studying the effects of soil pollution on trees, leaf analysis (32 citations) is by far the most applied technique. Combined with the rare application of bark (0 citations), bud (0 citations), wood (6 citations) and root analysis (6 citations) this review focussed on leaf analysis as a tool in describing the element status of trees grown on polluted lands.

2.5 Spatial and temporal variation of element concentrations in tree leaves

Several factors are known to cause variation in the element concentration in leaves. A sampling strategy should consider the effects of these factors. The variation due to the crown class, the vertical crown dimension i.e. sampling height, the horizontal crown dimension i.e. orientation, the number of trees sampled, the sampling time and the year of sampling among with *other* factors and method of expression are discussed.

2.5.1 Variation in element concentration by crown class

2.5.1.1 Effects of crown class

Generally five social positions or crown classes are distinguished, namely pre-dominant, dominant, co-dominant, intermediate and suppressed trees. Social position or crown class is opposed to the common or average situation in the stand. This makes the use of crown class or social position interesting. However, crown class or social position is a less objective parameter as it is partly measured and partly determined by the opinion of the observer.

Saarinen (1996) found that the leaf concentrations of the *Picea abies* (L.) Karst. understorey did not possess predictive worth in regard to the *Pinus sylvestris* L. overstorey. The element concentration in the dominant trees in the overstorey were found to show less variation than overtopped trees (Lavender, 1970). Morrison (1985) concluded that although weakly significant ($p = 0.1$) concentrations of most elements (N, P, Fe, K, and S) in *Betula alleghaniensis* Britton and *Acer saccharum* Marsh. were higher in foliage of intermediate than in foliage of co-dominant trees. Ovington & Madgwick (1959 b) found higher element concentrations in foliar of younger trees. These observations were confirmed for suppressed conifers (Leyton & Armson, 1955; Leyton, 1956; Wright and Will, 1958; Ovington & Madgwick, 1959 a). As exceptions to these general trends Leyton & Armson (1955) and Leyton (1956) reported lower N, P, K, and Ca concentrations in the foliage of smaller trees and Lowry & Avard (1968) found that in *Picea mariana* Mill. N, P, and Ca concentrations were unaffected by social position of the tree. Leyton & Armson (1955) remarked that in some cases the correlation between element concentration and social position possibly could be explained by the absence of suppression by shade. The variation in tree height might then be ascribed to heterogeneity in soil conditions. In that case the element status is a reflection of the soil conditions, not of the social position, due to light competition.

2.5.1.2 Sampling strategy by crown class

Foliar element concentrations differ between species, crown, diameter or height classes. The population should therefore be stratified in sub-populations based on diameter class (Ovington & Madgwick, 1959b; Kozlov et al., 1995), biomass (Prégent & Camiré, 1985), crown class (Morisson, 1985) or tree height (Koricheva & Haukioja, 1995). By stratifying the stand in sub-populations, each of these sub-populations becomes more homogeneous. In this way the variation within each sub-population decreases.

The objectives of the study should determine which crown, diameter or height classes have to be sampled and analysed. The beginning of foliar analysis in forestry was linked to diagnosing the need of fertiliser application. Dominant and co-dominant trees are due to their major economic importance better representatives for the site than overtopped trees (Leaf, 1973; van den Driessche, 1974). The stand was therefore stratified according to the crown class and generally sampling was restricted to the dominant and co-dominant trees in the stand (Table 2.2). From the number of citations in Table 2.2 it was concluded that stand stratification by crown class is common practise irrespective of the background of the study. Researcher studying the effects of soil pollution and atmospheric depositions on trees as well as those studying forest growth and treatment limited leaf sampling to dominant and co-dominant trees.

Table 2.2. Overview of 30 available citations concerning the sampled crown class in deciduous trees; arranged by research background and decade of study

Background of research	Crown class	Decade				Total
		<1960	1970	1980	>1990	
Soil pollution	(Co)dominant		5			5 [9]
	Intermediate					
	Suppressed					
Atmospheric pollution	(Co)dominant				1	1 [10]
	Intermediate					
	Suppressed					
Forest growth and treatment	(Co)dominant	2	7	5	3	17 [11]
	Intermediate			2		2 [12]
	Suppressed		5			5 [13]

Leaves were found to dominate as major trapping site for particles (Little and Wiffen, 1977). Efficiency of deposition of particles to plant surfaces was found to be proportional to wind speed (Little, 1977; Sehmel, 1980; Hosker & Lindberg, 1982), tree species (Little, 1973) and stand density (Hager & Kazda, 1985). As a consequence the social class and stand density in the vicinity of a tree could be of influence on the trapping efficiency and thus the element concentration of leaves. The relation between social position and metal concentrations in the leaves could be of importance in risk assessment and food web studies. It is conceivable that animals feed exclusively on suppressed trees or that the higher concentration of pollutants compensate for the lower biomass of the suppressed class. When all crown classes are representatively sampled the proportion of each class to the total burden of pollution could be determined. Despite these considerations, no literature dealing with the metal uptake in relation to the social position of trees was found.

2.5.2 Variation in element concentration by the vertical crown dimension

2.5.2.1 Effects of the vertical crown dimension

The element distribution in the crown of a tree was found to show a considerable variation. Chapman (1966) encountered ranges in the foliar concentration of N: mean ± 25 %; P mean ± 50 %; K mean ± 40 %; Ca mean ± 60 %; Mg mean ± 35%; Zn mean ± 40 % and Mn mean ± 75%. Ellis (1975) reported variations of 30-40% for Ca and Mg and 20% for K and Zn in *Acer saccharum* Marsh., *Fraxinus americana* L., and *Prunus serotina* Ehrh.. Some authors concluded that the variation was not random but followed a trend. These trends could be classified in distinct distribution patterns (van den Driessche, 1974). An overview of the distribution patterns found in nutrient (N, K, P, K, Mg, Na and Mn) concentrations is given in Table 2.3. Guha and Mitchell (1966) linked the vertical variation in the crowns with the characteristics of the element. They stated that the vertical variation in element concentration is only important for immobile micro-elements since they did not observe significant differences in macro-elements with the height. But, this explanation did not hold very long. Ever since the beginning many similar or opposite research findings have been reported. Potassium is given as an example, Table 2.3 shows that pattern I, II, III, IV and V were found for this element. The distribution pattern in the same tree species e.g. for *Acer saccharum* Marsh and *Quercus alba* L. was found to differ.

Distribution patterns of heavy metals within the crown are given in Table 2.4. The studies referred to in the table considered the metals as essential micro-elements. No literature was found dealing with the distribution pattern of heavy metals (Cd, Cu, Pb, Zn) in the crown of a tree affected by pollution.

2.5.2.2 Sampling strategy by vertical crown dimension

Table 2.5 reveals two strategies to account for the vertical distribution pattern of nutrients and heavy metals in tree crowns. The first strategy accounts for the

Table 2.3. Patterns of element distributions within tree crowns; (**I**) uniform, (**II**) increasing to the top of the crown, (**III**) decreasing to the top of the crown, (**IV**) maximum in the middle of the crown, and (**V**) minimum in the middle of the crown.

Author	Tree species	N	P	K	Ca	Mg	Na	Mn	Other
Wallihan, 1944	*Acer saccharum* Marsh.	I	I	I	I				
McVickar, 1949	*Quercus alba* L.	I	I	I	I	I			
Ovington & Madgwick, 1958	*Quercus robur* L.		II	II			II		
	Castanea sativa Mill.		II	II			IV		
Guha & Mitchell, 1966	*Acer pseudoplatanus* L.		I	I	I	III	I		III Fe, III Al
	Aesculus hippocastanum L.		III	III	I	III	I		III Fe, III Al
	Fagus sylvatica L.		I	I	III	III	I		III Fe, III Al
Le Tacon & Totain, 1973	*Fagus sylvatica* L.	III	I	III	III	III		III	III Fe
	Fagus sylvatica L.	III	III	IV	III	III		III	III Fe
Auchmoody & Hammack, 1975	*Quercus rubra* L.	I	I	III	III	III			
	Q. alba L.	I	I	III	III	III			
	Q. coccinea Muenchh.	I	I	III	III	III			
Ellis, 1975	*Acer saccharum* Marsh.	I	I	III	III	III		IV	
	Fraxinus americana L.	I	I	V	I	I		I	
	Prunus serotina Ehrh.	II	I	V	I	I		III	
Verry & Timmons, 1976	*Populus tremuloides* Michx.	I	I	III	III	I	I	I	I B, I Fe, I Al
Ponder et al., 1979	*Juglans nigra* L.	I	I	I	I	I			
Insley et al., 1981 b	*Tilia spp*.	I	I	I	III	III			
Heilman 1985	*Populus trichocarpa* Torr. & Gray clone #1	II							
	Populus trichocarpa Torr. & Gray clone #2	III							
Morrison, 1985	*Acer saccharum* Marsh.	I	I	III	III	III	III		III Fe, I S
	Betula alleghaniensis Britton	I	I	I	III	I	I		I Fe, I S

Table 2.3. Continued

Author	Tree species	N	P	K	Ca	Mg	Na	Mn	Other
Bernier & Brazeau, 1988	*Acer saccharum* Marsh.		III						
Erdmann et al., 1988	*Acer rubrum* L.	III	III	III	III	I			
McLennan, 1990	*Populus trichocarpa* Torr. & Gray ex Hook.	II	II	II	III	II		III	II S, II SO_4, III Fe

Table 2.4. Patterns of heavy metal distributions within tree crowns; (**I**), (**II**), (**III**), (**IV**), and (**V**) according to Table 2.3; Seasonal changes in metal concentration in leaves; (**I**) continuous decrease in leaf concentration, (**II**) continuous increase, (**III**) gradual decrease followed by a period with a constant concentration, (**IV**) very little variation without seasonal trend, (**V**) strong variation without seasonal trend, and (**VI**) strong variation with seasonal trend both increasing and decreasing, (†) samples taken in the frame of a nutrient study (‡) reference to study found, changes not described

		Distribution pattern				Seasonal changes			
Author	Tree species	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
† Guha & Mitchell, 1966	*Acer pseudoplatanus* L.		I	III	I		III	II	III
	Aesculus hippocastanum L.		I	III	I		III	II	III
	Fagus sylvatica L.		I	III	I		III	II	III
† Ellis, 1975	*Acer saccharum* Marsh.				III				
	Fraxinus americana L.				I				
	Prunus serotina Ehrh.				I				
Hall et al., 1975	*Acer pseudoplatanus* L.						‡	‡	‡
† Verry & Timmons, 1976	*Populus tremuloides* Michx.		I		I		VI		VI
Hall, 1977	*Ligustrum vulgare* L.						‡	‡	‡
	Crataegus monogyna Jacq.						‡	‡	‡
† Lea et al. 1979 b	*Acer saccharum* Marsh.						I		V
	Betula alleghaniensis Britton						I		II
† Morrison, 1985	*Acer saccharum* Marsh.		I		III				
	Betula alleghaniensis Britton		I		I				
Capelli et al., 1989	*Populus nigra* L. cv. Italica							II/VI	
† McLennan, 1990	*Populus trichocarpa* Torr. & Gray ex Hook.		II		III				
Kim & Fergusson, 1994	*Aesculus hippocastanum* L.					IV	I	II	I

Table 2.4. Continued

		Distribution pattern				Seasonal changes			
Author	Tree species	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
Riddell-Black, 1994	*Salix viminalis* L.					II	II	II	II
	Salix triandra L.					II	I	II	II
	Salix dasyclados Wimm.					II	II	II	II
Duncan et al. 1995	*Betula pendula* Roth.								II
Alfani et al. 1996a	*Quercus ilex* L.						VI	VI	
Dinelli & Lombini, 1996	*Salix* spp.						I		I
	Populus nigra L.						I		I

distribution pattern by exclusion. Leaf samples are taken from a single specified height. This approach is common practice when studying the effects of soil and atmospheric pollution on trees (Table 2.5). The second strategy accounts for the distribution pattern by inclusion. Leaf samples are taken from at least two, most often three and sometimes more than three specified heights. This allows the description of the variation in element concentration along the tree height. Both strategies contain methodological problems.

Table 2.5. Overview of 90 available citations concerning the number of sampling heights in deciduous trees; arranged by research background and decade of study

Background of research	# of sampling heights	Decade				Total
		<1960	1970	1980	>1990	
Soil pollution	1				1	1[14]
	2					
	3					
	>3					
Atmospheric pollution	1		7		16	23[15]
	2					
	3					
	>3					
Forest growth and treatment	1	2	9	4	3	18[16]
	2		1			1[17]
	3	7	3	4	2	16[18]
	> 3	2	3	2		7[19]

When the first strategy is followed, a single height from where the samples will be taken needs to be specified. For the elements K and Mg the concentrations in the lower third of the crown varied the least (Erdmann et al., 1988). By this mean the lower part would be the most favourable part of the crown to sample. Only sampling the lower canopy to reduce the variance will lead to an overestimation of K, Ca and Mg (Erdmann et al., 1988). So, a different part of the crown had to be sampled. Restricting sampling to branches in the mid-crown diminished the coefficients of variation for Ca, Mg and K and thus improved the efficiency of sampling (Ellis, 1975). Morrison (1985) and Erdmann et al., (1988) showed that the lowest variation in element concentration occurred in the mid-crown. Morrison (1985) found that the foliage in the upper crown somewhat differed from foliage in the mid- and lower-crown. Inasmuch as upper-crown foliage is more variable in its composition and is difficult to reach, it was recommended to restrict sampling to mid- or lower-crown positions rather than the upper-crown position. Also Erdmann et al. (1988) could in no case show that the upper third of the crown was best i.e. had the lowest coefficient of variation. But, the element concentrations relevant for tree growth are found in the shoots and these are mainly located in the upper third of the crown. Therefore, when interested in the element status affecting the growing conditions the upper third of the crown should be sampled.

As forwarded by Guha & Mitchell (1966) the element concentration in leaves is the outcome of physiological processes. Therefore it is expected that different individuals from the same tree species exposed to the same growing conditions will show, given the element, the same distribution pattern. The unequivocal result of Table 2.3 and Table 2.4 might be explained by the conventional sampling strategy to describe the element distribution pattern. The limited number of sampling heights generally three or less was thought to be insufficient to grip the variation in element concentrations as observed by Chapman (1966) and Ellis (1975)(see 2.5.3.1). The strategy of including the variation in element concentration is only meaningful when the distribution pattern is correctly described.

In pollution effects studies a leaf sample to describe the element concentration is commonly taken from a single specified height. Till present no publications were found describing the distribution pattern of heavy metals originating from pollution in tree crowns. As a consequence it is not known how the results of sampling at a single height relate to the distribution pattern in the crown. In other words : is the result from sampling at a single height the minimum, average or maximum concentration that can be found in the tree crown?

Sampling the effects of soil and atmospheric pollution the problems presented above should be diminished. The conventional sampling strategy i.e. sampling three heights can be optimised. The optimised variant is then applied to describe the element distribution pattern in the crown. In agreement with the aims of the study, the description of the distribution pattern is used to derive a single sampling height. The rest of the stand is then sampled at this height. Within this approach it is known how the concentration at the specified height relates to the concentrations that can be found in the crown. A sampling height can be chosen in accordance with the aims of the study.

2.5.3 Variation in element concentration by the horizontal crown dimension

2.5.3.1 Effects of the horizontal crown dimension

Most authors found no significant difference between element concentrations from leaves with a different aspect. This was true for Wallihan (1944), Tamm (1951), Auchmoody & Hammack (1975), Insley et al. (1981 a, b) and Erdmann et al. (1988). Tamm (1951) observed that K concentrations were slightly higher in southerly exposed leaves. Except in autumn, when it was found that northerly exposed leaves had slightly higher element concentrations than southerly exposed.

Watterson et al. (1963) and Leroy (1968) showed that there are differences in element status between sun leaves and shade leaves. Leroy (1968) found that shaded leaves were richer in N, P, Mg, and Ca than sun leaves. Emerging from the distribution of sun and shade leaves the element concentration in the inner crown of deciduous trees will differ from the outer crown. Without references to experiments Farago (1994) stated that the leaves from the outer crown of trees accumulate more heavy metals because of differences in transpiration rates with the inner crown. Likewise not supported by research, leaf ageing could result in an inner and outer crown with different heavy metal concentrations due to a different exposure time. The inner crown consists of the oldest leaves from spring flush, the outer crown of leaves on newly growth shoots. Although three processes explain the differences between concentrations in the inner and outer crown, no publications were found showing beyond doubt the existence of an inner and outer crown. When sampling a cross section of an inner and an outer crown at a single height the element concentration in the leaves would show a horizontal variation.

2.5.3.2 Sampling strategy by horizontal crown dimension

Because horizontal variation is rarely reported (exception Tamm, 1951) it is considered as being of minor importance. Nevertheless 63 citations anticipated horizontal variation by bulking leaves from the same height but from a different aspect in a single sample.

Table 2.6. Overview of 49 available citations concerning the number of aspects that were bulked in a sample of deciduous trees; arranged by research background and decade of study

Background of research	# of aspects Bulked	Decade				Total
		<1960	1970	1980	>1990	
Soil pollution	Unspecified				6	6[20]
	4					
	Whole crown				5	5 [21]
Atmospheric pollution	Unspecified		2	5	2	9 [22]
	4		2		3	5 [23]
	Whole crown				5	5 [24]
Forest growth and treatment	Unspecified	1	4			5 [25]
	4		2	3	4	9 [26]
	Whole crown	4		1		5 [27]

2.5.4 Variation in element concentration between trees

2.5.4.1 Effects of variation between trees

The magnitude of the variation in element concentrations between trees from the same species is given in Table 2.7. Macro-elements show a lower coefficient in variation than micro-elements. This is, at least partly, explained by the analytical method. At low concentrations the analytical method gets near to the detection limit. Therefore, it will be more difficult to obtain reproducible results for samples with a low concentration. The lower reproducibility increases the variation on the analytical result.

Table 2.7. Coefficient in variation in element concentration between trees from the same species; (†) trees from the same plot

Author	Tree species	Coefficient of variation (%)						
		N	P	K	Ca	Mg	Cu	Zn
Ellis, 1975 (†)	*Acer saccharum* Marsh.	12.1	12.9	15.0	12.6	13.9		19.4
	Fraxinus americana L.	14.9	12.9	17.1	13.1	13.4		14.5
	Prunus serotina Ehrh.	10.8	10.9	22.6	12.6	13.9		16.2
Erdmann et al. 1988	*Acer rubrum* L.	7.6	14.5	21.4	31.2	27.2		
Morrison, 1985	*Acer saccharum* Marsh.	12						
	Betula alleghaniensis Brit.	12						
McLennan, 1990	Populus trichocarpa Torr. & Gray ex Hook	12.3	14.6	14.0	16.8	15.0	26.5	29.9

2.5.4.2 Sampling strategy by variation between trees

To determine the element status of a stand, bulking leaves from several trees is common practice (Table 2.8).

Table 2.8. Overview of 14 available citations concerning bulking leaves from several trees in a single sample; arranged by research background and decade of study

Background of research	Decade				Total
	<1960	1970	1980	>1990	
Soil pollution					
Atmospheric pollution				4	4 [28]
Forest growth and treatment			9	1	10 [29]

The number of trees to sample depends on the aimed precision and the accepted chance to wrongly reject the tested hypothesis. Both criteria are determined by the study's objective. The authors referred to in Table 2.7 proposed a precision of 5 % or 10 % to calculate the number of trees that should be sampled. A precision with physiological instead of statistical relevance should preferably be used. No references were found elaborating on the precision needed when sampling with the objective to determine the effects on the element status caused by soil or atmospheric pollution.

Emerging from the observed variation in element concentration between trees, Table 2.9 gives the number of samples that should be bulked before analysis. Also averaging the proposed number of samples after analysis can be used to determine the stand's average element concentration with a given precision.

Table 2.9. Number of samples needed to estimate the element concentration; Min refers to the lowest number of samples needed to estimate the element concentration with a precision of 10%. Max refers to the highest of samples needed to estimate the element concentration with a precision of 5 %

Background of research		Number of samples						
		N	P	K	Ca	Mg	Cu	Zn
Soil pollution	Min							
	Max							
Atmospheric pollution [30]	Min	4	5	11	15	30		
	Max	8	13	36	52	111		
Forest growth and treatment[31]	Min	3	8	5	10	10	110	17
	Max	100	100	100	131	57	962	280

The results cited above should be considered as a directive rather then a standard recipe. In reality, the relation species-region is important. In differently polluted regions e.g. air versus soil pollution, different numbers of trees from the same species can be needed to obtain results with the same precision and accepted risk. The best way to determine the number of trees needed for an assumed precision and accepted risk is the use of a pilot study (Browne, 1995).

2.5.5 Seasonal variation in element concentration

2.5.5.1 Effects of seasonal variation

Forty-six citations showed that foliar element concentrations in woody plants varied seasonally[32]. Summarising the literature upon this subject seven types of seasonal variation were distinguished, each type characterised by its own trend within a growing season (Table 2.1). Ricklefs & Matthew (1982) concluded that the seasonal variation in chemical composition was not uniform among species. Surprisingly as shown in Table 2.1 several authors found general trends for the seasonal variation of N, P, K, Ca and Mg.

Evidence of seasonality and variability in foliar metal concentration in trees was found. The seasonal trends are described in Table 2.4. No consistent seasonal changes were found. Several reasons for the lack of consistency can be suggested : (i) differing heavy metal deposition patterns from one forest to another, (ii) differences in the heavy metal vector (dust, precipitation,…) (iii) differences in heavy metal concentrations in soils and their parent material, (iv) differences in sample treatment (unwashed leaves (Alfani et al., 2000) versus washed leaves (Dinelli & Lombini, 1996)), and (v) error propagation by the use of an inadequate sampling strategy. Nevertheless, the seasonal trends suggest that either the availability of metals in the soil show a seasonal pattern and/or growth dilution and/or metal shunting occurs in plant tissues (Ross, 1994). Kim & Fergusson (1994) explained increasing foliar metal concentrations within a growing season by contamination due to dry deposition. Decreasing foliar element concentrations could be caused by removal of surface deposits due to precipitation (Keller et al., 1994).

Kozlov et al. (1995) and Alfani et al. (1996a) detected a relationship between the amount of metals present and the intensity of the seasonal changes. Pronounced seasonal variations of Ni, Pb, Cu, and Fe in foliage at polluted sites were measured, whereas less seasonal variation was measured at less polluted sites and no pronounced variation was observed at the control site.

Table 2.10. Seasonal changes in element concentration in leaves; (**I**) continuous decrease in leaf concentration, (**II**) continuous increase, (**III**) (gradual) decrease followed by a period with a constant concentration, (**IV**) very little variation without seasonal trend, (**V**) strong variation without seasonal trend (**VI**) strong variation with seasonal trend both increasing and decreasing, and (**VII**) sharp increase followed by a period with a constant concentration or decrease

Author	Tree species	N	P	K	Ca	Mg	Other
McVickar, 1949	*Quercus alba* L.	III	I	I	II	IV	
Tamm, 1951	*Betula verrucosa* Ehrh.	I	I	I	II		
Hoyle, 1965	*Betula alleghaniensis* Britton	I		I	II	V	I (S)
Guha and Mitchell, 1966	*Acer pseudoplatanus* L.		III	III	II	IV	I (Fe, Cr, Al); II(B, Mn); III (Cu, Na)
	Aesculus hippocastanum L.		III	III	II	IV	I (Fe, Cr, Al); II(B, Mn); III (Cu, Na)
	Fagus sylvatica L.		III	III	II	IV	I (Fe, Cr, Al); II(B, Mn); III (Cu, Na)
Leroy, 1968	*Quercus robur* L.	I	I	I	II	I	
Le Tacon & Totain, 1973	*Fagus sylvatica* L.	I	I	III	II	I	II (Fe, Mn)
Grigal et al., 1976	*Acer spicatum* Lam.	I	I	I	II		
	Alnus crispa Ait.	I	I	I	II		
	Corylus cornuta Marsh.	I	I	I	II		
	Salix spp.	I	I	I	II	II	
	Amelanchier spp.	I	I	I	II	I	
Verry & Timmons, 1976	*Populus tremuloides* Michx.	I	I	I	II	IV	IV (Na); VI (Fe, Mn)
Bowersox & Ward, 1977	*Prunus serotina* Ehrh.	I	I	I	V	III	
Lea et al., 1979 a, b	*Acer saccharum* Marsh.	I	I	I	II	VI	I (Na); II (Mn, Fe, Al); V(Co)
	Betula alleghaniensis Britton	I	I	VII	II	IV	I (Na); II (Mn); VI (Co, Fe, Al)
Ponder et al., 1979	*Juglans nigra* L.	I	III	VI			
McColl, 1980	*Populus tremoloides* Michx.	I		I	II	IV	
Insley et al., 1981 b	*Tilia* spp.	I	I	I	II	V	

Table 2.10. Continued

Author	Tree species	N	P	K	Ca	Mg	Other
Dawson & Funk, 1981	*Alnus glutinosa* L.	VII					
Ricklefs & Matthew, 1982	*34 species of broad-leaved trees*	I	IV	I	II	II	
Alban, 1985	*Populus tremoloides* Michx.	I	III	III	II	VII	
Heilman, 1985	*Populus trichocarpa* Torr. & Gray	I					
Nilsson & Ericsson, 1986	*Salix viminalis* L.	I	I	IV	I	I	
Rytter & Ericsson, 1993	*Salix viminalis* L.	I	VI	IV	II	VII	
Robert et al., 1996	*Quercus suber* L.	I			VII		

2.5.5.2 Sampling strategy by seasonal variation

From 100 available citations no clear generally accepted sampling period could be extracted. Table 2.11 shows that leaf samples were taken at all times in the growing season.

Table 2.11. Overview of 100 available citations concerning the time of sampling deciduous trees; arranged by research background and decade of study

Background of research	Season of Sampling	Decade				Total
		<1960	1970	1980	>1990	
Soil pollution	Spring					
	Summer		3		4	7[33]
	Autumn					
	Growing season				6	6[34]
Atmospheric pollution	Spring				5	5[35]
	Summer			1	7	8[36]
	Autumn		4	2	4	10[37]
	Growing season		2	6	2	10[38]
Forest growth and treatment	Spring					
	Summer	3	4	15	2	24[39]
	Autumn					
	Growing season	6	15	5	4	30[40]

Again the optimal sampling time should be determined by the aims of the study. Spring and early summer foliage analysis shows the mobile elements during the physiologically important period of 'nutrient stress' (Smith et al. 1970). Erdmann et al. (1988) supported this idea. As a drawback they mentioned that spring and early summer sampling would be problematic since changes in leaf nutrient concentration are rapid by this time. McVickar (1949) and Ponder et al. (1979) found the opposite to be true. They found the minimal fluctuation of elemental concentrations in June and July. McColl (1980) remarked that the time of maximum ‘nutrient stress’ just after spring flush varies every year. This change makes the prediction of the time of maximum ‘nutrient stress’ very difficult. Also Alban (1985) rejected the idea of spring sampling. He found clear evidence that most of the spring uptake of N, and P came from perennial tissues, which act as a buffer to soil nutritional limitations that might show up later. For both reasons spring and early summer sampling was abandoned in favour of late summer sampling. Late summer is characterised by fairly stable concentrations of most nutrients in the leaves. This has the practical advantage that comparisons between different stands are still valid even when dates of sampling are not exactly the same (van den Driessche, 1974). But then again late summer may not be the best time for representing the nutrient status of the mobile elements, such as N, P, and K. However, it is advised against sampling in autumn. It is the time following the retranslocation of the mobile elements out of the leaves in preparation of the next year's growth season, and the time following the accumulation of the non-mobile elements in the leaves (Leaf, 1973).

Woodwell (1974), and Lea et al. (1979 a) concluded that the optimal periods for foliar sampling were not solely determined by physiological processes but were also influenced by the methods of expressing foliar data (e.g. concentration per unit dry weight, concentration per surface unit,…) as well as elements studied, species, soil, stand condition and silvicultural treatments.

Since the beginning of 1990 there is a tendency to sample in late summer (Table 2.11). A recent description for the optimal sampling time is given by UN/ECE-EC (1998) : 'sampling must be done when the new leaves are fully developed, and before the very beginning of the autumnal yellowing and senescence'. For an optimal sampling period, leaf elemental levels should be relatively stable and should reflect the overall elemental status of the tree.

No discussion on optimal sampling time for heavy metals was found in the international literature. The above made considerations on the optimal sampling time do make sense for essential elements but does not necessarily hold for heavy metals. For monitoring heavy metal concentrations in leaves UN/ECE-EC (1998) recommends -by not mentioning a different period- the same period as for sampling nutrients. It seems that this recommendation is solely based on practical considerations; samples for both nutrients and metals are obtained within the same sampling campaign.

2.5.6 Annual variation in element concentration

2.5.6.1 Effects of annual variation

In contrast with the variation within a season, only 15 citations tackled the problem of variation between seasons (Table 2.12).

Table 2.12. Overview of 15 available citations concerning annual variation in leaves; arranged by research background and decade of study

Background of research	Decade				Total
	<1960	1970	1980	>1990	
Soil pollution					
Atmospheric pollution			2	7	9 [41]
Forest growth and treatment	1	5		1	7 [42]

In leaves, the nutrient levels are expected to be variable since physical environmental characteristics of the site which influence these levels vary from year to year. Insley et al. (1981 b) sampled the same trees during four growing seasons. The foliar concentrations of K, Ca and Mg were very consistent but poor correlations were found for N and P. Leroy (1968) sampled the same trees in two successive years and found different seasonal trends in both years. Opposed to these conclusions Ponder et al. (1979) and Alban (1985) found fairly similar concentrations for P, K, Ca and Mg between two or three growing seasons.

The magnitude of the between-season variation depends on the nutrient species (van den Driessche, 1974). Duquesnay et al. (2000) give inter-year CV's of 5 % for N and 7 % for P. The observed coefficient of variation for Mn was found to be 20 % for all consecutive years. Oppositely no inter-year variation was found for Mg. In a study of the variability between sampling times 27 years apart from each other Duquesnay et al. (2000) found changes of +12 % for N, -23 % for P, -16% for Ca, -38 % for Mg and -6 % for K. Tree and stand ageing during this 27-year period may partly explain the observed changes in foliar composition together with environmental changes.

In non-polluted areas the concentrations of metals was found to be extremely stable at five sampling sites over nine years (Djingova et al., 1996). In the vicinity of a waste incinerator Meneses et al. (1999) found no significant variations in heavy metal concentrations in herbage samples collected over two years. Exceptions were Cr, V and Hg that varied significantly within this two-year period. This probably indicates a change in composition of the waste.

In regions with high pollution stress Kozlov et al. (1995) found clear annual variation in the foliage concentration. They observed in one year metal concentrations two to threefold the concentration of other years. Alfani et al. (2000) reported a decrease of Pb, Fe and Cr concentrations in leaves in a seven year span. In the same period no significant difference was found in the leaf concentration of Zn, V whereas the leaf Cd concentration increased. The authors attribute the changes to changing pollution sources e.g. use of unleaded petrol and closure of a steel mill. No literature, studying the annual variation of heavy metals from soilborn pollution was found.

2.5.6.2 Sampling strategy by annual variation

The variation caused by the annual differences in element status is only a problem if the study aims to describe environmental changes. The variation between growing seasons then contributes to the imprecision of foliar analysis as a diagnostic tool and therefore calls for a long-term approach.

2.5.7 Variation in element concentration by *other* factors

2.5.7.1 Effects of *other* factors

Even though this literature study deals only with deciduous trees this paragraph mixes the results of conifers and deciduous trees. Findings of conifer trees are cited when similar research is not yet available for deciduous species. The factors

discussed in references that look at conifers are indicative but not necessarily proven to be true for deciduous trees.
Mader & Thompson, (1969) believed that both N availability, uptake and foliar concentration were restricted during droughty periods. Earlier Miller (1966) could explain the variation in nutrient levels (N, P, K) in conifers by weather factors. More recently Hippeli & Branse (1992) showed that increasing rainfall and higher mean temperatures during the growing season increased foliar concentrations of N, P, Ca and Mg in *Pinus sylvestris* L.. Weather conditions could be an important factor in explaining the annual variation in element concentrations in leaves.

DeBell & Radwan (1984) and Heilman (1985) described a negative correlation between the age of the tree and the N, P, Ca, S, Fe, and Al concentrations in the foliage of *Alnus rubra* Bong. and *Populus trichocarpa* Torr. & Gray ex Hook.. Morrison (1972) found a more complex relation between nutrient concentration and tree age. In the needles of *Pinus banksiana* Lamb. The P, K, and Mg concentrations decreased with increasing age, whilst foliar Ca, Fe, and Mn concentrations were shown to increase. The results suggested that declining leaf concentration with age may be related, at least in part, to limiting supplies of essential elements. Translocation and retranslocation of nutrients could explain the ability of trees to grow despite limited nutrient supply (Miller, 1984). These processes can then be held responsible for changing foliage nutrient concentrations with ageing of the tree.

Nuorteva & Kurkela (1993) studied the nutrient status in *scleroderris*-canker-diseased *Pinus sylvestris* L. Compared with the control trees, concentrations of foliar B, Ca, N, Mn and S were significant higher whereas foliar Fe and Mg concentrations were significant lower in diseased trees. Raitio & Merilä (1998) found higher K, Zn, B, Fe and Al concentrations in with spruce needle rust infected foliar. As a result foliar analysis of defoliated trees or unhealthy foliage can give poor estimates of element deficiencies and toxicities.

Also stand management influences foliar concentrations e.g. depending on the initial K concentration, removal of *Betula pubescens* Ehrh. shelterwood decreased (Saarinen, 1996) or increased (Saarinen, 1999) the K concentration. In both experiments the N and P concentrations increased in the foliar of the *Picea abies* (L.)

Karst. understorey (Saarinen, 1996; Saarinen, 1999). Nuorteva & Kurkela (1993) related pruning of *Pinus sylvestris* to increased B, Ca, N, S, Na and Cu concentrations in the needles. Hager & Kazda (1985) concluded that when leaf S concentrations are used to evaluate the influence of atmospheric S pollution, one should consider that the leaf S concentrations may be altered by usual forest management practices, like heavy thinning.

Hoyle (1965) concluded that foliar nutrient levels were not affected by soil moisture but determined primarily by the supply of soil nutrients. McVickar (1949) and Wells & Metz (1963) demonstrated the influence from the soil as did McLennan (1990). It was demonstrated by Chen et al. (1998) that *Populus tremuloides* Michx. grown on sites with a higher site index had higher total N, B, and Mn concentrations in their foliage than individuals grown on less suitable sites. Similar results for N, P, and Mg were found earlier in conifers by Kayahara et al. (1995), Wang (1995), and Wang & Klinka (1997). For metals Jung & Thornton (1996) studied the factors affecting the bioavailability and the uptake by plants. Factors studied were total metal concentration in soil, soil pH, cation exchange capacity, organic matter concentration, soil texture and interaction among elements. It was concluded that the total metal content in soil and pH are the main factors that influence the metal concentration in plants.

Eltrop (1991) showed the difference in element concentrations in the leaves between *Betula pendula* Roth. and *Salix caprea* L.. Brieger et al. (1992) sampled 48 plants grown on the same site. Both found different metal concentrations between species. Raitio & Sarjala (2000) found significant differences in nutrient concentrations between provenances of *Pinus sylvestris* L. Heilman (1985) demonstrated the influence of *Populus* spp. clones on the element uptake. Landberg & Greger (1994), Riddel-Black (1994) and Punshon (1996) compared willow clones and species. All three studies came to the same conclusion: there is a significant difference in heavy metal uptake and tolerance between species and clones.

Comparing the element concentration of washed leaves and unwashed leaves (10 citations[43]) washed leaves show lower element concentrations than unwashed leaves.

Several researchers showed a relation between the pollution level of leaves and the distance from a source of atmospheric pollution (22 citations[44]).

2.5.7.2 Sampling strategy by *other* factors

The aims of the study should determine which factors are controlled. The influence of a factor can be limited by careful selection of the study object. If element concentrations are compared the stands have to grow in the same weather conditions. The stand should have been subject to the same treatment and soil conditions should be similar. The distance and direction to the source of atmospheric pollution should be comparable. Stand and trees should be of the same age. Leaves should be healthy and taken from the same species or clone. Finally, leaves samples should receive the same preparation.

The introduction of this paragraph can be misleading by insinuating that the so called 'Effects of other factors' are extra problems in standardising leaf sampling. Some of the above mentioned factors will express as a spatial variation i.e. site index. Others will cause seasonal variation i.e. weather. Some of the above mentioned factors can be and are used for the advantage of the researcher. The influence of the soil composition, pollution source and the combination of distance and direction of the pollution source are the reason for existence of leaf sampling as a diagnostic tool in studying environmental pollution. Without these influential factors all analytical results of leaf analyses would be equal and of no diagnostic value. Because the composition of soil, water and air influences the composition of the leaves, leaves can be used as biomonitors in forest health and pollution studies. Factors that contribute to, at first sight, unwanted variation in the leaf composition, e.g. species, crown class, and weather contribute to the surplus value of leaf sampling. Whereas soil analyses can show a similar degree of pollution, leaf analysis of e.g. different species with the same social position can show a different element concentration in their leaves and therefore call for a different management approach.

Some studies aimed to distinguish between superficially absorbed and biomass incorporated elements e.g. studying the internal pool of elements (Wyttenbach et al.

1985) or studying metal distribution in plants (Dinelli & Lombini, 1996). In these cases it is strongly recommended that the foliage samples are washed for a short time in a washing agent. Raitio (1995) reviews sample formation, washing techniques, washing agent, time and effects of washing. Especially when studying the effects of atmospheric pollution samples are washed (Table 2.13). In some cases factor analysis can be used as an alternative for washing (Kuik & Wolterbeek, 1994).

Table 2.13. Overview of 40 available citations concerning sample preparation by washing of the leaves; arranged by research background and decade of study

Background of research		Decade				Total
		<1960	1970	1980	>1990	
Soil pollution	Washed		3		4	7 [45]
	Unwashed					
Atmospheric pollution	Washed		7	2	6	15 [46]
	Unwashed		2	2	9	13 [47]
Forest growth and treatment	Washed	3			1	4 [48]
	Unwashed				1	1 [49]

2.5.8 Variation in element concentration by method of expression

To start with there is the misuse of the terminology *concentration* and *content* of elements in plant tissues. *Concentration* is the amount of a particular element present in a unit amount of plant material (Timmer, 1991) expressed in percent (%), part per million (ppm), or in the equivalent S.I. unit mg.(kg DW)$^{-1}$ (Dry Weight). *Content* is the amount of element in a specific amount of plant material (Timmer, 1991) expressed in g, per 100 leaves, 1000 needles, or 1 ha (10000 m²). In 1974 van den Driessche defined the terminology, articles published before 1974 should be interpreted with care but even nowadays authors often use the wrong terminology.

The expression of element concentrations as a percentage of the leaf tissue dry weight frequently offers problems in interpretation of the data. Elements as N, K, P often show a decrease in concentration when the growing season progresses whereas their content increased (Hoyle, 1965). The author concluded that the use of percentage values reduces measurement sensitivity, is an obstacle to correct evaluation of seasonal gains and losses of elements and posses problems in detecting differences in foliage element levels.

Smith (1962) compared, based on literature, alternatives for the concentration method and concluded that most alternatives had no advantage. The method that used milli-equivalents (meq) was at that time popular to express the amount of elements in leaf tissue. The explanation for its popularity was given by (i) meq give a more accurate picture of nutrient interrelations e.g. cations and anions, and (ii) it is desirable to use the same basis of expression for plant material as is used for soils. The use of the meq-method is mainly useful when comparing the concentration of one element with that of another within the same plant. When studying metals which function with several different valences (e.g. Cr, Fe, Al) there is no advantage of using equivalents as method of expression. While there is some logic to these arguments, it should be noted that no publication using meq as method of expressing element concentrations in leaves was found.

A number of empirical methods of handling the data have been used e.g. totalling the percentage of K, Ca, and Mg in the leaf has been found useful when studying oil palm grown under different conditions (Smith, 1962). Ratios of elements in various combinations are also used e.g. N/P, N/K, N/Mg, Mg/Ca (Duquesnay et al., 2000) but they were not found to be more effective or constant than the percentage method.

Some authors compared different methods of expression on the same data set. Ovington & Madgwick (1958) expressed the concentration of Na, K, and P on an ash weight basis. Due to an increase of ash weight with increasing crown heights, the increase in element concentration were greatly reduced when the elements were expressed as percentages of the ash weight. Claussen (1990) suggested that the concentrations of heavy metals are better related to the dry ash of a plant, than to the dry weight. Heavy metal concentrations in ash of *Populus nigra 'Italica'* L. were reported to correlate better with values in air and soil than those in dry plant material. Woodwell (1974) found that the nutrient concentrations for two *Quercus spp*. were equal if expressed per unit of area. If leaf weights were used leaves of one species appeared throughout the season 10-20 % richer in nutrients than the other species. He supposed to express nutrients as a function of the leaf area because the latter changes only little after leaf expansion. In this way the dilution caused by the increase in leaf mass does not mask the real change in element content. Lea et al. (1979) used percent dry weight, mg per leaf, percent ash or mg per cm² of surface area to express the element concentrations. After examination of the four methods of expression the results did not support the use of one expression over another. They can usually complement each other when interpreting responses treatments.

A wide use of leaf analysis is in comparing element status of plants grown under different conditions (fertilised-unfertilised, polluted-unpolluted,…). In these cases the vector analysis is a favourable technique that allows for simultaneous comparison of plant growth, nutrient concentration and nutrient content (Haase & Rose, 1995).

2.5.9 Conclusion

Despite the differences in objectives, no difference was found between the common practice in leaf sampling to study the effects from soil pollution, the effects from atmospheric pollution or the effects of forest treatment. Dominant or co-dominant trees are preferred. The leaves are taken from a single height location, generally the upper third of the crown. Samples consist of leaves from an unspecified number of aspects from the sampling height. Within a stand leaf samples are taken from at least five trees. Samples are analysed separately or bulked before analysis. The sample campaign takes place in late summer well before leaf yellowing and senescence. Milligram element per kilogram dry weight tissue is the common unit to express the nutrient and metal concentration in leaves. The sampling strategy as described above is nearly the same as the strategy imposed on the participants of the ‘European Programme on Assessment and Monitoring of Air Pollution Effects on Forests’ (UN/ECE-EC, 1998). UN/ECE-EC (1998) limits the use of the sampling strategy to detect the effects from unfavourable chemical conditions in the rooting zone and from intoxication or from high immission levels but in environmental and forestry research it is the generally used sampling strategy. Therefore in the continuation of the study it is called the generally used sampling strategy and referred to as UN/ECE-EC (1998).

Some major problems remain for using leaf analysis in pollution effect studies. (i) Unless all the importance in the forest ecosystem intermediate and suppressed trees are excluded from leaf analysis. (ii) By sampling a single height the vertical element variation is removed but not described. Description of the vertical trend could be used to derive a sampling location that accounts for the aims of the study. The conventional sampling strategy to describe trends in tree crowns produces unequivocal results. (iii) The physiological relevance of the sampling time for metals originating from soil or atmospheric pollution is not known. (iv) Due to many factors probably affecting the element concentration in leaves study objects should be selected after profound deliberation.

2.6 Cadmium concentrations in tree leaves

2.6.1 Background concentration

A lot of research was done on the background Cd concentrations in plants for human and livestock consumption. The concentration in plant material ranged from 0.1 to 1.0 mg.kg^{-1} (Mengel & Kirkby, 1982). Kabata-Pendias & Pendias (1992) reported concentrations of 0.013 to 0.22 mg.kg^{-1} dry weight (DW) for cereal grains, 0.07 to 0.27 mg.kg^{-1} DW for grasses and 0.08 to 0.28 mg.kg^{-1} DW for legumes. Extended research on vegetables all over the U.S.A. confirmed this range (Wolnik et al. 1985). Even background concentrations as low as 0.0031 mg Cd kg^{-1} for sweet corn were reported (Wolnik et al., 1983). The background concentrations for Cd in trees are given in Table 2.14. Memon et al. (1980) investigated 60 plant species at 9 sites. Fifty nine species had foliar concentrations lower than 2.49 mg Cd kg^{-1} DW and 49 species had foliar concentrations below 1 mg Cd kg^{-1} DW. Stoeppler (1991) reports that the background value for Cd in plants is below 0.5 mg Cd kg^{-1} DW, Riddell-Black et al. (1997) considered concentrations of 0.05 and 0.2 mg Cd kg^{-1} as normal. Kabata-Pendias & Pendias (1992) gave an overall background value for plants ranging from 0.1 to 2.4 mg Cd kg^{-1} DW.

Table 2.14. Background concentration for Cd in leaves of trees; (†) results expected to be classified differently

Author	Tree species	Cd concentration mg.kg^{-1} DW
Buchauer, 1973	*Betula lenta* L.	< 3.00 (†)
	Quercus prinus L.	< 1.00
	Quercus rubra L.	< 3.00 (†)
van Hook et al., 1977	*Acer rubrum* L.	0.17
	Pinus echinata Mill.	0.30
	Quercus alba L.	0.12
	Quercus prinus L.	0.11
	Quercus rubra L.	0.35
	Quercus velutina L.	0.69
Parker et al., 1978	*Quercus velutina* L.	0.40-0.60
	Populus tremuloides Michx	0.70-1.30
Heinrichs & Mayer, 1980	*Fagus sylvatica* L.	0.66
	Picea abies Karst.	0.24-0.39

Table 2.14. Continued

Author	Tree species	Cd concentration mg.kg^{-1} DW
Mankovska, 1980	*Fagus sylvatica* L.	0.57
Memon et al., 1980	*Abies* spp.	0.19-0.21
	Acer spp.	0.55-1.32
	Aesculus spp.	0.52
	Carpinus spp.	0.26-0.74
	Castanea spp.	0.22-0.78
	Fagus spp.	0.14-1.06
	Fraxinus spp.	0.24-0.86
	Pinus spp.	0.31-0.99
	Prunus spp.	0.46-0.69
	Quercus spp.	0.33-0.73
	Sorbus spp.	0.56-0.86
	Tsuga spp.	0.20-0.24
Freedman & Hutchinson, 1981	*Betula* spp.	0.64
Martin et al., 1982	*Corylus avellana* L.	0.15
Burton et al., 1984	*Picea sitchensis* Carr.	0.50
Thomas et al., 1984	*Corylus avellana* L.	0.05
Linzon, 1986	*Acer Saccharum* Marsh.	0.30-0.40
Løbersli & Steinnes, 1988	*Betula pubescens* Ehrh.	0.06-0.17
Landolt et al., 1989	*Picea abies* Karst.	0.05-1.60
Breckle & Kahle, 1992	*Fagus sylvatica* L.	< 0.30
Kurczynska et al., 1997	*Pinus sylvestris* L.	0.23
Walkenhorst & Hagemeyer, 1997	*Picea abies* Karst.	0.35
Santamariá & Martin, 1998	*Fagus sylvatica* L.	0.07
	Quercus spp.	0.05-0.08
	Pinus nigra Arnold	0.04
	Pinus sylvestris L.	0.05
Alfani et al., 2000	*Quercus ilex* L.	0.007-0.08
Monaci et al., 2000	*Quercus ilex* L.	0.02-0.05

The background concentration calculated from Table 2.14 was with a confidence limit of 95% lower than 1.12 mg Cd.kg^{-1} DW.

2.6.2 Elevated concentration

Elevated concentrations of some metals in soils may produce toxic symptoms in trees and in the forest as a whole. Metals operate as stress factor in the trees environment when they cause changes in physiological reactions. In extreme metals can reduce vigour or inhibit tree growth. Many authors found indications that trees and the forest as a whole are affected by the presence of heavy metals:

- The nutrient status of tree foliage was altered by enhanced leaching and competition between divalent cations and Cu and Ni (Rautio et al, 1998). Disruptions in nutrient pools were caused by less efficient retranslocation during autumnal senescence (Nieminen & Helmisaari, 1996). For one specific *Quercus* species Santamaria & Martin (1998) found a correlation between defoliation and increased foliar concentrations of P and N.
- Litter was shown to be one of the major sinks of heavy metals in forest ecosystems (Strojan, 1978; Løbersli & Steinnes, 1988; Huvenne et al., 1997). The breakdown of litter polluted with heavy metals was observed to be retarded (Rühling & Tyler, 1973, Tyler, 1974; Tyler, 1975 a, b; Hutchinson & Whitby, 1977; Coughtrey et al., 1979; Freedman and Hutchinson, 1980; Zwolinski, 1994). In this way metals accumulating in the litter and disturb nutrient cycling in the forest (Friedland et al. 1984).
- Influence of heavy metals on tree and seedling growth was observed (Little & Martin, 1972; Jordan, 1975; Hutchinson & Whitby, 1977; Burton et al., 1983; Smith & Brennan, 1984; Sutherland & Martin, 1990; Dickinson et al., 1991 a, b, Lamersdorf et al., 1991; Breckle & Kahle, 1992; Watmough & Dickinson, 1995).
- A shift in the available species and an impoverishment of vegetation was recorded by Buchauer (1973), Folkeson & Andersson-Bringmark (1988), Heliövaara & Väisänen (1990), Eltrop (1991), Kozlov et al. (1995), Dinelli & Lombini (1996) and Stjernquist et al. (1998).

Disorders in the physiological processes compared to growth disorders were demonstrated at lower or approximately the same Cd concentrations in the growth medium and/or the plant organs (Balsberg Påhlsson, 1989). Although the variation between species is great, the lower limit to affect growth ranges from 3 to 10 mg Cd kg^{-1} DW of leaf tissue (Beckett & Davies, 1977). Reported foliar Cd concentrations for trees are given in Table 2.15 and Table 2.16.

Table 2.15. Concentration of Cd in leaves of trees grown on polluted sites (mg.kg^{-1} DW); (†) results expected to be classified differently; (‡) expressed in mg.kg^{-1} dry ash

Author	Tree species	Cd concentration mg.kg^{-1} DW
Little & Martin, 1972	*Ulmus glabra* Huds.	15-50
Buchauer, 1973	*Betula lenta* L.	3.0-15.0
	Quercus prinus L.	1.0-40.0
	Quercus rubra L.	3.0-38.0
Smith, 1973	*Acer platanoides* L.	0.5-2.0
	Acer saccharum Marsh.	0.5-1.5
	Quercus palustris Muenchh.	1.5-3.0
Jordan, 1975	*Populus grandidentata* Michx.	5.0-12.0
	Populus tremuloides Michx.	5.0-12.0
	Quercus rubra L	5.0-12.0
Jackson & Watson, 1977	*Quercus* spp.	1.9-3.9
Parker et al., 1978	*Quercus velutina* L.	0.2-0.4 (†)
	Populus tremuloides Michx.	2.9-18.9
Martin & Coughtrey, 1981	*Acer campestre* L.	3.4-3.7
	Quercus robur L.	2.7
Martin et al., 1982	*Corylus avellana* L.	2.3
	Acer campestre L.	4.6
	Fagus sylvatica L.	2.0
	Fraxinus excelsior L.	2.8
	Quercus robur L.	1.2
Schebek et al., 1984	*Betula pendula* Roth.	0.1-0.9 (†)
Thomas et al., 1984	*Betula verrucosa* Ehrh.	0.2 (†)
Trlica et al., 1985	*Populus sargentii* Dode	0.87-1.66 (†)
Borgegård & Rydin, 1989	*Betula* spp.	4.0-4.4
Claussen, 1990	*Populus nigra 'italica'* L.	3.6-27.0 (‡)
Turner, 1991	*Acer pseudoplatanus* L.	5.4-6.0
Bache et al., 1992	*Populus tremuloides* Michx	1.92
Kim & Fergusson, 1994	*Aesculus hipocastanum* L.	0.4-0.1 (†)
Watmough, 1994	*Acer pseudoplatanus* L.	<0.3 (†)
Punshon, 1996	*Salix caprea* L.	5.0-60.0
Kurczynska et al., 1997	*Pinus sylvestris* L.	0.6 (†)
Punshon & Dickinson, 1997	*Salix cinerea* L.	33.0-53.0
Monether, 1998	*Salix viminalis* L.	2.2-2.9
	Salix viminalis L.	7.3

Table 2.16. Concentration of Cd in leaves of trees grown on Cd enriched solution culture (mg.kg^{-1} DW); (†) results expected to be classified differently

Author	Tree species	Cd concentration mg.kg^{-1} DW
Burton et al., 1984	*Picea sitchensis* Carr.	4.8
Breckle & Kahle, 1992	*Fagus sylvatica* L.	> 0.3 (†)
	Fagus sylvatica L.	3.6
Turner & Dickinson, 1993	*Acer pseudoplatanus* L.	5.5-5.9
	Acer pseudoplatanus L.	3.6-4.0
	Betula pendula Roth.	5.0-6.0
Landberg & Greger, 1996	*Salix spp.*	1.8-5.7

2.6.3 Conclusion

To evaluate the cadmium concentration in the sampled willow leaves background and elevated Cd concentration in tree foliage have to be known. Although the tables were compiled from different references discussing different species, analysed with a different methodology, grown on a different soil, under different conditions and polluted from different sources the proposed background and elevated concentrations were surprisingly similar. Concentrations lower than 1.12 mg Cd.kg^{-1} DW were in 95 % of the cases background concentrations. Elevated concentrations are concentrations higher than 1.12 mg Cd.kg^{-1} and were found to amount to 60.0 mg Cd.kg^{-1}.

3 Material and methods

3.1 Study area

The study area is located on the banks of the Schipdonk Canal between Nevele and Deinze at Bachte-Maria-Leerne, Belgium (Fig. 1).

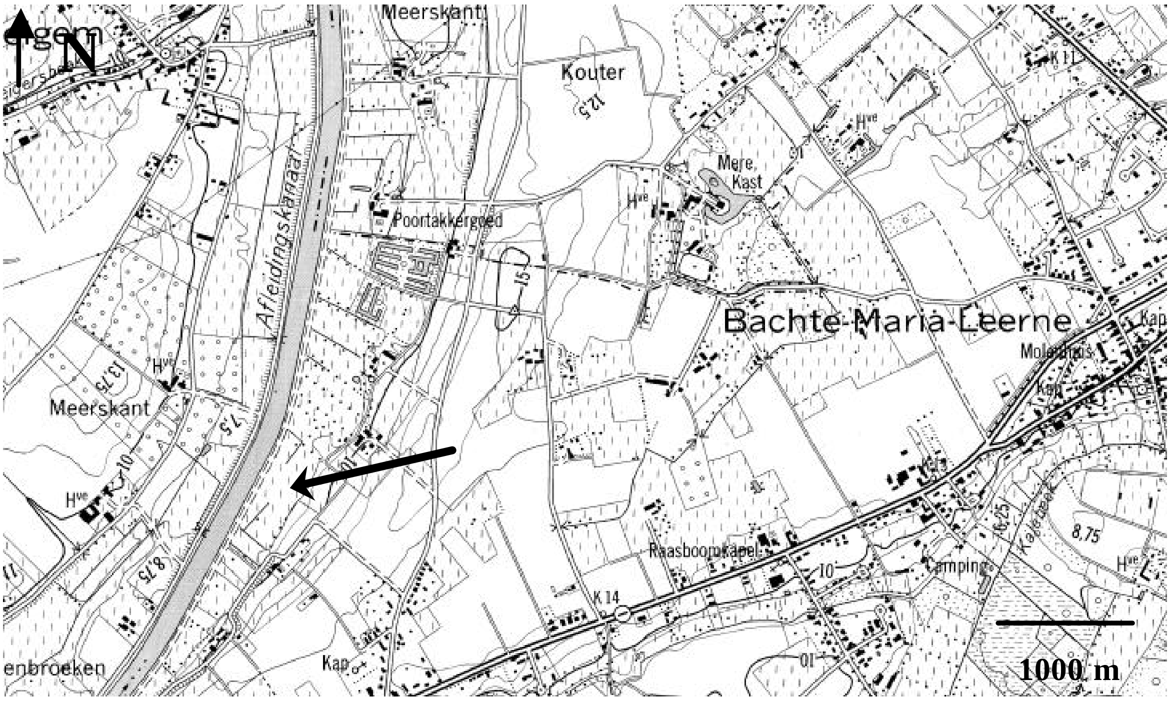


Fig. 1. Location of the study area. The arrow indicates the location of the experimental site

Between 1986 and 1988 dredging sludge from the neighbouring river Leie was salvaged and disposed on this area. The confined disposal site has a surface area of 59,000 m^2 and was after settling raised three meters. The sludge originated from two locations : the Leie and a swing basin located at the Noorderwal both in Deinze. Cadmium concentration of the sediment at these locations ranged from 5.5 to 11.5 mg.kg^{-1} DW. These concentrations were considered as critical. Also the Zn, fluorantheen and benzo(b)fluorantheen concentrations were considered as critical by the author (Van den Eede, 1997).

Van Grieken (1996) measured the aerial heavy metal concentration in Flanders and did not reported a specific source of aerial Cd pollution near the experimental site. But their still is an average annual atmospheric deposition of 0.7 mg Cd, 7.3 mg Cu, 11.0 mg Pb and 44.0 mg Zn per square meter (Van Grieken, 1996). The average annual atmospheric cadmium deposition on the canopy of the experimental site was estimated by assuming :

- (i) A uniform deposition throughout the year and a growing season of 6 months. At the end of the growing season a total amount of 6 months . 0.7 mg Cd m^{-2}.year^{-1} / 12 months.year^{-1} = 0.35 mg Cd is deposited per square meter.
- (ii) That all atmospheric deposition occurs above the zero plane displacement. The zero plane displacement indicates the mean level at which wind energy is absorbed by crowns of trees in forest stands. The zero plane displacement is located between 0.6 and 0.8 times the tree height (Grace, 1983). An average tree height of 8.2 m causes a zero plane of displacement to occur at 8.2 m . 0.7 = 5.6 m above ground level.
- (iii) A leaf biomass above the zero plane of displacement of 326 g.m^{-2} ground surface. 0.3 = 97 g.m^{-2}, a leaf area of 3.8 m^{2}.m^{-2}. 0.3 = 1.2 m^{2}.m^{-2} and thus a deposition area of 2 . 1.2 m^{2}.m^{-2} = 2.4 m^{2} leaf surface.m^{-2} ground surface (for the initial values of leaf biomass and leaf area see § 3.2).

Working on these assumption the deposition on the leaves amounted : 0.35 mg Cd m^{-2} . 2.4 m^{2}.m^{-2} / 97 g DW m^{-2} = 0.009 mg Cd g^{-1} DW or 9 mg Cd kg^{-1} DW leaf biomass. Most of the airborne polluted Cd particles remain on the surface of the leaves and eventually are washed off (Hagemeyer et al., 1986). A month drought before sampling would result in a 9 mg Cd kg^{-1} DW / 6 = 1.5 mg Cd kg^{-1} DW on the leaves caused by atmospheric deposition. If atmospheric deposition would be the main source of elevated Cd concentrations, concentrations should be higher above than below the zero plane of displacement and Cd concentrations below the zero plane of displacement should not exceed 1.5 mg. kg^{-1} DW. These conclusions were not confirmed by the data (see chapter 5) and therefore on the experimental site, atmospheric deposition was considered a less important Cd source than soil cadmium.

Between 1990 and 1991, after settling and dewatering, the whole surface was afforestated. The site of interest for this study was a fenced area of 2500 m^{2}, planted with 21 *Salix fragilis* L. and completed with *S. viminalis* L.. The stand within the fence was planted according to a 1.5 m within row and 4 m between rows spacing.

After planting, it was left unmanaged. All *S. fragilis* L. appeared to be vital. Trees were from the same age class and no distinction between social classes could be made. In 1999 an average tree height of 8.2 m, an average stem diameter of 14 cm and a basal area of 5.8 m^2 per 10 000 m^2 was measured. That year the *S. fragilis* L. had a leaf biomass of 326 $g.m^{-2}$, in 1998 the leaf area amounted 3.8 $m^2.m^{-2}$. Shrubs were absent, and the herb layer was dominated by *Urtica dioica* L.. The humus form taxa is mull.

3.2 Study object

Over the last years, *Salix* species, which are among the fastest and highest producers of biomass (Perttu, 1993), have been widely planted on derelict land. These lands were used for sewage sludge and wastewater disposal, disposal of mining wastes and industrial wasteland (Eltrop et al., 1991; Kahle, 1993; Perttu & Kowalik, 1997; Hasselgren, 1998). Moreover, the preference of *Salix* to wet habitats, flooding areas and rivers banks, together with a fast vegetative reproduction, easy hybridisation and efficient nutrient uptake (Weeda et al., 1994) suggest what experiments have shown: dredged material is a suitable substrate for tree growth (Huvenne et al., 1997; Luyssaert et al., 2001; Vervaeke et al., 2001). Expectations are that an alternative for dumping dredged materials in covered sealed confined sites will be found in forests managed for biomass production, phytostabilisation, phytoextraction or with combined management objectives. Species tested so far for growth and survival on hydraulic raised fresh water sludge were *Quercus robur* L., *Fraxinus excelsior* L., *Alnus glutinosa* L., *Salix* species and different *Populus* species. Especially *Salix* has shown, by high yields and plant survival, to take advantage of the high concentrations of plant nutrients present (Huvenne et al., 1997) while withstanding high metal concentrations (Perttu, 1993; Labrecque et al., 1995). On brackish sludge *Fraxinus excelsior* L. and *Salix* spp. favoured from the growth conditions (Luyssaert et al., 2001). The produced woody biomass is characterised by large quantities of light flexible wood which has many economic uses such as bio-fuel, landscape mulching, bedding, composite boards, chemicals and raw material in the pulp and paper industry (Ledin & Willebrand, 1996).

Because heavy metal uptake is known to be species and clone dependent (Eltrop, 1991; Brieger et al., 1992; Landberg & Greger, 1994; Riddel-Black, 1994; Koricheva & Haukioja, 1995; Punshon, 1996) the stand manager can choose between phytostabilisation or phytoextraction. If wanted, both options can be combined with biomass production. In phytostabilisation, the choice is made to keep the pollution in the soil. This way the risk of foodweb contamination by animals feeding on the trees is reduced. Soil conditions have to be kept stable or changed in favour of reduced mobility of the heavy metals (Mench et al., 1986; Gworek, 1992; Mench et al., 1994; Dickinson, 2000; Tordoff, 2000). By afforestation a dispersal of metals by water and wind erosion and percolation of the metals to the groundwater can be prevented (Ettala, 1988; Vangronsveld et al., 1995 a, b; Glimmerveen, 1996 a, b). It could be a suitable management objective on sites used for recreation or in the neighbourhood of valuable or sensitive forests, pasture, etc. In phytoextraction, metals are transferred to the plant, establishing a long-term sanitation of the soil (Baker et al., 1988; Baker et al., 1994; Östman, 1994; Duncan et al., 1995; Ernst, 1996; Greger & Landberg, 1997; Rulkens et al., 1998). Cadmium and Zinc give reasonable perspectives. Therefore, nowadays phytoextraction in general and with willow in specific, targets a decrease of Cd and Zn concentrations of the soil (Baker et al. 1994). Although in this technique, the risk of foodweb contamination is expected to be larger (Ernst, 1996; Glimmerveen, 1996 a). In phytoextraction, care has to be taken when processing the biomass because of the presence of the toxic metals (Punshon & Dickinson, 1997). Soil conditions have to remain stable or change in favour of less mobility of the heavy metals or a higher bioavailability for the plants. Phytoextraction could be a suitable management objective on land farming, in-situ reclamation of contaminated soils or remediation of industrial areas.

Upon consideration of the above *Salix spp.* is an obvious study object within the context of a methodological study using leaf sampling to reveal soil pollution. Of Cd and Zn, the elements of major interest in forests on polluted sites, Cd was chosen because as a non-essential element for plant growth Cd it is toxic to both plants and animals.

3.3 Sample collection

Two 8-year-old *Salix fragilis* L. trees were used for sample collection. The first tree was chosen randomly from the fenced area. The second tree was chosen randomly from the two neighbours in the same row of the first tree.

In August 1998 at both sides of the first tree, a scaffold was erected. On both towers, the shop floor was 4 m long and 9 m above ground level. The towers stood at a distance of 4 m from each other. The towers were joined by four gangplanks (Fig. 2). By moving the gangplanks, the entire crown could be reached.

Fig. 2. Scaffolds joined by gangplanks erected around the *Salix fragilis* L. tree

In a vertical half of the tree the specific leaf area was determined at eight heights each separated by one meter. At each height three samples, one at the top, one in the middle and one at the base of a branch, were taken (Fig. 3). A sample consisted of ten leaves. Leaves were put in paper bags. Back in the laboratory, the leaf area was determined with an optical area scanner. After drying at 70 ± 2 °C until constant weight the samples were weighed and the specific leaf area calculated. The following year (see further) leaf biomass of the second *Salix fragilis* L. was determined and multiplied with the specific leaf area resulting in the leaf area of the tree. By using the stand density the leaf area was calculated to be 3.8 m^2.m^{-2}. Leaf area was used to estimate the importance of the atmospheric deposition (see § 3.1).

Fig. 3. Outline of the collected data on the stand and tree level. Data collection is ordered by the chapter in which they are used

Stand	Tree	
Data used in chapter 3 to describe the stand and estimate the influence of atmospheric deposition		
- *stand density, basal area, diameter and height*	- *specific leaf area of 3 x 10 leaves from 8 heights*	Willow # 1
Data used in chapter 4 to calculate the size requirements of a representative elementary sampling unit		
	- *Cd concentration in individual leaves contained in a 0.4 by 0.4 by 0.4 m unit*	
	- *Number of leaves in 0.05 by 0.05 by 0.05 m units contained in 1.8 by 0.6 by 1.1 m volume*	
Data used in chapters 5 and 6 to describe the spatial variability of cadmium concentrations in the crown of Salix fragilis and to optimise the sampling strategy		
	- *Leaf biomass in 1136 0.3 by 0.3 by 0.3 m units contained in a whole crown*	Willow # 2
	- *Cd concentration in 292 units selected from the 1136 units*	
Data used in chapter 7 to determine the representativity of willow #2 within the stand		
- *Cd concentration in 0.3 by 0.3 by 0.3 m units from 20 trees each at 5 height locations*		

The smallest sampling unit that yields a stable variation in accordance with the aims of the study is called a representative elementary sampling unit. To determine its size the variability in Cd concentration between individual leaves was studied (Fig. 3). Leaves were collected from an unsampled vertical quart of the crown. To avoid the presence of unwanted variation a single branch with side branches was used. For sampling convenience the branch was selected at chest height on the first shop floor, so 3.3 m above ground level. It was postulated that individual leaves from the branch and side branches would be sampled until the sampling unit contained at maximum 60 leaves. Sixty leaves corresponds with twice the minimum number of repeats needed to estimate the variance of a normal population (Neter, 1996). Sampling was stopped when the block reached dimensions of 0.4 m by 0.4 by 0.4 m this block contained fifty-eight leaves. Individual leaves were put in paper bags, dried and analysed on Cd concentration. On these data the size of a representative elementary sampling unit was determined using an analytical approach, bootstrap approaches and a geostatistical approach. To compare the results from the different approaches, leaf clustering data and weight were used to transform all results in metric units. Sampling did not yet disturb at this point one vertical quart of the crown. This quart was used to measure leaf clustering (Fig. 3). A volume of 1.8 m long, 0.6 m wide and 1.1 m high was centred at 3.3 m above ground level. The volume was centred at the same height but in the adjacent quart at which the individual leaves were analysed to determine the size of a representative elementary sampling unit. A movable frame materialised sampling blocks with a dimension of 0.05 m by 0.05m by 0.05 m. The measured volume included 45,360 blocks of which 520 contained petioles. The number of petioles within each block was recorded. Based on these data a representative elementary sampling unit for this study was found to have dimensions of 0.3 m by 0.3 m by 0.3 m (see chapter 4). In the continuation of the study, sampling units with these dimensions were used.

In August 1999 at both sides of the second tree a similar scaffold as previous year was erected. The crown was sampled in a non-systematic (Fig. 4). The first four days the upper right quart, the next four days the upper left quart and in the last four days the lower half was sampled. So on the third, ninth and last day samples were taken from approximately the same height. On days 3, 9 and 15; 1 and 7; 2 and 8; 4, 10 and

14 and days 10, 11, 13 and 14 samples were also taken from approximately the same height.

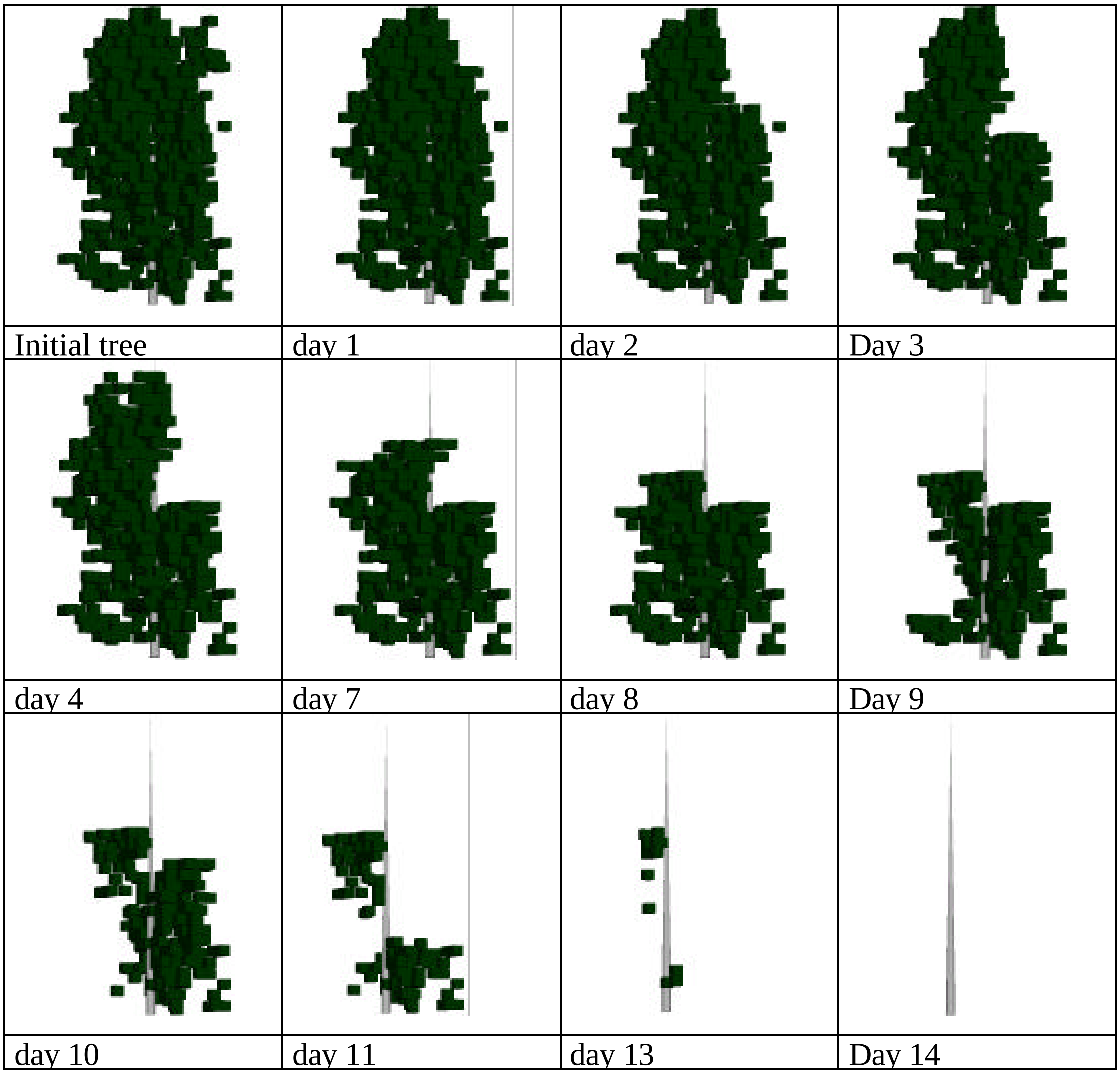

Initial tree
day 1
day 2
Day 3
day 4
day 7
day 8
Day 9
day 10
day 11
day 13
Day 14

Fig. 4. Progress of sampling of the second *Salix fragilis* L. during a 15 day span. As in the field samples collected at a given day are removed from the initial tree of the image

Representative elementary sampling units of 0.3 m by 0.3 m by 0.3 m were used to sample the crown (Fig. 3). These units were materialised with a movable frame. The entire crown covered 8721 blocks of which 1136, which were all sampled, contained leaf biomass. Immediately after sampling, twigs were stripped of leaves. Leaves were put in paper bags and dried. Dry weight of all 1136 samples was determined enabling the description of the leaf biomass distribution along the tree height. By summation, the leaf biomass of the entire tree was known. Multiplication with the stand density resulted in a leaf biomass of 326 $g.m^{-2}$ used to estimate the importance of the atmospheric deposition (see § 3.1).

A stratified random sampling design with a separation distance of 0.6 m was applied on the entire crown of 8721 blocks. Because only 1136 blocks contained leaf biomass 142 samples were withdrawn by this stratified random design. From the remaining 994 samples 150 were withdrawn according to a simple random design. A total number of samples of 292 ensured to reveal a spatial structure if present (Webster & Oliver, 1992). By using both designs, an even spatial distribution of the samples was combined with the possibility to analyse samples on a distance smaller than the smallest separation distance of the stratified sample. Solely, on these 292 samples Cd concentration and the ratio dry ash to dry weight were measured. Being inspired by the results of Ovington & Madgwick (1958) and Claussen (1990) (see § 2.5) the influence of the method of expression was studied. The Cd distribution within the crown was described using concentration per unit dry weight and concentration per unit dry ash.

To enable spatial analysis the sample location of the petioles of the 58 individual leaves, the 520 leaf clustering blocks and the 1136 representative elementary sampling units were recorded in Cartesian co-ordinates. The scaffold, levelled by using the scaffold spindles, was used as a reference to record the Cartesian co-ordinates.

In August 2000 the remaining 20 *S. fragilis* L. were positioned within the stand. Their leaves were sampled at five heights: 1.5, 3.0, 4.5, 6.0, and 7.5 m (Fig. 3). Because some trees had no leaf biomass at the sampling height 97 leaf samples instead of the expected 100 could be collected. These 97 leaf samples had the same dimensions as the representative elementary sampling unit. The samples were taken close to the stem, by this mean the Cartesian co-ordinates of the samples within the stand were known. To illustrate the relation between soil and leaf Cd concentrations the NH_4-EDTA extractable fraction of Cd in the soil was used. The analytical results were available from the study of Mertens et al., (2001). Ammonium-EDTA extractable Cd was determined on 12 locations at a depth of 0.3 m.

3.4 Chemical analyses

Immediately after sampling, twigs were stripped of leaves. Leaves were put in paper bags and dried at 70 ± 2 °C until constant weight. The 58 individual leaves, the 292 sampling units from the second willow tree and the 97 sampling units from the stand were analysed on Cd concentration. To avoid excess heating, and contact with metals possibly containing cadmium, samples were manually ground with mortar and pestle. Samples were sieved on a 1-mm sieve, homogenised, labelled and stored in a closed recipient till analysis for Cd. An entire leaf or if available approximately 0.5 g dry leaf material was weighed in a microwave vessel, 5 ml HNO_3 p.a. 65% was added. On a CEM Microwave Sample Preparation System MDS 2000 a pressure controlled closed vessel digestion program was applied. During a 14 minute span, the power of the microwave system was increased from 250 Watt to 600 Watt while the pressure in vessel is kept at a constant level of 25 bar. Vessels were left to cool down. Quality of the digestion was controlled by weighing the vessels before and after digestion. Vessel content was filtered over a 0.45 µm membrane filter in a 50-ml flask. The flask was then filled till 50 ml. The method run on a Varian ICP-AES VISTA was validated with the certified reference material CRM 060 containing *Lagarosiphon major* Moss and CRM 281 containing *Elymus spp.*. The analytical quality of the inductive coupled plasma (ICP) was checked by including a method-blank and a laboratory control sample every 10 samples. The relative standard deviation of the determination of Cd in the laboratory control sample was 3.6 % on 34 repetitions. Concentrations were reported with a precision of 0.1 $mg.kg^{-1}$ DW.

From the 292 samples, the ratio dry ash to dry weight was determined. The sample was once more dried for 72 hours at 70 ± 2 °C. Approximately 1 g of ground and sieved sample was weighed and put in porcelain crucibles. The crucibles were gradually heated until 450 ± 10°C and ashed for 4 hours, at this temperature. The analytical quality of the method was controlled by including method-blanks. The relative standard deviation of the determination of the dry ash of the laboratory control sample was less than 1% on 30 repetitions. The Cd concentration expressed on dry ash (DA) was calculated according to :

$$[3.1] \quad Cd\ (mg.kg^{-1}DA) = \frac{Cd\ (mg.kg^{-1}DW)(kg\ DW)}{(kg\ DA)}$$

with :

$Cd\ (mg.kg^{-1}DA)$ = Cd concentration expressed on dry ash

$Cd\ (mg.kg^{-1}DW)$ = Cd concentration expressed on dry weight

$\frac{(kg\ DA)}{(kg\ DW)}$ = ash ratio of the sample

3.5 Computational methods

To attain the readability of the text, the main methods used for exploring and computing are given at the beginning of the chapter in which they are used. In this section an overview is given. Computations of the geostatistical methods were conducted in the Geostatistical Library software package (GSLIB). The bootstraps, the transfer function and the Monte Carlo simulation were computed with self-written Fortran programs.

Four mathematical approaches were compared to determine the size of a representative elementary sampling unit. These approaches include an analytical approach, two bootstrap approaches and a geostatistical approach.

The spatial variability of the Cd concentration in the crown was described by a deterministic trend. The residuals of this trend were modelled with a geostatistical method i.e. indicator variograms. These variograms contain essential information to estimate the Cd concentration at unsampled locations in the crown. The Cd concentrations at these locations were estimated using a sequential indicator based stochastic simulation algorithm.

It was found that the generally used sampling procedure yielded a biased estimate of the average Cd concentration. Therefore, the generally used sampling procedure was optimised to reveal the average Cd concentration in the crown of a willow. The sampling procedure was parameterised in a transfer function. The input data were randomly selected within the limits of constrains given by the sampling procedure. This approach is known as the Hit and Miss Monte Carlo simulation. The Monte Carlo simulation approach provided a practical way to determine the distribution of the average Cd concentration given by a certain sampling procedure. The distributions of the average Cd concentration obtained by the generally used and the optimised sampling procedure were compared.

Last, a bootstrap approach was used to test the representativiness of the Cd distribution pattern in the crown and the Cd concentration of the single tree within the stand.

4 Size requirements of a representative elementary sampling unit for estimating the Cd concentration in the crown of *Salix fragilis*

4.1 Abstract

The size of the representative elementary sampling unit is a measure to control (i) the processes that can be studied, (ii) the number of samples, (iii) the variation and (iv) practical considerations as in field sampling and sample preparation. The smallest meaningful sampling unit for leaf analysis is a single leaf. Leaves on the same twigs and twigs on the same branch showed a variation in Cd concentration ranging from 1.3 to 6.3 mg Cd kg^{-1} DW. Accounting for this small-scale variation would mask the large-scale variation within the entire crown. Therefore a number of leaves had to be bulked to smooth the small-scale variation. In this pilot study a block with dimensions 0.4 m by 0.4 m by 0.4 m contained 58 leaves. On these data four different approaches were used to determine the dimensions of a representative elementary sampling unit. When the small-scale variance was given by the variance between the 58 leaves, the analytical or classical approach resulted in a unit of 0.35 m by 0.35 m by 0.35 m. A distribution free approach i.e. the bootstrap resulted in a sampling unit with dimensions 0.40 m by 0.40 m by 0.40 m. It was clear that the Cd concentration in leaves on the same twig were not independent. So, a dependent bootstrap, accounting for the spatial correlation of the Cd concentration was used. Now a sampling unit of 0.45 m by 0.45 m by 0.45 m described the variance. In both bootstrap methods and the analytical approach the estimated variance (s^2) of the 58 leaves was used as variance (σ^2). The reliability of this assumption can not be tested and was therefore ones guess. The geostatistical approach, free of this assumption lead to the conclusion that the dimensions of sampling unit should be 0.30 m by 0.30 m by 0.30 m to smooth the small-scale variance without affecting the large-scale variance. This block size was adopted as the representative elementary sampling unit in the continuation of the study.

4.2 Introduction

Sampling a perfect homogeneous medium would make the considerations about the sample size redundant. Any sample would give exact analytical results irrespective of its size. It is the heterogeneity that is responsible for the fact that sampling is a non-exact process requiring a probabilistic approach (Gy, 1992). The size of the representative elementary sampling unit should be considered during the design of a sampling campaign. It is a measure to control (i) the processes that can be studied, (ii) the number of samples that should be taken to describe the population at hand, (iii) the variation of the results and (iv) practical considerations as in field sampling and sample preparation.

The conceptual and quantitative analysis of heterogeneity is a prerequisite to determine the optimal size of a sampling unit. The smallest meaningful sampling unit to investigate the element concentration in the crown of a tree is a single leaf. Since leaves from the same location show an important variation in element concentration several leaves have to be bulked to ensure that a sample is representative for its location. By bulking leaves from different locations in a single unit the sample will give a better representation of the average element concentration in the tree crown but information on the variability of the element concentration within the crown is lost. Opposed to a single leaf sample all leaves from the entire crown could be bulked in one unit. This sample would allow determining the average element concentration, but does not hold any information on the variability. The optimal size of a representative elementary sampling unit for an experiment, somewhere in between a single leaf and a single sample of all foliage, should be determined by considering the precision and significance levels thought to be necessary for the study (Marshall et al., 1992).

In this chapter a pilot study of the Cd concentration in a block with dimensions 0.4 m by 0.4 m by 0.4 m was used to determine the optimal dimensions of a representative elementary sampling unit which smoothes the variation between leaves. Variability between leaves was described and the size of a sampling unit obtained by an analytical, a dependent and an independent bootstrap and a geostatistical approach were compared. At the start of this study the pros and cons of each approach could have been weighed against each other, based on considerations about the validness of the statistical assumptions e.g. normal distribution, independent data, etc. a single method to determine the sample size could have been selected. Besides statistical assumptions each approach contained assumptions to incorporate the aims of the study in the computations i.e. estimator of the variance, scale of the process to describe, etc. By testing different methods the influence of the method-related assumptions on the size of a representative elementary sampling unit was analysed.

4.3 Data processing

4.3.1 Analytical approach

The analytical approach is often denoted as the *a postiori* method to determine the sample size. It has been used in the determination of the number of samples in forest sampling in general (Stauffer, 1982; Marshall et al., 1992), for foliar analyses (Marshall & Jahraus, 1987), in forest hydrology (Houle et al., 1999) and forestry herbicide trials (Zedaker et al., 1993). Due to its frequent application in forest studies and its general inclusion in statistical textbooks from the early sixties (Freese, 1962) till present (Rosner, 2000) it can be considered as the classical approach to determine the sample size.

The references illustrated that the approach is most often used to determine the sample size, i.e. the number of samples, in a sampling campaign. Beside the scale difference there was no fundamental difference to the problem at hand : a sample was an individual leaf and the sample size the number of leaves needed to make up a representative elementary sampling unit. Statistically the problem of determining the sample size is summarised as follow (Rosner, 2000): given that a significance test will be conducted at level a and that the true alternative mean is m_1, what sample size is needed to be able to detect a significant difference with probability $1-b$? The null hypothesis $H_0 : m_0 = m_1$ with m_0 the true mean is tested versus the alternative hypothesis $H_1 : m_0 \neq m_1$, assuming that the Cd distribution is normal in both cases and that the standard deviation s is known. The test will be conducted with a significance level a , the probability that H_0 will be rejected although this hypothesis is true. Also $1-b$, the power of the test, has to be known. This is the probability of rejecting H_1 if this hypothesis is true. At last, some idea of the magnitude of $|m_0 - m_1|$, the effect size, is needed to estimate the sample size. Because it is not known whether m_1 is bigger or smaller than m_0 the two-sided test was adopted to determine the sample size for a normal distribution, given by :

[3.2] $$n = \frac{s^2 \left(z_{1-b} + z_{1-a/2}\right)^2}{\left(m_0 - m_1\right)^2}$$

One question that arises is how to estimate the parameters necessary to compute the sample size. By convention the type I error (a) is usually set to 0.05. What the level of the power should be is somewhat less clear. Cohen (1977) gives, with his concept of relative seriousness something to go on. He defines the relative seriousness as the ratio between the risk of false null rejection and the risk of false null acceptance. With $a = 0.05$ and $1 - b = 0.80$ the relative seriousness is given by (1-0.80)/0.05 and equals 4. Thus, with these parameter values, mistaken rejection of the null hypothesis is considered four times as serious as mistaken acceptance.

An important issue brought in by Cohen (1977) and recalled by Rosner (2000) is the assessment of what a scientifically important absolute effect size $|m_0 - m_1|$ would be in the context of the problem studied. Again Cohen (1977) gives something to hold on to : the relative effect size (*d*), defined as:

[3.3] $$d = \frac{|m_0 - m_1|}{s}$$

Under the assumption of normality of the populations, the relative effect size (*d*) can be transformed in percent non-overlap between the two populations with average m_0 and m_1. A large effect size is then defined as d= 0.8 or two populations with almost half of their areas not overlapping (Cohen, 1977). A medium effect size is given by d=0.5. In terms of measures of non-overlap, 33% of the combined area covered by two normal equal-sized equally varying populations is not overlapped. Cohen (1977) defines a small effect size as d=0.2. This means that the two populations have only 14 % of their combined area not overlapped.

4.3.2 Resampling approach: the bootstrap

As the approach discussed in the preceding paragraph, the majority of the classical statistical methods, used in forestry, assume that the error variances are homogeneous and the observations normally distributed. Combined with the common prevalence of non-normality in ecological data (Hampel et al., 1986; Austin, 1987; Biondini et al., 1988), one rarely does have sufficient data to test adequately the assumption of normality. It is apparent that, if the assumption of normality does not hold, significance levels may be distorted, the methods may suffer loss of power, and the estimates obtained based on this assumption may be inaccurate (Huber 1964, 1973). Therefore, in addition to the analytical approach, a distribution-free method known as the *bootstrap* was used to determine the sample size. Bootstrapping can be viewed as a method that simulates alternative sample sets. Such simulations make it possible to explore the average statistical properties of alternative samplings and their variability.

The general approach to construct a bootstrap sampling distribution for a parameter is :

- (i) Draw a random sample of size X_i^* with $i = n$, with replacement from X_i with $i = N$. Each X_i^*, called a bootstrap replicate, is used to calculate the bootstrap parameter of interest $\hat{q}^{*b}$.

- (ii) Repeat step (i) B times and obtain the corresponding estimates $\hat{q}^{*1},...,\hat{q}^{*B}$. The bootstrap estimate of q is given by $\hat{q}(.)$:

$$\hat{q}(.) = \sum_{b=1}^{B} \hat{q}^{*b} / B$$

with:

$\hat{q}^{*b}$ = the bootstrap estimate of q for the b th replicate

B = number of bootstrap replicates

The variance is calculated in the usual manner as

$$\operatorname{var}\hat{q}^{*} = \frac{\left\{ \sum_{b=1}^{B} \left[\hat{q}^{*b} - \hat{q}^{*}(.) \right]^2 \right\}}{(B-1)}$$

Dane et al. (1986) and Starr et al. (1992) formulated the problem to determine the minimum sample size required as follows: There is one set of N observations (N =

58), how many of these observations are needed to obtain a reliable estimate of the mean value of the 58 observations? Calculation of the number of observations and thus the minimum size of a sampling unit are given in Fig. 5.

Fig. 5. Flowchart of the computer program to determine the minimum size of a sampling unit using an independent bootstrap. The flowchart is concretised with a step by step example

Flowchart of an independent bootstrap	Example
- Read all observations (*N*)	- Make a table (T_1) with the Cd concentration of the 58 individual leaves
- Do $n = 2$ to N	- Repeat for $n = 2$ to 58
- Do $b = 1$ to B	- Repeat for $b = 1$ to 5,000
- Select *n* at random observations called a bootstrap replicate X_i^*	- Make a table with *n* Cd concentrations selected at random with replacement from T_1
- Calculate the bootstrap parameter $\hat{q}^{*b}$	- Calculate the average Cd concentration of the n selected observations
- Save the value of the bootstrap parameter $\hat{q}^{*b}$	- Make a table with the average Cd concentration as value T_2
- End do	
- Calculate the bootstrap estimate $\hat{q}(.)$	- Calculate the average Cd concentration of the 5,000 bootstrap parameters from T_2
- Calculate the bootstrap variance $\mathrm{var}\,\hat{q}^*$	- Calculate the variance of the 5,000 bootstrap parameters from T_2
- Sort T_2	- Calculate the fraction of the *B* replicates having means within a confidence interval of the average Cd concentration for the 58 observations(*)
- Plot these data points on the graph	- Create a graph as Fig. 9 and Fig. 10
- End do	

---

[*] Marshall et al. (1992) defined a 'good' sample was one which confidence interval width for the population parameter of interest was less than some specified maximum. For these bootstraps the confidence interval width was set at 0.5 mg Cd kg^{-1} DW.

Since ecological data were often found to be spatially correlated (Dutilleul & Legendre, 1993; Legendre, 1993) analysis of these data should account for this correlation. In problems, such as the sample size determination, the correlated structure of the data should be preserved. A resampling method as the independent bootstrap could destroy the spatial correlation between the Cd concentration in the leaves (Efron & Tibshirani, 1993). The resampling algorithm was redesigned in a way that it honours the spatial structure of the data, the method was then called the dependent bootstrap.

Shao & Dongsheng (1995) discussed that an appropriate bootstrap procedure should mimic the true sampling mechanism that generates the original data. Therefore in the dependent bootstrap the randomisation algorithm to select a replicate X_i^* with size *n* started with:

- (i) Draw up a contingency table describing the topology of the leaves and twigs;

and then proceeded as follows:

- (ii) select one leaf at random from the 0.4 by 0.4 by 0.4 m block;
- (iii) based on the contingency table all neighbours on the same twig of this leaf are determined, one of these neighbours is selected at random;
- (iv) step (iii) is repeated for the newly selected leaf;
- (v) step (iv) is repeated until a replicate of size *n* is selected or until all leaves from the same twig are selected. In the first case the algorithm continues with (vii);
- (vi) based on the topology table all neighbouring twigs are determined, one leaf from these twigs is selected and the algorithm proceeds with (iii);
- (vii) the bootstrap parameter is calculated.

Dependent bootstrap replicates of sizes $n = 2, 3, 4, \ldots, 58$ were generated *B* times. For each value of *n* the fraction was calculated of the *B* replicates ($B = 5{,}000$) having means within a given percentage of the mean calculated for the 58 observations.

4.3.3 Geostatistical approach: variogram

This approach supports the hypothesis that the combined action of biological, chemical and physical processes determines the Cd concentration in the leaves. The

Cd concentration can be expected to be more similar at locations close to each other. Therefore samples were expected to be spatially dependent. In geostatistics this spatial structure is commonly described through a measure of spatial continuity, the semivariance. The description of the spatial structure can be used as a frame for the determination of the sample size (Zhang et al., 1990; Gy, 1992; Starr et al., 1992; Lamé & Defize, 1993). The semivariance is given by eq. [3.4]:

$$[3.4] \quad g(\mathbf{h}) = \frac{1}{2N(\mathbf{h})} \sum_{a=1}^{N(\mathbf{h})} \left[z(\mathbf{x}_a) - z(\mathbf{x}_a + \mathbf{h}) \right]^2$$

with $g(\mathbf{h})$ = semivariance at a lag distance **h**

$z(\mathbf{x}_a)$ = attribute value (here Cd concentration) at location $\mathbf{x}_\alpha$

$z(\mathbf{x}_a + \mathbf{h})$ = attribute value at location $\mathbf{x}_\alpha+\mathbf{h}$

$N(\mathbf{h})$ = number of pairs of data locations a vector **h** apart

A plot of $g(\mathbf{h})$ *vs.* **h** is commonly called a variogram. A variogram of two dimensional data is determined by its (i) number of lags, (ii) lag separation distance, (iii) lag tolerance, (iv) azimuth angle, (v) tolerance on the azimuth angle, and (vi) azimuth bandwidth (Goovaerts, 1997; Deutsch & Journel, 1998). Two variograms were calculated. The variogram describing the spatial continuity within a branch was, calculated based on the observed angle of the branches. So an azimuth angle of 330°, a tolerance of 30° and an azimuth bandwidth of 0.07 m was used. The lag distance was an increasing series with a separation distance of 0.02 m for the first 4 lags and 0.03 m for the last 2 lags. So a total distance of 0.14 m was covered. To obtain the omnidirectional variogram for the block the azimuth angle was set to 0°, tolerance on this angle was set to 90° and the bandwidth to 0.4 m being the block's physical limit. Again, the lag distance was an increasing series with separation distances from 0.02 to 0.03 m for lags smaller than the range, widening to 0.05 for lags near the range of the semivariogram. For both variograms calculated tolerance on the lags was set to the conventional half of the separation distance.

4.3.4 Leaf clustering

A volume of 1.8 m long, 0.6 m wide and 1.1 m high was split in 45,360 equal blocks of 0.05 m by 0.05 m by 0.05 m. The volume included 520 blocks which contained petioles. The number of petioles as a measure for the number of leaves was recorded. The number of leaves within a bigger sampling unit could be calculated by summation of the number of leaves in the 0.05 m by 0.05 m by 0.05 m blocks. E.g. a sampling unit of 0.3 m by 0.3 m by 0.3 m could be calculated by combining 216 or six in each direction, neighbouring 0.05 m by 0.05 m by 0.05 m blocks. This was repeated for the whole volume of 45,360 blocks. So the data of the initial 45,360 blocks were re-organised in 45,360 / 216 = 210 sampling units of 0.3 m by 0.3 m by 0.3 m. The re-organisation was then repeated for different locations to start at. This raised the number of simulated sampling units from 210 to 3235. From the determination of the specific leaf area the average dry weight of single leaves was known. So beside the mean, median, standard deviation and kurtosis of the number of leaves the mean, median, standard deviation and kurtosis of the dry weight in a sampling unit of 0.3 m by 0.3 m by 0.3 m could be computed. The computations were repeated for sampling units ranging in size from 0.05 m by 0.05 m by 0.05 m to 0.5 m by 0.5 m by 0.5 m. Dimensions of the simulated sampling unit were increased by steps of 0.05 m in all three dimensions. In this way the number of leaves in a sampling unit could be translated in the sample weight or the metric dimensions of that sampling unit.

4.4 Results and discussion

4.4.1 Explorative data analysis

The 0.4 m by 0.4 m by 0.4 m block (Fig. 6) contained 58 mature leaves. The four side branches were approximately in a plain with each other and no relation between the Z-axis and the Cd concentration could be shown (correlation coefficient r = -0.05; probability p=0.00). Due to these features the three dimensional block could be reduced to a two dimensional projection in a 0.4 m by 0.4 m plain.

The null hypothesis that the Cd concentration of the 58 leaves came from a normal distribution was accepted with a probability of 0.86 derived from the Kolmogorov-Smirnov test (Neter et al., 1996). The symmetrical distribution around an average Cd concentration of 4.0 $mg.kg^{-1}$ dry weight (DW) and a coefficient of variation (CV) of 20% is shown in the histogram (Fig. 7). The Cd concentration ranged from 1.3 $mg.kg^{-1}$ DW to 6.3 $mg.kg^{-1}$ DW.

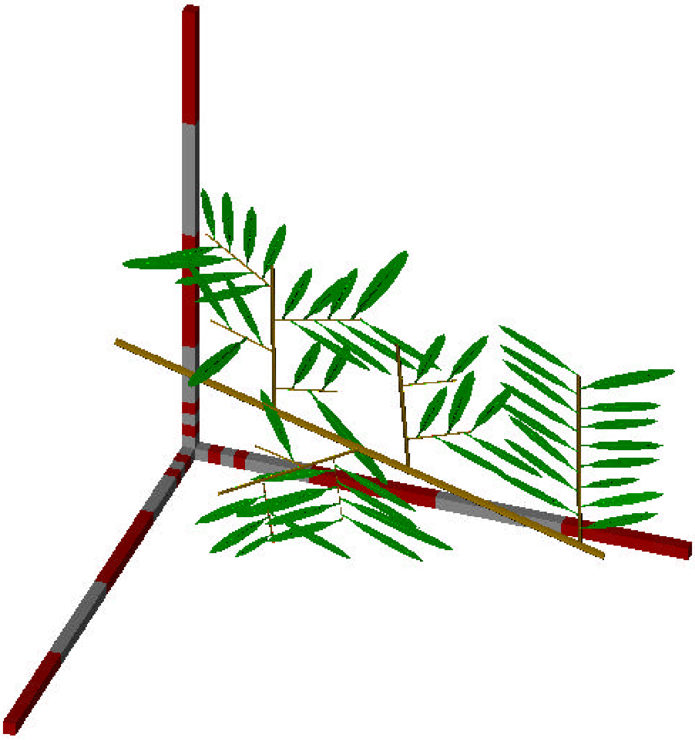


Fig. 6. 0.4 m by 0.4 m by 0.4 m block containing the individual sampled leaves, the markers represent 0.4 m

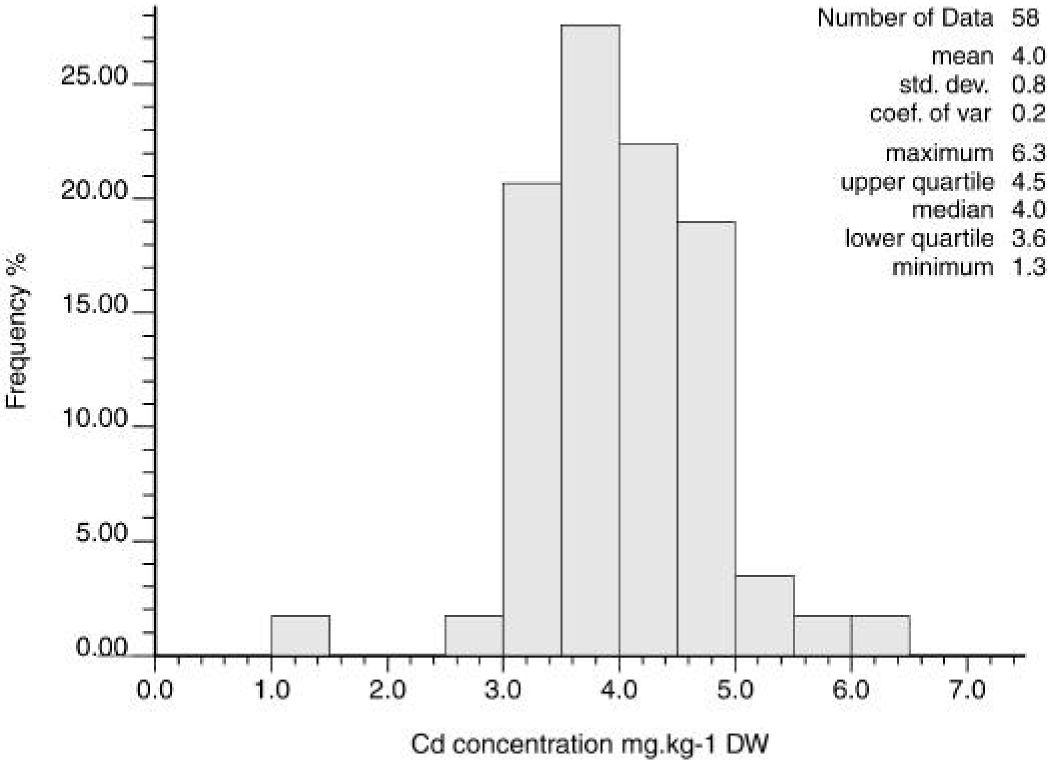


Fig. 7. Histogram of the Cd concentration in the 58 individual sampled leaves with some descriptive statistics

The spatial distribution of the number of leaves along the X-axis was found to be uniform with a probability of 0.22 according to a Kolmogorov-Smirnov test (Neter et al., 1996). At the contrary the null hypothesis of a uniform distribution of the number of leaves along the Y-axis was rejected with a probability of 0.00. Therefore, the location at the Y-axis was transformed in the absolute value of the distance between the base of the petiole and the main branch. The transformed parameter showed a uniform distribution along the Y-axis with a probability of 0.13. In other words, for all distances from the Y-axis an equal number of leaves were sampled.

In Fig. 8 the Cd concentration of all leaves within the block is shown. The Spearman correlation model was employed to study the relations between the variables (Neter et al., 1996). Spearman's model is free of assumptions of the parameter's distribution and uncovers beside linear, non-linear relations between the variables (Neter et al., 1996). Because some parameters were found to follow a uniform distribution where others followed a normal distribution Spearman's correlation model was adopted. A very significant (p=0.00) correlation of -0.70 was demonstrated between the Cd concentration in the leaves and their distance to the main branch. The sign of the correlation implies that leaves at the top of a side branch had a lower Cd concentration than leaves at the bottom of that side branch (Fig. 8).

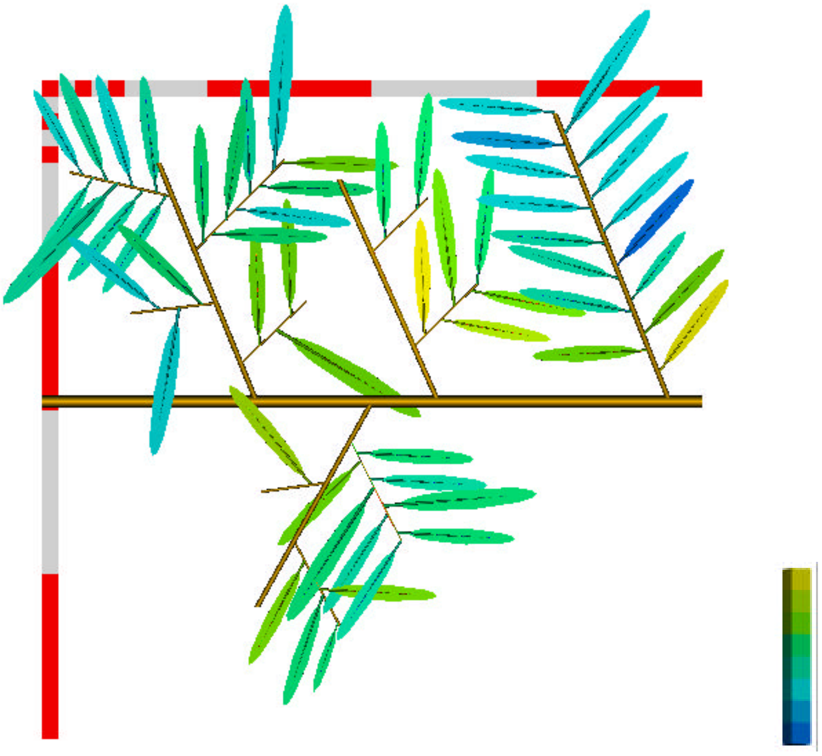


Fig. 8. Cd concentration of the sampled leaves within the block. Cd scale in mg.kg^{-1} DW ranges from 1.3 to 3.0 (blue), 3.0 to 4.0 (cyan), 4.0 to 5.0 (green) and 5.0 to 6.3 (yellow); the markers represent 0.4 m (top view)

4.4.2 Sample size based on the analytical approach

Our interest was to calculate the sample size needed to detect a large relative effect size *d* as defined by Cohen (1977; see 4.3.1). For this population a large relative effect size corresponded (Eq. [3.3]) with an absolute effect size of 0.5 mg.kg^{-1} DW. Opposed to the common definition of relative seriousness as defined by Cohen (1977; see 4.3.1), the relative seriousness was set to 0.25. The underlying idea of this choice was that it is less serious to take different samples if there are no differences in mean value then taking a single sample if there is a real difference in mean value. Therefore, accepting that $m = m_0$ when they did differ was considered four times as serious as rejecting $m = m_0$ when they were equal. To obtain this relative seriousness

a was set to 0.20 and $1-b$ to 0.95. At this point the variance was the last parameter needed to compute the sample size using equation [3.2], however its value was unknown and had to be estimated. For the entire block of 58 leaves the variance s^2 can be estimated by s^2 which equals 0.6 (mg.kg^{-1})^2 DW (Fig. 7).

Based on this set of parameters a sample size of 25 leaves was needed to characterise a population with a variance of 0.6 (mg.kg^{-1})^2 DW, given the significance levels. Since only 25 instead of 58 samples were needed, the estimate of the variance changed and the sample size problem could only be solved with an iterative algorithm. Next, when calculating the sample size a population of 25 leaves had to be characterised which raised the problem that a selection of 25 from 58 leaves cannot be characterised by a single variance. $58^{25}/25! \approx 2.5\times10^{20}$ Random selections with replacement are possible resulting in a population of as many variances.

The distribution of s^2 is known to be positively skewed, which means that over 50 % of the time, a random s^2 will be less than s^2 (Browne, 1995). The same author showed that using a 100-δ per cent upper one-side confidence limit on s will provide a sample size sufficient to achieve the planned power in at least 100-δ per cent of such trials. The upper limit of s is used since an overestimation of s will act to ensure sufficient sample size for the planned power (Browne, 1995). For the given sample size s^2 was determined from 10,000 bootstrap replicates. From this population the 95% (δ = 5 %) upper limit was used as variance in the calculation of the sample size. ? shows that after 6 iterations convergence occurred to a sample size of 32 leaves. The variance between the different realisations (s^2_{real}) of a sample consisting of 32 leaves was on average, a factor 1.6 higher than the best estimate (s^2_{best}). The ratio between s^2_{real}/s^2_{best} was introduced because s^2 differed between the different approaches due to the use of both resampling algorithms with and without replacement.

- Iterative algorithm to calculate the sample size in number of leaves. a = 0.20, $1-b$ = 0.95 and $m_0 - m_1$ = 0.5 mg.kg^{-1} DW.

Number of iterations	number of samples used	$\frac{S^2_{real}}{S^2_{best}}$	number of samples needed
1	58	1.0	25
2	25	1.9	35
3	35	1.6	30
4	30	1.7	32
5	32	1.6	31
6	31	1.6	32

Although, Kupper & Hafner (1989) demonstrated that formula [3.2] performed quite well, even for small sample size situations. They stressed that the sample sizes so obtained will generally be inadequate for the desired analysis goal. This is caused by the user's ignorance of the fact the sample sizes so computed are only appropriate for simple statistical analyses. It is often the case that a much more complicated statistical method is employed at the data analysis stage. The sample sizes required to ensure that such complicated procedures have adequate precision and/or power can be larger than those based on formula [3.2] (Kupper & Hafner, 1989)

4.4.3 Sample size based on the independent bootstrap

Settings for a , b and the effect size were the same as in the analytical approach. To account for both types of errors the sum of the corresponding *z*-values was used to derive an overall significance level. So the appropriate sample size was reached when 99.74 % of the bootstrapped means fell within a given percentage, corresponding to the effect size, of the mean calculated for the 58 observations. Fig. 9 shows that this approach resulted in a sample size of 21 leaves. A limited effect of sample size on sample mean was observed (not shown) which is similar to the observations in soil science (Rice & Bowman, 1988; Johnson et al., 1990; Starr et al., 1992).

By using fewer observations than the whole data set a variation between the realisations is generated (s_{real}^2). The lowest variation between the realisations (s_{best}^2) was generated when all 58 observations were used. To reduce the ratio s_{real}^2 / s_{best}^2 to a factor 1.6, by analogy with the ratio for which convergence occurred in the analytical approach, a sample size of 36 leaves was needed. The assumption of normality made in the analytical approach was valid. This was illustrated by the fact that both approaches reached the same conclusion i.e. a sample size of 32 leaves for the analytical versus 36 leaves for the bootstrap approach.

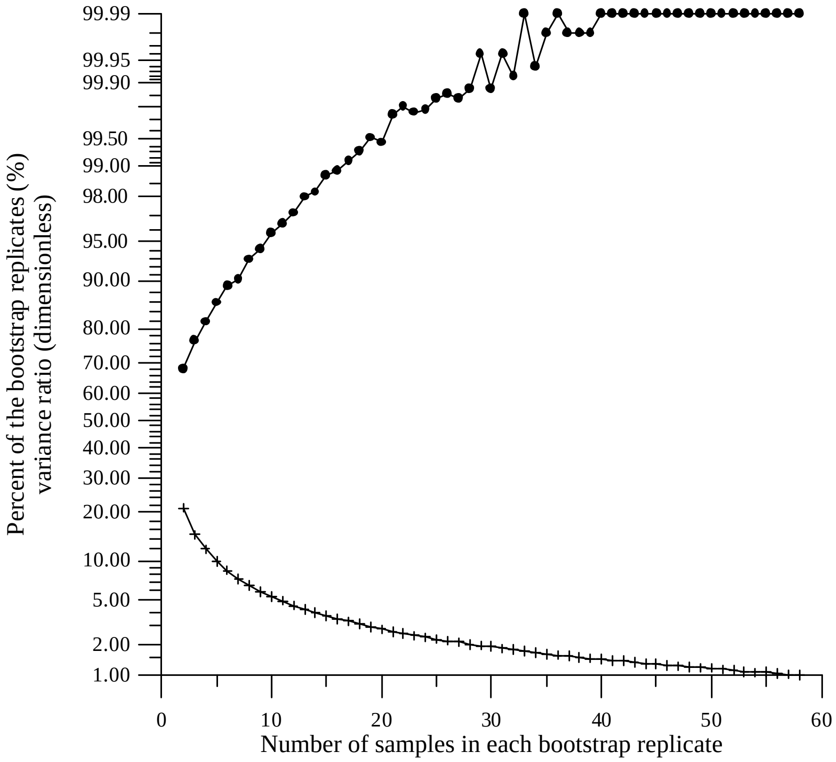


Fig. 9. An independent bootstrap. (-●-) Shows the percentage of the bootstrap replicates that fall within ± 0.5 mg.kg^{-1} DW limits of the true average and (+) shows the ratio s_{real}^2 / s_{best}^2 of the variance

4.4.4 Sample size based on the dependent bootstrap

The analytical as well as the bootstrap method treat the data as observations from an independent distribution. As seen in Fig. 8 leaves are correlated with their distance to the main branch. The bootstrap approach requires a randomisation procedure that mimics the true sampling mechanism that generated the original data. This randomisation procedure was easier to derive than the theoretical formula required by the analytical method, which made the bootstrap an attractive method to apply with spatial correlated data (Shao & Dongsheng, 1995).

The same settings were used for a, b and the effect size as in the analytical approach and the independent bootstrap. Again, the simulations were stopped when 99.74 % of the bootstrapped means fell within plus or minus 0.5 mg.kg^{-1} DW of the mean calculated for the 58 observations. Taking the dependency of the observations in account the number of leaves raised to 30 (Fig. 10). The biggest twig contained 21 leaves so at least leaves from 2 branches should be bulked to compose a sample that generates a reliable estimate of the Cd concentration. If besides a reliable estimate of the Cd concentration the control over the variation between the different realisations is aimed, a sample size of 56 leaves was needed. For this sample size the ratio s_{real}^2 / s_{best}^2 was reduced to the factor 1.6 by analogy with the preceding approaches.

Both bootstrap methods and the analytical approach used the mean value and variance of the 58 leaf samples as a reference. It was assumed that the variance of the 58 leaves came close to the small-scale variance i.e. the variance between leaves. In this way the aim of the study, smoothing the small-scale variance was incorporated in the computations. And so the sample size was sufficient if its mean value and variance came close to the values for the 58 leaves. But the result, the number of leaves that made up a sample, can be influenced by the number of samples taken in the pilot study (Marshall et al., 1992). The methods performed well if the variance of the 58 leaves was a good representation of the small-scale variance. Because the methods were applied on data from a pilot study, the true small-scale variance was not known (Cohen, 1977; Marshall et al. 1992; Browne, 1995) and therefore it was not known if the computed sample sizes will smooth the small-scale variance.

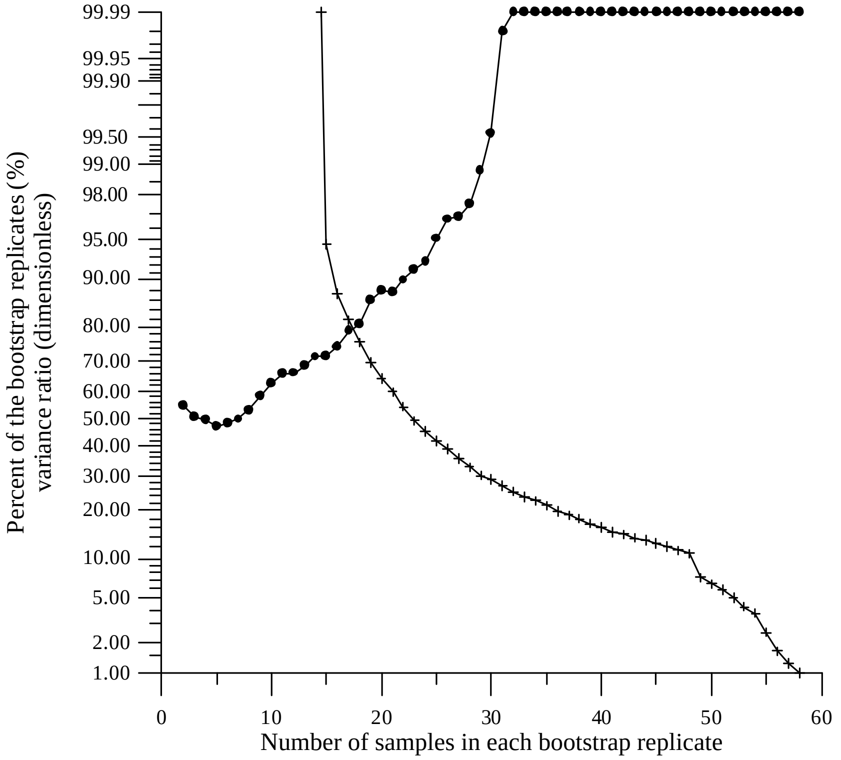


Fig. 10. A dependent bootstrap. (–●–) Shows the percentage of the bootstrap replicates that fall within ± 0.5 mg.kg^{-1} DW limits of the true average and (+) shows the ratio s^2_{real}/s^2_{best} of the variance

4.4.5 Sample size based on a geostatistical approach

The geostatistical approach discussed in this paragraph used the spatial structure of the data to determine the size of a sampling unit. The initial number of samples determines the number of data pairs for a given lag. As can be understood by equation [3.4] the number of pairs influences the semivariance without affecting the structure of the variogram. In- or decreasing the number of samples taken in the pilot study can influence the sill of the variogram, although this number should not affect the range.

The dashed line in Fig. 11 describes the directional semivariance within the branches. For a lag distance of 0.14 m no sill was reached. This means that the Cd concentration in leaves 0.14 m apart from each other is still correlated. To describe the spatial continuity within the entire block data pairs of leaves from different branches should be included in the variogram. Therefore the omnidirectional variogram was calculated. Its experimental data are given in Fig. 11 by bullets, the solid line represents a model of the experimental data. The lower semivariance of the omnidirectional variogram could be caused by the higher number of data pairs for the same lag distances or a true difference in semivariance between the directional and the omnidirectional variogram. The variogram reveals a range of 0.21 m, the Cd concentration in leaves separated by this distance are independent.

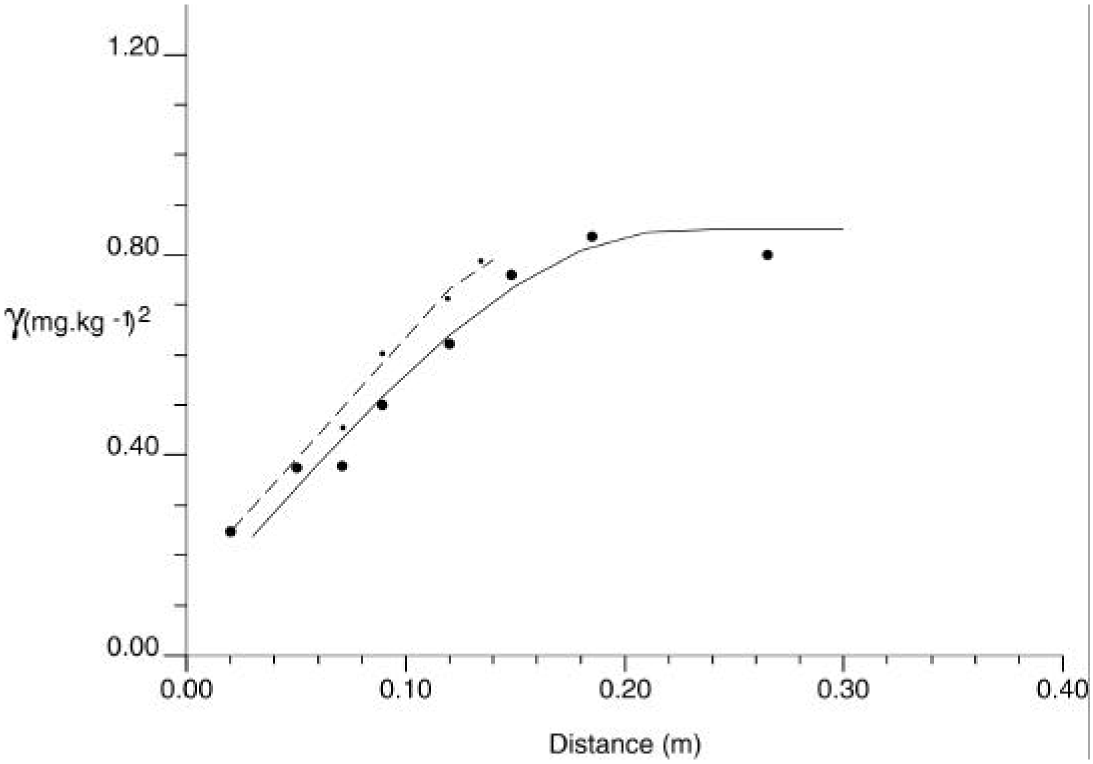


Fig. 11. Detail of the omnidirectional and directional variogram for the Cd concentration in leaves within a 0.4 m by 0.4 m by 0.4 m sampling unit. The full line represents the omnidirectional variogram, the bullets (•) the experimental semivariance. The dashed line represents the variogram within the branches, the bullets (•) the experimental semivariance

For lags between 0.28 and 0.40 m the semivariance showed a sharp increase (Fig. 12). The average Cd concentration of leaves separated by more than 0.28 m is not stationair. The loss of stationarity indicates that the variation in Cd concentration for leaves at a distance smaller than 0.28 m is determined by other spatial processes than the variation at a distance bigger than 0.28 m.

By confronting the variographic results with the aims of the study, the optimal sampling unit can be determined. In the continuation of the study, leaf samples will be used to describe the variation in Cd concentration within the crown of a willow. So the sampling unit should allow describing the large-scale spatial process that determines the variation within a crown. Since the loss of stationarity shown in Fig. 12 leads one to suspect that the sharp increase in semivariance is due to large-scale process a sampling unit should not contain leaves separated by more than 0.3 m. Otherwise the variation originating from this large-scale process will be smoothed.

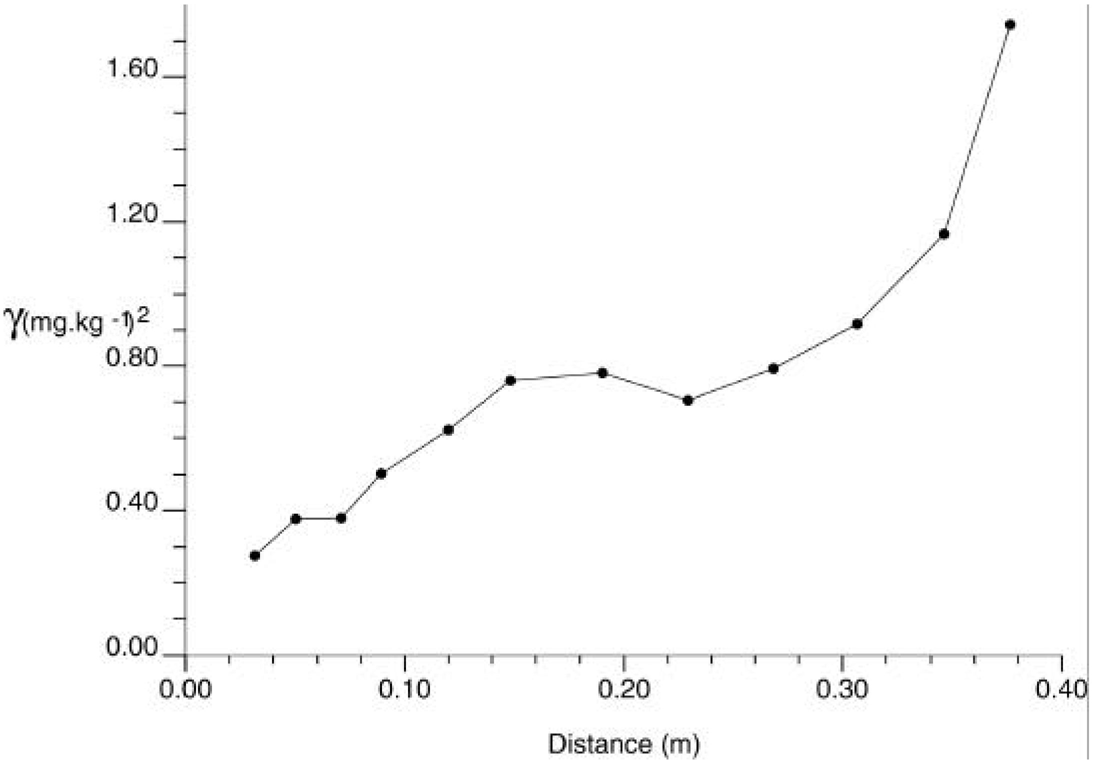


Fig. 12. Omnidirectional experimental variogram for the Cd concentration in leaves within a 0.4 m by 0.4 m by 0.4 m sampling unit. The bullets (•) represent the experimental semivariance, the full line shows the change in semivariance

In the next chapter (see chapter 5) the semivariance of the entire crown is calculated with data from a sampling unit which dimensions agree with a distance at which the sill is reached (0.3 m). Therefore, the semivariance of the entire crown will be lowered with the variance within the sampling unit. Due to the similarity of the Cd concentration in neighbouring leaves smaller sampling units will have a lower variance than bigger ones (Fig. 11). Therefore, lowering the semivariance of the entire crown will be less effective for smaller than for bigger sampling units. For this reason an optimal sampling unit for the continuation of this study should contain leaves that are separated by a distance bigger than the range because at this distance the sill or maximum variance is reached. Reckoning with the above made considerations the dimensions of the optimal sampling unit range between 0.2 by 0.2 by 0.2 m and 0.3 by 0.3 by 0.3 m. The latter was carried as the dimensions of the sampling unit for the study at hand to limit the number of samples.

4.4.6 Leaf clustering

The analytical and the bootstrap method expressed the sample size in number of leaves. For the problem at hand -sampling the entire crown of a tree- the use of a fixed number of leaves as a sampling unit would be inconvenient. Therefore the number of leaves, needed to describe the small-scale variability i.e. the variability between the leaves, had to be converted in a sampling volume likely to contain the specified number of leaves. Beside the sampling volume and number of leaves the related dry weight of each sampling unit is given (?).

- Mean, median, standard deviation and kurtosis of number of leaves and the related dry weight (g) in relation with increasing dimensions of the sampling unit (N=520)

Dimension	Mean	Median	Std. deviation	Kurtosis
Number of leaves				
0.05 x 0.05 x 0.05 m	3	3	1.4	1.1
0.10 x 0.10 x 0.10 m	5	4	3.1	2.3
0.15 x 0.15 x 0.15 m	8	7	5.8	2.4
0.20 x 0.20 x 0.20 m	12	9	10.0	2.0
0.25 x 0.25 x 0.25 m	19	14	15.6	1.6
0.30 x 0.30 x 0.30 m	27	20	23.4	1.3
0.35 x 0.35 x 0.35 m	38	29	33.3	1.1
0.40 x 0.40 x 0.40 m	53	42	46.2	0.7
0.45 x 0.45 x 0.45 m	70	53	62.6	0.4
0.50 x 0.50 x 0.50 m	89	64	81.1	0.1

Table 4.2 Continued

Dimension	Mean	Median	Std. deviation	Kurtosis
Dry weight				
0.05 x 0.05 x 0.05 m	0.6	0.7	0.3	1.2
0.10 x 0.10 x 0.10 m	1.2	1.2	0.7	2.2
0.15 x 0.15 x 0.15 m	1.9	1.6	1.3	1.1
0.20 x 0.20 x 0.20 m	2.9	2.3	2.2	1.3
0.25 x 0.25 x 0.25 m	4.2	3.0	3.7	1.8
0.30 x 0.30 x 0.30 m	6.1	4.0	5.5	1.3
0.35 x 0.35 x 0.35 m	8.7	6.5	7.4	0.3
0.40 x 0.40 x 0.40 m	11.2	8.3	10.3	1.0
0.45 x 0.45 x 0.45 m	15.2	10.4	15.2	1.2
0.50 x 0.50 x 0.50 m	18.3	12.6	18.4	1.1

Because the analytical and the bootstrap method expressed the sample size in number of leaves decisions are based on the number of leaves. The most obvious criterion was the mean number of leaves in a sampling unit. Using the analytical approach 32 leaves were needed to describe the variance, the independent bootstrap resulted in 36 leaves whereas the dependent bootstrap asked for 56 leaves per sampling unit, the corresponding dimensions of a sampling unit are given in ? . Due to the skewness of the number of leaves per sampling unit an important difference between the mean and median existed, especially for the bigger sampling units. The use of the median as a criterion resulted in units 0.05m bigger in all three directions than those obtained with the mean number of leaves per sampling unit. The use of the standard deviation as a criterion was justified by the idea that minimisation of the variance between the sampling units indicates sampling units with a more constant number of leaves. The minimum number of leaves for all dimensions of sampling units equalled one but the maximum number of leaves increased from 9 to 355 with increasing dimensions. This caused the standard deviation to be an increasing series (?) so the best result was obtained for the units of 0.05 m by 0.05 m by 0.05 m. Based on the same idea as minimising the variance, the kurtosis as a measure of the extent to which observations cluster around a central point was maximised. This resulted in sampling units 0.15 by 0.15 by 0.15 m.

- Dimension of a sampling unit optimised for four criteria: mean, median, standard deviation and kurtosis for the analytical, the independent and the dependent bootstrap

Metric dimensions	Mean	Median	Std. Deviation	Kurtosis
Analytical method	0.30x0.30x0.30	0.35x0.35x0.35	0.05x0.05x0.05	0.15x0.15x0.15
Independent bootstrap	0.35x0.35x0.35	0.40x0.40x0.40	0.05x0.05x0.05	0.15x0.15x0.15
Dependent bootstrap	0.40x0.40x0.40	0.45x0.45x0.45	0.05x0.05x0.05	0.15x0.15x0.15

The dimensions obtained by using the standard deviation and the kurtosis as a criterion were smaller than those obtained by the mean and the median (?). Using a sampling unit with small dimensions would likely result in an insufficient number of leaves to filter the variation between leaves, called the small-scale variance. Therefore it was concluded that the mean or the median should determine the dimensions of the sampling unit.

? show sizes of sampling units used to study the mean and variance of elemental concentrations in tree crowns. None of the authors elaborated on the determination of these sample size and therefore it is not known if these elementary sampling units were representative for the respective aims of the studies. The forthcoming motivation was suggestive of the fact that the size of a sampling unit was determined by the amount of tissue needed for the chemical analysis rather then by precision thought to be necessary for the goals of the study. In the cited work, no clear relation between the goals of the study and the size of the sampling unit was specified.

With the exception of some authors e.g. Ellis (1975), McLennan (1990) and UN/ECE-EC (1998) the sample weight of the representative elementary sampling units were bigger than the ones proposed for the description of Cd in *Salix fragilis* L.. The influence of the assumptions related with the different approaches on the size of a representative elementary sampling unit fall out of the range of the sample sizes referred to in ? . Some authors e.g. Morrison (1985), Erdmann et al. (1988), Koricheva & Haukioja (1995), Kozlov et al. (1995) and Alfani et al. (1996a) prescribe a number of leaves that fall within the range of a representative elementary sampling unit for the Cd concentration in the crown of *Salix fragilis* L. All tree species with the exception of *Betula spp.* investigated by these authors have big leaves compared to willow leaves. The specified number of leaves will result in a big leaf biomass compared to the biomass a representative elementary sampling unit for the Cd concentration in the crown of *Salix fragilis* L..

- Sample size in dry weight (DW), fresh weight (FW), number of leaves or surface area of the leaves

Author	Tree pecies	Sample size
Guha & Mitchell, 1965	*Acer pseudoplatanus* L.	50 g DW
	Aesculus hippocastanum L.	50 g DW
	Fagus sylvatica L.	50 g DW
Ellis, 1975	*Acer saccharum* Marsch..	15 g DW
	Fraxinus americana L.	15 g DW
	Prunus serotina Ehrh.	15 g DW
Bowerox & Ward, 1977	*Salix viminalis* L.	60 leaves
Insley et al., 1981	*Tilia spp.*	600 cm^2
Ricklefs & Matthew, 1982	34 deciduous tree species	30-40 g FW
Morrison, 1985	*Acer saccharum* Marsh.	30-40 leaves
	Betula alleghaniensis Britton	30-40 leaves
Trlica et al., 1985	*Populus sargentii* Dode	50 leaves
Nilsson & Ericsson, 1986	*Prunus serotina* Ehrh.	70 g FW
Bernier & Brazeau, 1988	*Acer saccharum* Marsh.	70-90 leaves
Erdmann et al., 1988	*Acer rubrum* L.	50 g FW
		± 50 leaves
McLennan, 1990	*Populus trichocarpa* Torr. & Gray ex Hook	30 g FW
Granier & Chevreuil, 1992	*Platanus vulgaris S.*	20 leaves
Keller et al., 1994	*Fagus sylvatica* L.	200 g FW
Kim & Fergusson, 1994	*Aesculus hippocastanum* L.	12 leaves
Koricheva & Haukioja, 1995	*Betula spp.*	30 leaves
Kozlov et al., 1995	*Betula pubescœns tortuosa* Ledeb.	50 leaves
Alfani et al., 1996a	*Quercus ilex* L.	30 leaves
UN/ECE-EC, 1998	*All species*	20-30 g FW

Assuming all other conditions equal to those given in this study the use of such sample sizes is, due to smoothing, likely to result in a poor description of the variation between branches within the crown, called the large-scale variation. Therefore, objectifying the determination of the size of a representative elementary sampling unit is an improvement irrespective of the mathematical approach used.

4.5 Conclusion

Fifty-eight leaves on the same twig and twigs on the same branch showed a variation in Cd concentration ranging from 1.3 to 6.3 mg Cd kg^{-1} DW. Given the aim of the study, the dimensions of a representative elementary sampling unit determined with the analytical or classical approach were 0.35 m by 0.35 m by 0.35 m such a sampling unit contained 30 leaves. Leaving the assumption of normality increased the number of leaves in a representative elementary sampling unit to 36. This number of leaves was found in a unit of 0.40 m by 0.40 m by 0.40 m. A dependent bootstrap, accounting for the spatial correlation of the Cd concentration resulted in a sampling unit including 56 leaves. The number of leaves found in a sampling unit of 0.45 m by 0.45 m by 0.45 m. The previous approaches assumed that the variance of the 58 leaves was a good estimator of the small-scale variance. The validness of this assumption could not be tested and was therefore ones guess. The geostatistical approach free of this assumption used instead the spatial structure of the data to determine the sample size. It was concluded that a sampling unit with dimensions of 0.30 m by 0.30 m by 0.30 m did not affect the large-scale variance in Cd concentration. In the continuation of the study these dimensions are used as a representative elementary sampling unit to describe the spatial variability of the Cd concentration variation in the crown of a willow tree. Thus far the reported determination of the size of a sampling unit is based rather on practical than on scientific deliberation. Most of the reported sample sizes were bigger than the ones calculated in this study. Therefore, objectifying the determination of the size of a representative elementary sampling unit is an improvement irrespective of the mathematical approach used. Violating the assumptions on which the methods are based can result in loosing part of the improvements made by objectifying the determination of the size of a representative elementary sampling unit. At the start of a sampling campaign a pilot study should gain insight in the heterogeneity and the sample size requirements.

5 Spatial variability of cadmium in the crown of a *Salix fragilis* : implications for leaf sampling

5.1 Abstract

Elevated metal inputs become toxic to trees and eventually distort the entire forest ecosystem. Plant analysis is a valuable tool to evaluate the pollution level. However, leaf sampling is complicated due to the high variability within the crown. To investigate the variability of cadmium in the leaves of a tree, leaves of one willow tree (*Salix fragilis* L.) were sampled at 292 locations. The Cd concentration was found to be normally distributed within a range from 2.4 to 10.5 $mg.kg^{-1}$, with an average of 6.3 $mg.kg^{-1}$ dry weight or expressed per unit dry ash 23.1 to 73.0 $mg.kg^{-1}$, with an average of 51.2 $mg.kg^{-1}$. A clear trend was found with high Cd values in the lower parts of the crown and low concentrations in the top. After removal of this trend, with both methods of expression, the residuals showed a clear and analogous spatial structure modelled by an omnidirectional variogram. The Cd distribution in the leaves of the entire tree was predicted by sequential indicator based simulations. These results were used to evaluate the current sampling strategy for tree leaves i.e. sampling the sun leaves of the upper third of the tree crown. Differences between dry weight and dry ash as methods of expressions were not such that they add to the fundamentals of the discussion. For both methods it was found that the current sampling strategy could yield biased estimates of the average Cd concentration. If the goal of the sampling would be to evaluate the risk of contamination by Cd, the current sampling procedure was also found unsatisfactory. An alternative sampling procedure is proposed. This procedure investigates whether a trend is present. Once the height where sampling will result in a correct statement of the tree's pollution is located, the rest of the stand is sampled at this height.

5.2 Introduction

Little is known about the distribution of elements in tree crowns, although it represents indispensable information for methodological studies. Consequently, research papers on the methodology and procedures for taking a representative leaf sample in deciduous trees are rare (exceptions include Guha & Mitchell, 1965; Ellis, 1975; Morrison, 1985). Traditionally, studies on chemical composition of leaves target sun leaves from the upper third of the crown (Leaf, 1973; van den Driessche, 1974; UN/ECE-EC, 1998).

This chapter focuses on the description of the spatial distribution of Cd concentrations in leaves of a willow tree. It was thought that geostatistical tools could improve the prediction of the Cd concentration at non-sampled locations. Therefore, one willow was sampled intensively enough to allow a geostatistical analysis of the Cd spatial distribution within this tree. A set of equiprobable realisations of Cd concentration both expressed on dry weight and dry ash, obtained through conditional stochastic simulations, was used to analyse the leaf sampling strategies for two different goals. The first goal was taking one representative sample and the second goal was assessing the risk that a Cd threshold for leaves is exceeded. To enable this evaluation it was tested whether the time span of sample collection had an effect on the Cd concentration and whether the samples were spatially even distributed.

5.3 Data processing

5.3.1 Variograms

Variograms were calculated according to equation [4.3] given in chapter 4. The data set used in the previous chapter was simplified from a three to a two dimensional data set. Because the data subject to processing in this chapter were three dimensional, the variograms were determined by their (i) number of lags, (ii) lag separation distance, (iii) lag tolerance, (iv) azimuth angle, (v) tolerance on the azimuth angle, (vi) azimuth bandwidth, (vii) dip angle, (viii) dip tolerance and (ix) the dip bandwidth (Goovaerts, 1997; Deutsch & Journel, 1998). All variograms were calculated for 12 lags ranging from 0.3 to 5.6 m. The lag distance was an increasing series with separation distances from 0.1 to 0.3 m for lags smaller than the range widening to 0.6 and finally 0.9 for lags near the range of the semivariogram. Tolerance on the lags was set to the conventional half of the separation distance. To obtain an omnidirectional variogram azimuth and dip angle were set to 0°, tolerance on both angles was set to 90° and both bandwidths to 8.2 m being the tree height and so a physical limit. Following calculation of the variogram, to these experimental data a continuous curve was modelled allowing a continuous description of the semivariance over a range of **h** values (McBratney & Webster, 1986).

5.3.2 Stochastic simulation

To compute the Cd concentration in leaves at non-sampled locations the more complex simulation approach was preferred above Kriging. Goovaerts (1997) demonstrated that the Kriging variance is independent of the data values. Due to this independence its suitability as an estimator of the local uncertainty is questioned. As an alternative the local uncertainty can be calculated from a series of simulations, calling for the simulation approach. The sequential indicator algorithm was withdrawn; it is able to reproduce the connectivity between extreme values, it honours the true data and it allows to account for K+1 class-specific patterns of spatial continuity through an indicator coding according to a set of K threshold values z_k. Therefore each observation was coded as an indicator according to:

[3.5] $$i(\mathbf{x}_a; z_k) = \begin{cases} 1 & \text{if } z(\mathbf{x}_a) \leq z_k \\ 0 & \text{otherwise} \end{cases} \qquad k = 1, 2, \ldots, K.$$

with $i(\mathbf{x}_a; z_k)$ = indicator for the attribute value at location $\mathbf{x}_a$ with a threshold z_k

Then, the indicator semivariance $\gamma_I(\mathbf{h}; z_k)$ is obtained from :

[3.6] $$g(\mathbf{h}; z_k) = \frac{1}{2N(\mathbf{h})} \sum_{a=1}^{N(\mathbf{h})} \left[i(\mathbf{x}_a; z_k) - i(\mathbf{x}_a + \mathbf{h}; z_k) \right]^2$$

with $\gamma_I(\mathbf{h}; z_k)$ = indicator semivariance

$i(\mathbf{x}_a; z_k)$ = indicator for a threshold z_k for the attribute value at location $\mathbf{x}_a$

$i(\mathbf{x}_a + \mathbf{h}; z_k)$ = indicator for a threshold z_k for the attribute value at location $\mathbf{x}_a + \mathbf{h}$

$N(\mathbf{h})$ = number of pairs of data locations a vector **h** apart

The sequential indicator based simulation proceeds as follows (Goovaerts, 1997):

- A random path is defined visiting each node of the grid only once.
- At each node:

1. Determine the *k* conditional cumulative distribution function (ccdf) values using the indicator kriging algorithms. The conditioning information consists of indicator transforms of neighbouring original attribute data and previously simulated attribute values.
2. Correct for any order relation deviations. Then build a complete ccdf using interpolation and extrapolation algorithms.
3. Draw a simulated value from that ccdf.
4. Add the simulated value to the conditioning data set.
5. Proceed to the next node along the random path, and repeat steps 1 to 5.

- Repeat the entire procedure with a different random path to generate another realisation.

Order relation deviations are supposed to be kept at a reasonable level if sampling sparsity is avoided (Goovaerts, 1997). Therefore, the number of classes should be as low as possible. A low number of classes also limits the variogram modelling time and reduces the computation cost. At the other hand, smoothly varying variogram parameters -nugget, sill and range- from one threshold to the next reduces the number of order relation deviations (Goovaerts, 1997). But, this characteristic demands a series of variograms. As an optimum between these two opposite demands the number of indicator variograms was limited to four (k = 4) i.e. five classes each with 20 % of the data were distinguished.

The ccdf was interpolated between the minimum and maximum observed data values using the ccdf values for the four threshold values z_k. The interpolation models were based on the behaviour of the cdf of the data. The lower tail and the values in between two classes of the ccdf were interpolated using the tabulated bound model (Deutsch & Journel, 1998), the upper tail was modelled with a nearly linear hyperbolic model (Deutsch & Journel, 1998).

5.4 Results and discussion

The majority of publications used dry weight as method of expression. To be significant for present sampling practices, this study concentrates on the variability of Cd expressed per unit dry weight. Nevertheless some authors (e.g. Ovington & Madgwick, 1958; Woodwell, 1974; Lea et al., 1979 a, b; Claussen, 1990) were aware of the effects of growth dilution on the element concentrations expressed per unit dry weight. For this reason, Ovington & Madgwick (1958) used dry ash as a reference to express nutrient concentrations, as did Claussen (1990) to express Cd concentrations. For completeness both methods of expression i.e. concentration referenced to dry weight and concentration referenced to dry ash are applied on the data set. The results are presented and discussed in separate sections (see § 5.4.1 and § 5.4.2).

5.4.1 Cd concentration referenced to dry weight

5.4.1.1 Explorative data analysis

The null hypothesis was formulated that due to the 15 days period needed to collect the samples, the Cd concentration was influenced by the day of sampling. By a one way Anova with unequal variances, it was shown that the average cadmium concentration for samples taken from approximately the same height at different days was equal. This was true for the samples taken at days 3, 9 and 15; 1 and 7; 2 and 8; 4, 10 and 14 and 10, 11, 12, 13 and 14. Fig. 13 shows the relation between the Cd concentration and the day of sampling. Combining Fig. 13 with Fig. 3.4 the results of the one way Anova are confirmed. It was concluded that the Cd concentration at a given sampling location was consistent within a 12-day period and so the null hypothesis was rejected. The lack of a fast influence of crown reduction on the Cd concentration can be supported by the results for N in conifers (Piene, 1980; Piene & Percy, 1984; Ericsson et al. 1985). An increased N concentration occurred 1 to 3 years after strong crown reduction.

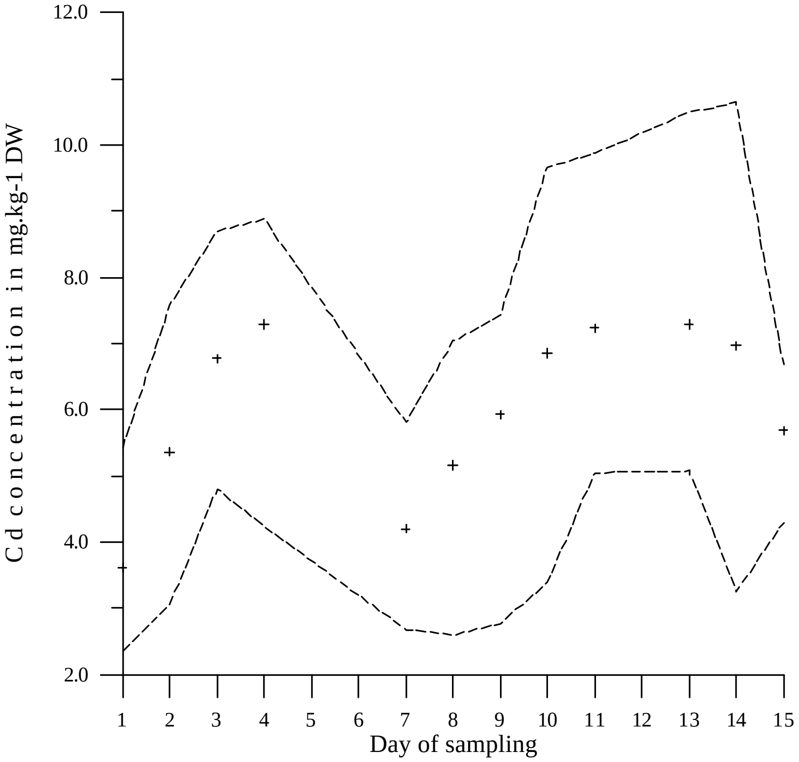


Fig. 13. Daily average (+), minimum and maximum (---) Cd concentration in mg.kg^{-1} DW versus the day of sampling

A Kolmogorov-Smirnov test was used to test the null hypothesis that the 292 Cd samples came from a normal distribution (Neter, 1996). This could be accepted with a probability of 0.59. Also the histogram (Fig. 14) indicates a symmetrical unimodal distribution around an average Cd concentration of 6.3 mg.kg^{-1} DW and with a coefficient of variation of 26 %. The Cd concentration varied between a minimum concentration of 2.4 mg.kg^{-1} DW and a maximum of 10.6 mg.kg^{-1} DW.

Sampled locations have to be spatially representative for all possible locations within the crown. A visual comparison between the distribution of the 1136 possible sampling locations with the positions of the 292 analysed locations was performed using a so called Q-Q graph (Isaaks & Sirvastava, 1989). Therefore the quantiles of the position at a given axis were calculated and plotted against each other. This was repeated for the three main directions, called X, Y and Z-axes (Fig. 15). Only small departures of the bisector were found indicating that both distributions were very similar. This means that sampling was not preferential so it was concluded that there was no need to spatially decluster the 292 samples.

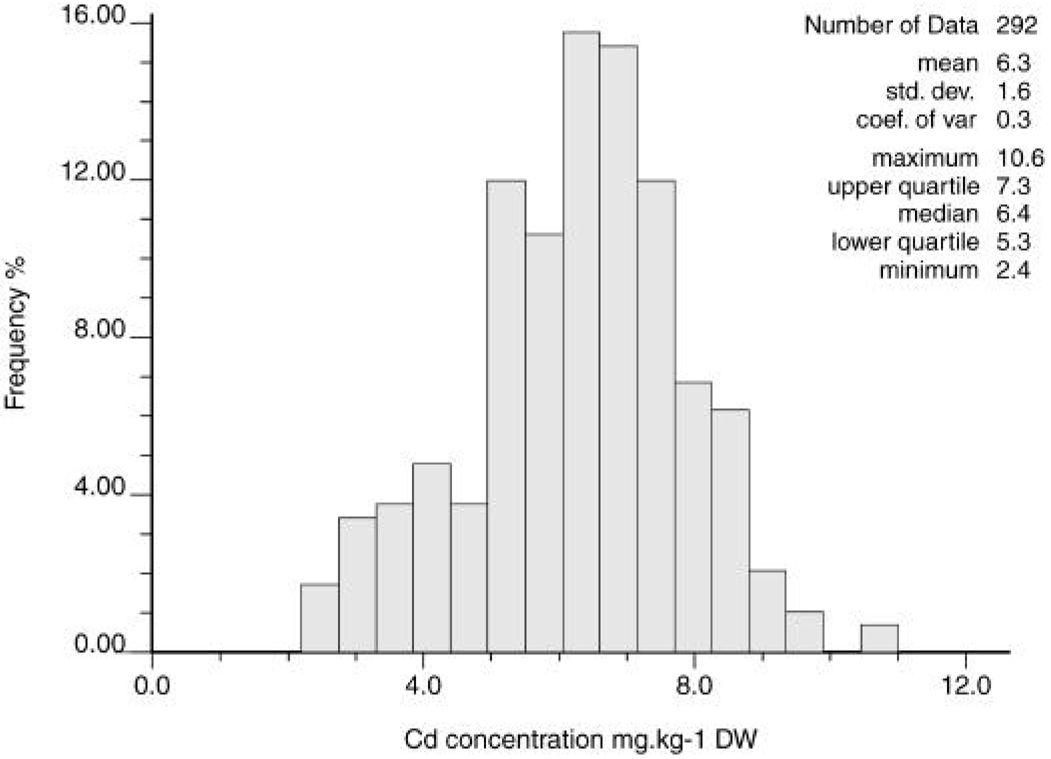


Fig. 14. Histogram of the 292 Cd concentrations with some descriptive statistics

To use the variogram as a measure of the spatial structure of the attribute it is important that the spatial behaviour of the variable is stationary, i.e. free of a trend. Because the tree stood at the western edge of the stand influence from its position on the Cd concentration could be expected. To check for the presence of a trend, the observations were plotted according to the three major orientations and the Pearson correlation coefficient was calculated. A weak correlation coefficient was found within the XY-plain. Along the X-axis (corresponding with the W-E direction) a correlation coefficient of 0.10 was found. The Y-axis (corresponding with the S-N direction) showed a correlation coefficient of -0.01. So for both orientations the absence of a trend was assumed. However, the Z-axis (represented height above ground) revealed a correlation of -0.65, indicating high Cd values in the lower part of the tree. The shape of the plot indicated a second order polynomial (eq. [3.7]) was most suitable to represent the relationship between leaf Cd concentration and height. This was confirmed by its correlation coefficient (0.47).

[3.7] $\mathrm{Cd} = 7.31 + 2.34.H - 9.28\ 10^{-2}.H^2$

with:

Cd = Cadmium concentration ($\mathrm{mg.kg^{-1}}$ DW)

H = tree height above ground (m)

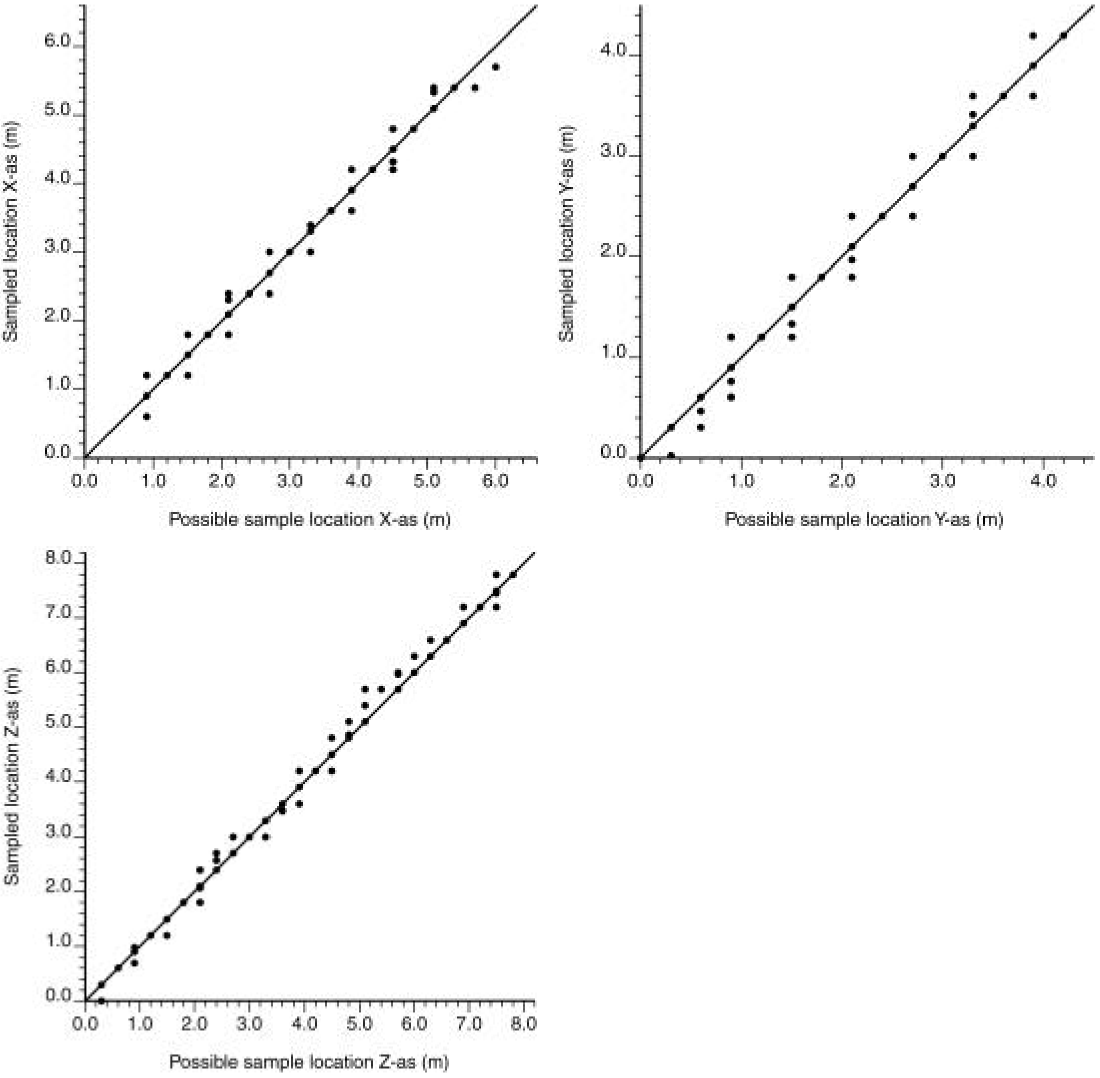
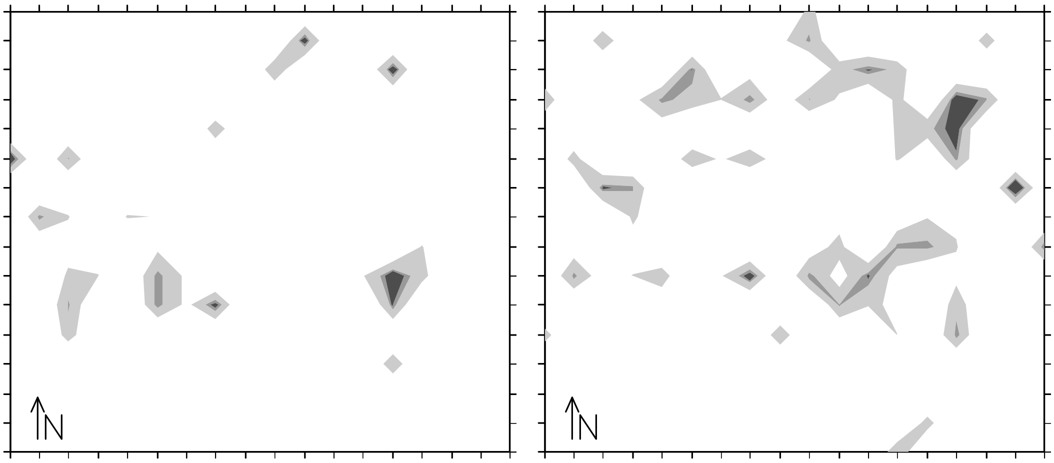
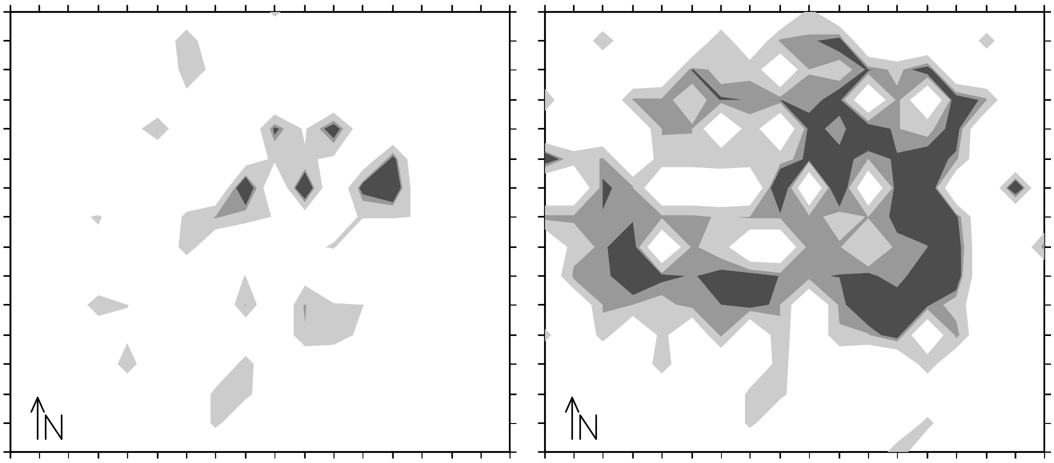


Fig. 15. Q-Q graphs showing possible sampling locations (m) versus sampled locations (m) along the X,Y and Z-axis

The above used approach would, if present, smooth a local correlation between the Cd concentration and the light-character of the leaves. The existence of distinct sun- and shade-leaves is thought to result in the presence of an inner and outer crown (Farago, 1994). To test whether differences in Cd concentrations could be explained by the presence of an inner and outer crown, relative Cd concentrations were used. Each observation was divided by the average Cd concentration for that height. Using the Nearest Neighbour algorithm surfaces were interpolated from the data points. Within this algorithm duplicate data were averaged. These way areas with a lower or a higher Cd concentration than expected at that height were delimited. The presence of an inner and outer crown would be confirmed by a circular pattern of the relative Cd concentrations. No clear evidence was found to confirm the existence of an inner and outer crown for the lower, the middle, the upper third or the whole crown (Fig. 16). So in the XY-plain isotropic behaviour of the Cd concentration was assumed. The anisotropic behaviour along the Z-axis was best described by the second order polynomial given in equation [3.7].



Lower third of the crown (0 - 2.7 m) Middle third of the crown (3.0-5.4 m)



Upper third of the crown (5.7-8.1 m) Whole crown (0 - 8.1 m)

Fig. 16. Relative Cd concentrations in the crown of *Salix fragilis* L. Dark gray delimites parts of the crown with a relative Cd concentration bigger than 1, gray parts equal to 1 and light gray parts smaller than one (Top view)

The vertical trend can be explained by dilution and translocation, processes often found responsible for the changing distribution of essential elements within the growing season (Guha & Mitchell, 1966). A decreasing concentration accompanied with an increasing or stable mineral content can be explained by *dilution*. Because the determination of the element concentration is a destructive process an additional condition has to be fulfilled. The initial element concentration in tissues that will be sampled at different times has to be equal. The increased supply of one nutrient result in an increased dry matter production and therefore the concentration, which is expressed per unit of dry matter, of other nutrients is reduced. This type of observations is not solely explained by dilution as is confirmed by the fact that the relative decrease in the same leaves is often not the same for all elements (Guha & mitchell, 1966). No research was found to question the use of dilution in explaining changes in heavy metal concentrations during growing season or within the crown of a single tree.

An increasing nutrient concentration and/or content can be due to nutrient *translocation*. Again it has to be assured that the initial element concentration in tissues that will be sampled at different times was equal. Within a season nutrients can be transported or retranslocated to regions of new growth. At the end of the growing season nutrients are retranslocated to new meristematic regions, for next year's growth (Combes, 1926; in Insley, 1981 b). Also nutrient loss during the growing season e.g. leaching by precipitation, can be compensated by retranslocation (Leaf, 1973, van den Driessche, 1974; Grigal et al., 1976). Nutrients are retranslocated at the end of the growing season from the leaves to the twigs (Tamm, 1951). At bud break the nutrients are translocated to the expanding leaves. In this way an internal nutrient recycling is established satisfying a significant proportion of the demand for nutrients for the production of new biomass (Chapin & Kedrowski, 1983; Meier et al., 1985; Lim & Cousens, 1986). Switzer and Nelson (1972) estimated that, in a 20 year old plantation of *Pinus taeda* L., internal recycling accounted for 20 to 30% of the trees' requirements for N, P, and K and 7 % for Ca. Similar estimates for N and K in *Alnus rubra* Bong. suggest contributions from internal recycling of 50-60% of the trees' requirements 5 to 10 years from planting (Miller, 1986). For Mg Helmisaari (1992) reported internal recycling amounts of 7 to 20 % of the demand in *Pinus sylvestris* L.. Because non-essential elements are not known to have a physiological function in the plant, there is no reason for the plant to retranslocate these non-essential elements to new meristematic regions. Plants could have other reasons to translocate heavy metals. It was observed that heavy metals were retranslocated from the roots to the leaves just prior to abscission. Leaf fall was then used as a way to remove excessive heavy metals (Ford, 1986; Sanità di Toppi & Gabbrielli, 1999).

Sander and Ericsson (1998) give a start to explain the observed Cd trend. They found one month prior to bud break a nearly uniform Cd distribution in the woody biomass of *Salix viminalis* L. which could lead to an initial uniform Cd distribution in the leaves. Then the increased supply of a nutrient could have resulted in an increased dry matter production, which for its part results in a reduction of the Cd concentration per unit of dry weight. The highest dry matter production is expected in the top of the crown because of the more favourable light conditions. Due to dilution of Cd within the tree during the growing season, the top leaves should display the lowest Cd concentrations, as can be observed in Fig. 17. The tree tries to maintain favourable growth conditions in the metabolic active part of the crown. Therefore Cd is retranslocated from the metabolic active top of the crown to the bottom of crown. This process reinforces the trend in Cd concentrations.

Besides translocation and dilution changing nutrient concentrations can be allocated to accumulation, leaching, uptake, luxury consumption, deficiency, sufficiency, toxic excess, antagonistic excess, water availability, and treatment (Haase & Rose, 1995). Although, the effects of some of these processes on metal concentrations in leaves are till present unknown, time dependent accumulation can explain the trend in Cd concentration. Leaves on the same tree differ in age. Mayer (1976) observed that in springtime leaves in the lower part start to develop earlier than leaves in the upper part of the crown. Therefore, leaves in the lower part are older than leaves in the upper part of the crown. This effect is reinforced by the fact that during the growing season new shoots develop mainly in the top of the crown. If accumulation is time related, the older leaves will show a higher Cd concentration than the younger leaves, which would result in a Cd distribution as observed in Fig. 16.

Atmospheric Cd deposition was considered but found unsatisfactory to explain the observed Cd trend (see § 3.1).

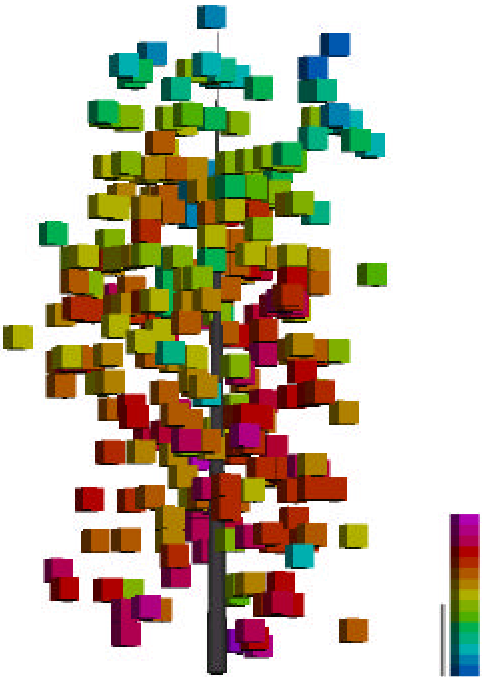


Fig. 17. Sampled Cd concentrations within the studied tree. Cd scale ranges linearly from 2.4 (blue) to 10.7 mg.kg^{-1} DW (purple); the grey bar next to the color scale represents a height of 1 m (North-western view) n = 292

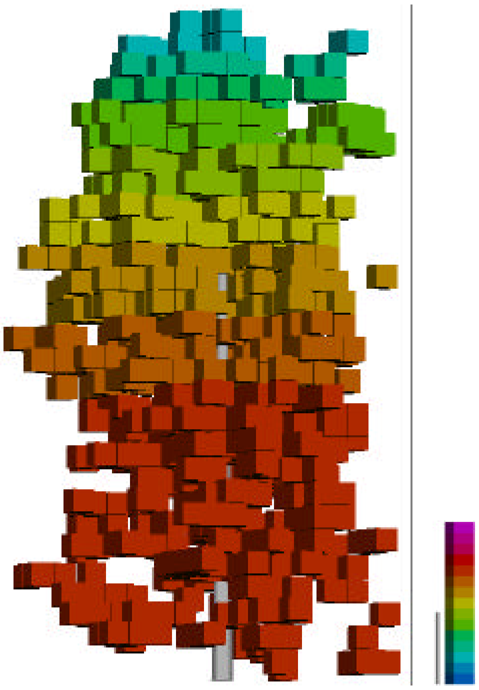


Fig. 18. Cd concentration calculated with deterministic trend given by equation [3.7] at all possible sampling unit locations. Scale ranges linearly from 2.4 (blue) to 10.7 (purple) mg.kg^{-1} DW; the grey bar next to the color scale represents a height of 1 m (North-western view) n = 1136

Fig. 18 shows for every sampling unit the Cd concentration calculated according to the fitted deterministic trend (Eq. [3.7]). The uncertainty of the predicted value can be obtained from the standard errors of the model parameters (not given). This resulted in a prediction interval widening from 1 mg Cd.kg^{-1} DW at ground level to 5.3 mg Cd.kg^{-1} DW at the top of the crown. But, as Fig. 19 shows, a large variation around the fitted trend is present in the true Cd values. The root mean square error was 1.2 mg Cd. kg^{-1} indicating a rather imprecise prediction of the Cd concentration in leaves of the studied willow leaves. This endorsed the hypothesis that a part of the information, although present in the data set, was not used. As with many natural phenomena spatial continuity was believed to be an essential feature. To study, and if present, to incorporate, the spatial continuity in our analysis geostatistical procedures were used. Our aim was to improve the prediction of the Cd concentration at non-sampled locations.

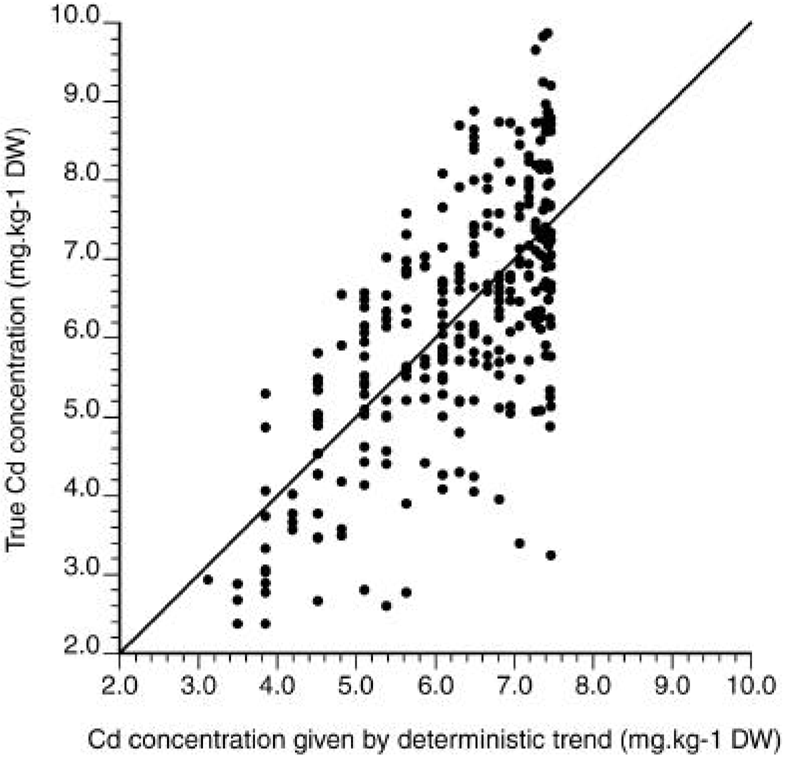


Fig. 19. Cross validation of the predicted values given by the deterministic trend versus the values at the sampled locations ($mg.kg^{-1}$ DW)

In order to obtain stationary residual concentration values, Cd concentration predicted by Eq. [3.7] were subtracted from the measured concentrations. The residuals could be considered to follow a normal distribution ($p = 0.623$) with $\mu = 0.0$ and $s = 1.2$ (Fig. 20). The correlation coefficient between height and these residuals was 0.00, so it was decided that detrending was successful. All further calculations were conducted on these residuals.

From a statistical point of view, detrending might not have been necessary. Because sampling locations are evenly distributed over the entire tree and the range of the variogram is rather small compared to the size of the entire tree, the simulation algorithm would probably not have been affected by the trend. But from a biological point of view we decided to model the trend explicitly to draw the attention to the existence of both a vertical trend and spatial dependency between Cd concentrations.

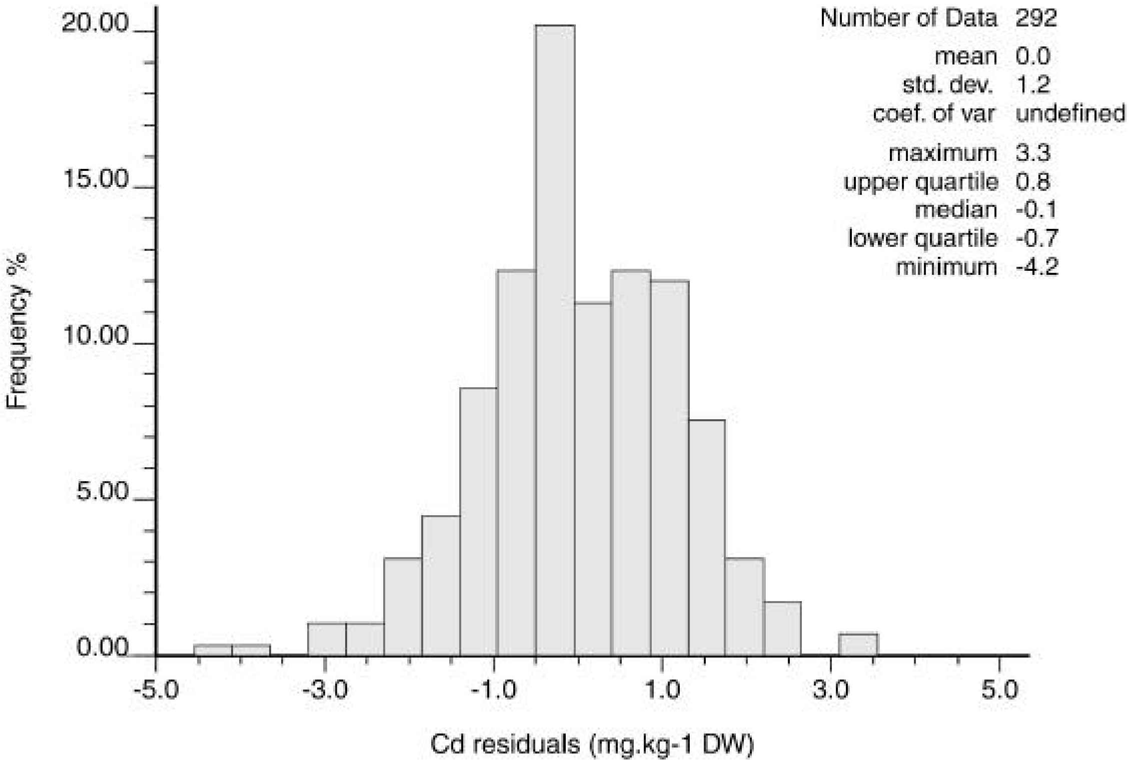


Fig. 20. Histogram of the Cd residuals with some descriptive statistics

5.4.1.2 Variograms

No difference was found between the directional variograms along the three major axes of the residuals, so the removal of the trend resulted in an isotropic behaviour of the spatial variance of the Cd concentration. Therefore, the experimental omnidirectional variogram of the residuals was calculated (Fig. 21).

A nested model, combining a nugget effect with an exponential and a spherical model, was fitted through the experimental values :

$$[3.8] \qquad \gamma(\mathbf{h}) = 0.15 + 1.03\left[1.5\frac{\mathbf{h}}{0.81} - 0.5\left(\frac{\mathbf{h}}{0.81}\right)^3\right] + 0.22\left[1 - \exp\left(-\frac{3\mathbf{h}}{3.63}\right)\right]$$

The model (Fig. 21) shows a nugget effect of 10 % of the total variance. This indicates that random sources of variation e.g. measurement errors, as well as variation at lag distances smaller than the shortest sampling interval were rather small. However, at a lag distance of 0.8 m already 80 % of the total variance, expressed by the sill, was encountered. This indicates the relatively small scale over which the spatial distribution of the Cd residuals is strongly autocorrelated.

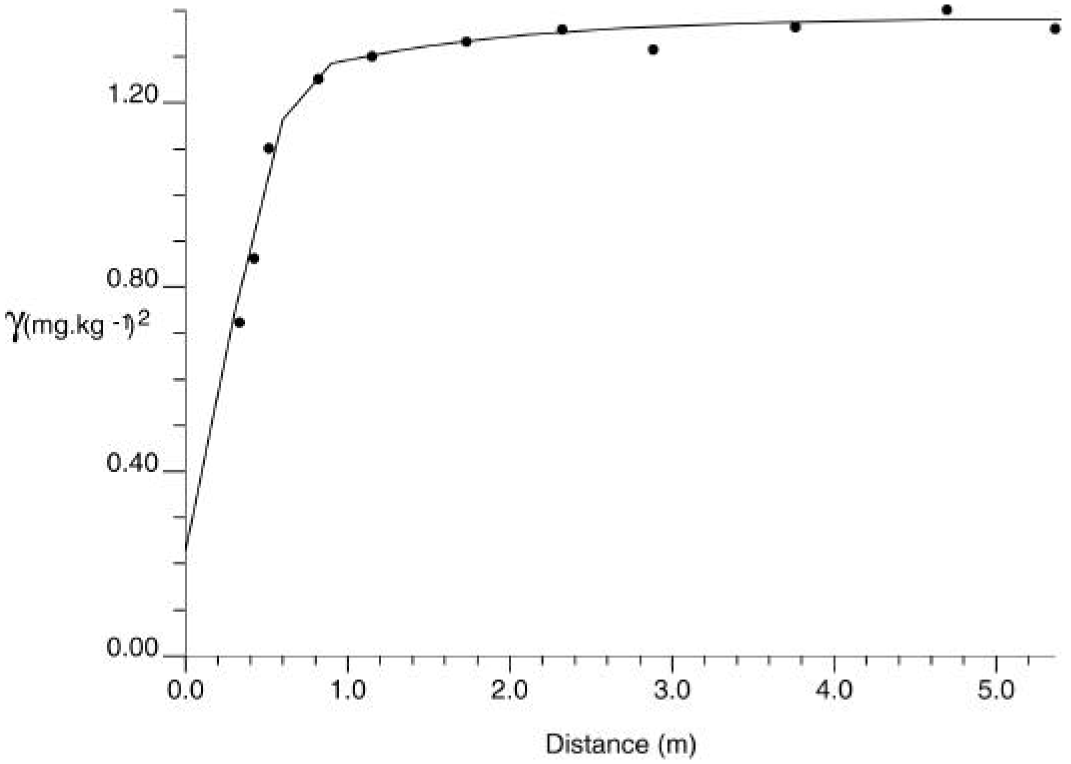
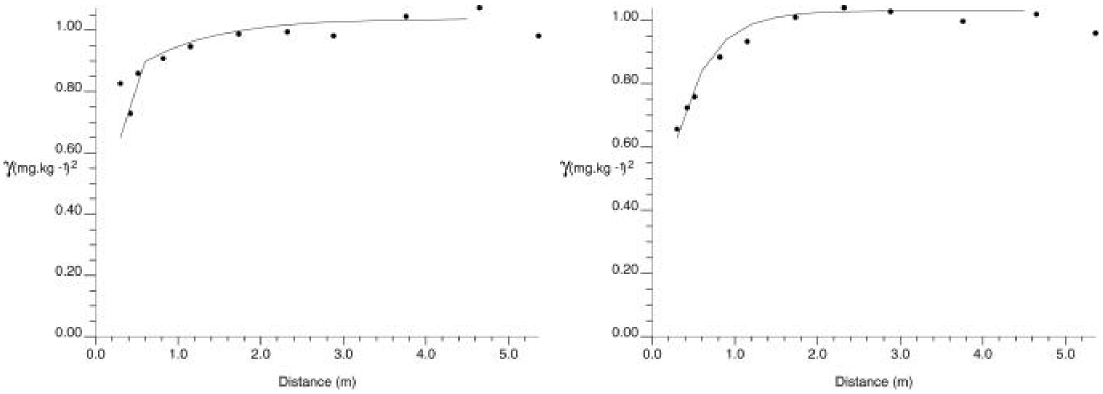


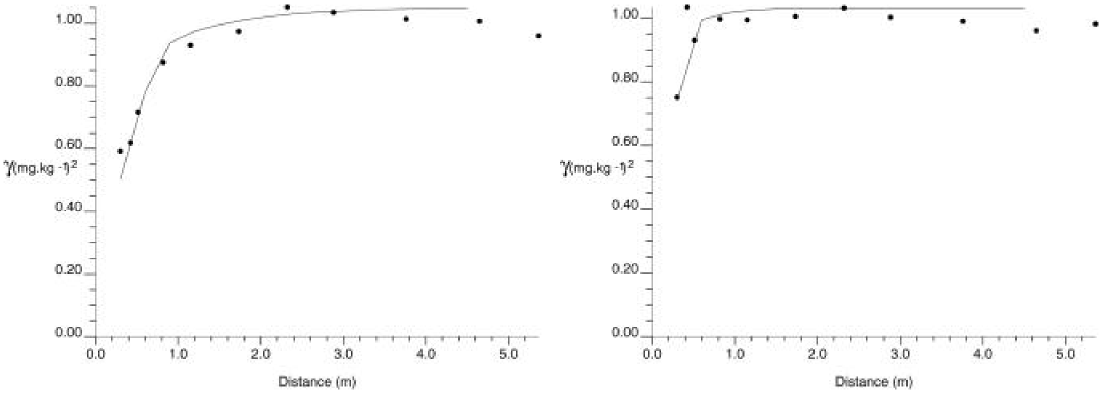
Fig. 21. Omnidirectional variogram for Cd concentration residuals; the curve represents the fitted model, the bullets are the experimental semivariances

Based on the cumulative distribution function (cdf) of the Cd residuals, threshold value z_k were obtained at the 0.2, 0.4, 0.6 and 0.8 quantiles : -0.91, -0.30, 0.29 and 1.04 respectively. Using Eq.[3.5] every residual ($z(\mathbf{x}_\alpha)$) was transformed into four indicators $i(\mathbf{x}_\alpha\ ;\ z_k)$. Four omnidirectional indicator variograms were calculated (Eq.[3.6]). These were modelled with a nested structure of an exponential and a spherical model for the indicators of the 0.2, 0.6 and 0.8 quantiles, and with a spherical model for the indicators of the 0.4 quantile (Fig. 22). The models of the 0.2 and 0.8 quantile indicator variogram are similar indicating that the small and large residuals show a similar spatial structure. Compared to these variograms the 0.4 and

0.6 quantile indicator variograms show a bigger range, indicating that the spatial similarity of the intermediate residuals is larger than the ones of the extreme values.



1 st Threshold at the 0.2 quantile 2 nd Threshold at the 0.4 quantile



3 rd Threshold at the 0.6 quantile 4 th Threshold at the 0.8 quantile

Fig. 22. Omnidirectional indicator variograms of the 0.2, 0.4, 0.6 and 0.8 quantiles of the Cd concentration residuals, the curve represents the fitted model, the bullets are the experimental semivariances

Cross validation allows evaluating the accuracy of the variogram models (Isaaks & Srivastava, 1989). The idea of cross validation consists of removing one datum at a time from the data set and re-estimating this value from the remaining data using the different variogram models. Interpolated and true values are compared, and the model that yields the most accurate predictions is retained (Goovaerts, 1997). The cross validation of the indicator variograms given in Fig. 22 is shown in Fig. 23.

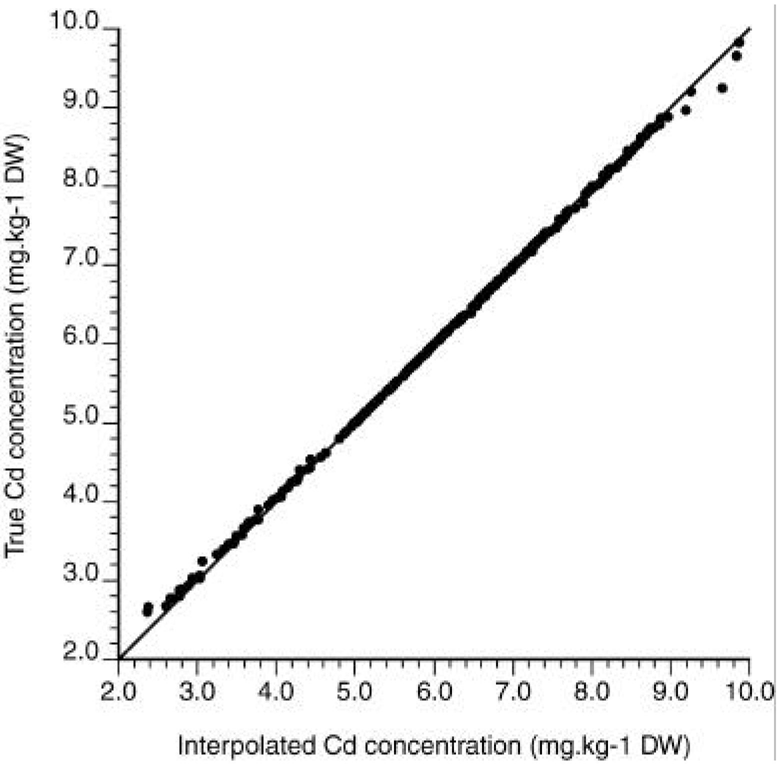


Fig. 23. Cross validation of the Cd values (mg.kg $^{-1}$ DW) interpolated with the variogram models of Fig. 22 versus the measured values at the same locations

The close match between the true and the interpolated Cd concentrations (Fig. 23) indicates that the models of the observed spatial structure yield an accurate prediction of the true Cd concentrations and are therefore retained. However, the accuracy given by a cross validation should not to be confused with the accuracy to predict concentrations at unsampled locations (Goovaerts, 1997).

5.4.1.3 Stochastic simulation

The indicator variograms described the class-specific patterns of spatial continuity in the sequential indicator simulation algorithm. The algorithm was characterised by the following settings: (i) interpolations between the conditioning data were computed linearly between tabulated bounds when a conditional cumulative distribution function (ccdf) was build, (ii) the measured minimum and maximum values were used as the extreme values for the simulation, (iii) the upper tail was simulated with a hyperbolic model ($\omega = 1$) whereas the lower tail was extrapolated linearly between tabulated bounds when building the complete ccdf (Deutsch & Journel, 1998). This simulation was repeated 100 times. As a result 100 realisations of the possible Cd distribution in the crown of a willow are now available. Overall, 20 % order relation deviations had to be corrected.

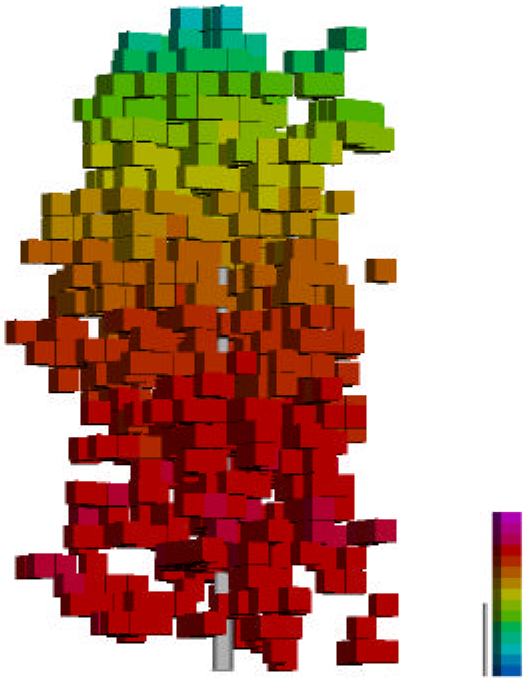


Fig. 24. Average Cd concentration (mg.kg-1 DW) computed from 100 realizations obtained from sequential indicator simulation and simulated annealing. 2.4 (blue) to 10.7 (purple) mg.kg^{-1} DW; the grey bar next to the color scale represents a height of 1 m (North-western view)

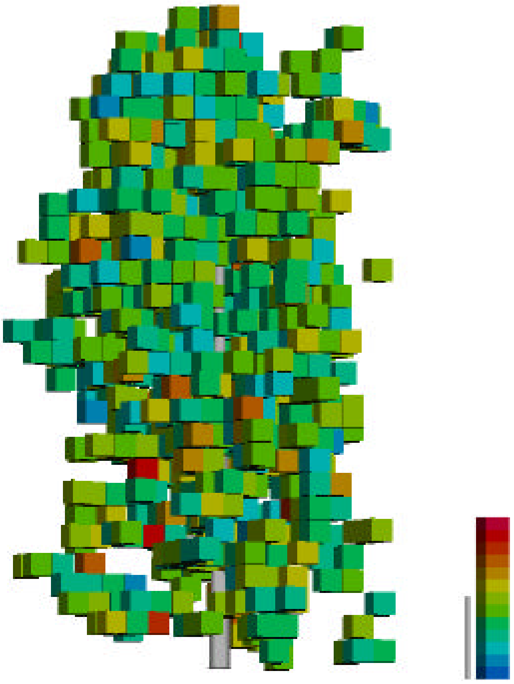


Fig. 25. Variance of the 100 realizations obtained from sequential indicator simulation and simulated annealing. Scale ranges linearly from 0.9 (blue) to 2.0 (red) (mg.kg^{-1})^2; the grey bar next to the color scale represents a height of 1 m (North-western view)

The expected value at every location was computed as the average of the 100 realisations (Fig. 24). This shows dominantly low Cd concentrations in the top of the crown and high concentrations in the lower part of the crown, as modelled by the trend. As observed (Fig. 17) fluctuations in the Cd concentration exist, motivating the geostatistical approach. The added value of this approach is shown in Fig. 24 by honouring these fluctuations. Ellis (1975) and Morrison (1985) distinguished areas in the crown with low and high variation. As a result both authors recommended restricting sampling to the areas with a low variation and avoiding sampling in the areas with a high variation. Computation of the variance at every location called for the use of a stochastic simulation. As Fig. 25 shows no systematic pattern of areas with high or low variances, it was concluded that the variance, within the limits of this study, is not a constrain in the choice of a sampling location.

A Q-Q plot comparing the quantiles of the average histogram of the 100 simulated realisations with the quantiles of the true, measured, histogram is given in Fig. 26. Deviations from the bisector show that the lowest values are overestimated up to 1.2 mg.kg^{-1} whereas the highest values can be underestimated by 2.3 mg.kg^{-1}.

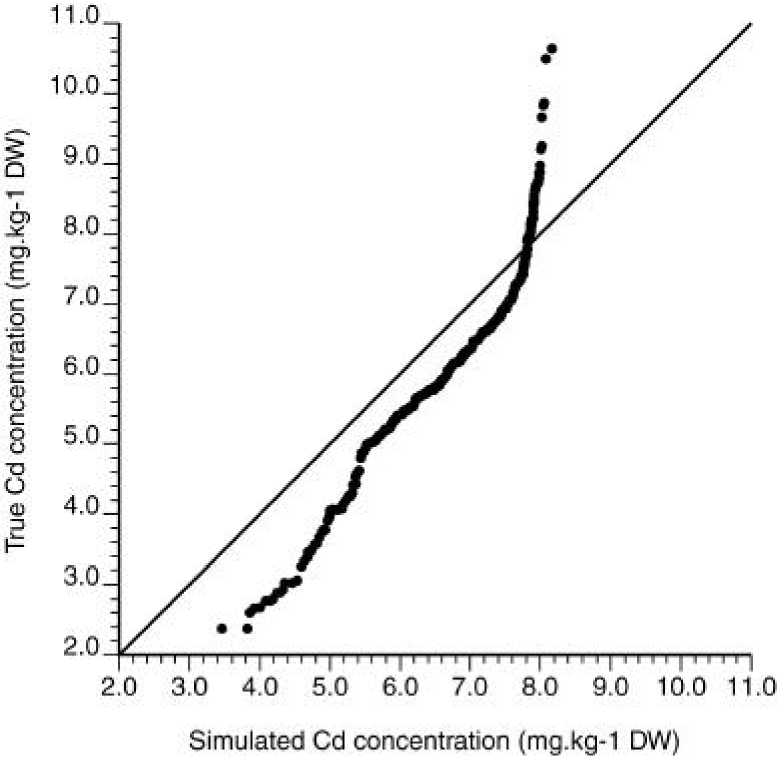


Fig. 26. Q-Q plot of the average of 100 simulated realisations of the Cd concentration (mg.kg^{-1} DW) versus observed Cd concentrations (mg.kg^{-1} DW)

The figure illustrated that the histogram of the most probable realisation was smoothed compared to the histogram of the true Cd concentrations. The simulation algorithm was set to predicted extreme residuals (i.e. -4.2 and 3.3) because the most probable realisation gives the average value at each location of the 100 realisations at that location smoothing was to be expected. A higher similarity between both histograms could have been obtained by guessing the distribution outside the observed range (i.e. setting the predicted extreme values lower than -4.2 and higher than 3.3). Extrapolation of the tails is supported by the fact that only 1 out of 3.8 possible samples was analysed and so their is a good chance that the real maximum and minimum Cd concentration were not observed. Leaving the distribution undefined outside its range and setting the simulation parameters in accordance with the observations resulted in a biased simulation. This seemed to be more reasonable than guessing the tails and simulation parameters to end with a perfect but unexplainable fit between both histograms.

The average simulated Cd concentrations (Fig. 24) are used in the continuation of this study. The bias does not affect the fundamentals of the discussion. In fact, because the simulated distribution is smoothed compared to the observed distribution and due to the character of the conclusions made, the conclusions made for the smoothed distribution are surely true for the observed distribution.

5.4.1.4 Evaluation of the current sampling strategy

In the early seventies, Leaf (1973) and van den Driessche (1974) proposed to standardise the sampling procedure for studies on chemical composition of tree leaves. They advised to target leaves from the upper third of the crown, and up to present this is still current practise (e.g. UN/ECE-EC, 1998). But studies can have different objectives, requiring different sampling procedures and different types of information required. Therefore, the performance of the current sampling strategy was confronted with two different goals : (i) a study targeting a representative average leaf sample and (ii) a study aimed to assess the risk of exceeding a chemical threshold anywhere in the leaves of a tree.

To evaluate the current sampling strategy for both study objectives a target value is needed. A target value for the second objective, the risk to exceed this threshold, was based on the Flemish legislation and literature compiled in Tables 2.4. and 2.5.. The background Cd concentration in tree leaves ranged till 2 mg.kg^{-1} DW. The reported range of the Cd concentration observed in leaves of *Salix spp*. grown on sites thought to be polluted extends from 1.8 to 60.0 mg Cd.kg^{-1} DW (Table 2.5.). The table was compiled independent of the authors criteria of pollution, therefore the concentration of Cd in the leaves was called *elevated* instead of *polluted*. The threshold to decide whether the concentration in tree leaves is elevated or not should be higher than the background and fall within the range of elevated concentrations. The annual leaf fall in a forest can be seen as a natural application of fertilisers to the forest floor. Therefore the threshold value of 6.0 mg Cd.kg^{-1} DW in raw material used as a fertiliser, imposed by the Flemish legislation (VLAREA, 1998) can be seen as a useful directive. When leaves contain more Cd than this threshold, due to the annual leaf fall a pollution problem will develop. Because this value satisfies the imposed conditions it was adopted as the threshold value for the second objective. Further, the value of 6 mg Cd kg^{-1} DW is close to the average Cd concentration of the willow under study (6.3 mg Cd.kg^{-1} DW) and will therefore be used as the target value for the first objective namely taking a representative average sample.

The distribution of the variance of the Cd in the leaves of the tree described earlier (Fig. 25) indicates that the selection of a sampling location should not be influenced by the considerations of sampling preferentially locations with a low variance or a high precision. Also the availability of leaf biomass to withdraw a sample from should be considered in choosing a sampling location. Whereas 28 % of the total leaf biomass is found in the lower third of the crown, 35 % is found in the middle and 37 % in top third. This indicates that in this tree the distribution of leaf biomass is as well not a limiting factor in the choice of the sampling location.

Sampling aim I : taking a representative leaf sample

At the conventional sampling location, i.e. the upper third of the crown of a tree, the average Cd concentration (per layers of 0.3 m) increased from 3.0 to 5.9 mg.kg^{-1} DW with decreasing height from the top (Fig. 27). When the additional limitation to sample only sun leaves is added, this average Cd concentration ranges from 3.0 to 5.0 mg.kg^{-1} DW. So it was found that, for the studied tree, the recommended sampling procedure would return Cd concentrations which are not at all representative for the average Cd concentration of the entire tree crown, since the average Cd concentration was 6.3 mg Cd.kg^{-1} DW. Because the target concentration was set at 6.0 mg Cd.kg^{-1} a representative sample could be taken approximately between 5.1 and 5.7 m above ground level where the average Cd concentration ranges from 5.9 to 6.1 mg.kg^{-1} DW.

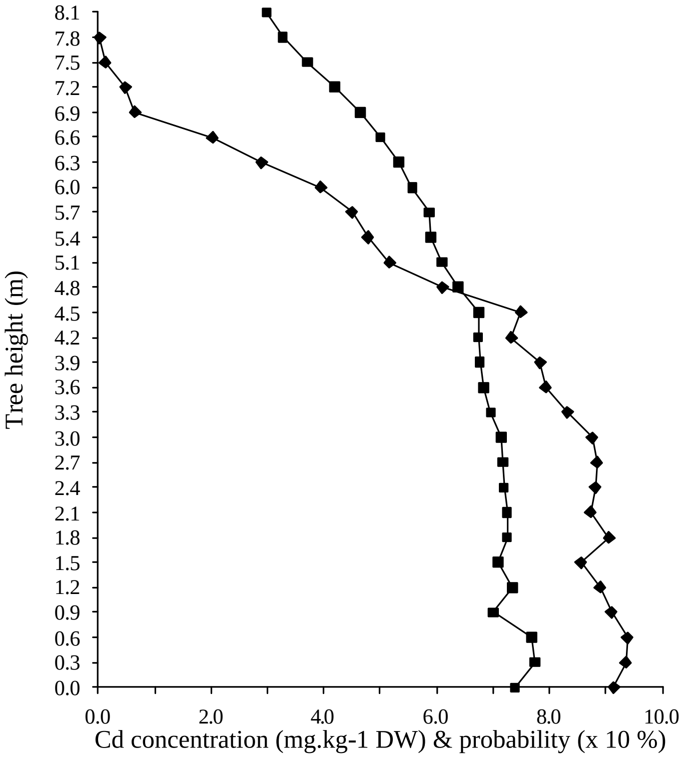


Fig. 27. Average Cd concentration in mg.kg-1 DW (■) and average probability of exceeding a 6 mg.kg-1 DW threshold in x 10 % (◆) versus tree height[SL28]

Due to the trend, this inference is tree specific and the trend has to be known to suggest the sampling location for taking a representative sample. Once it is known whether there is a trend, and if so it must be described, a representative, or average, Cd concentration can be determined. So, if the aim is to save analytical costs and sampling time by taking only one representative leaf sample per tree one is confronted with a paradox because several samples need to be taken to investigate whether a trend is present and if so to describe it. In practice, one is limited in describing the trend to only a few trees of a stand. Therefore their selection must be representative for the entire stand calling for a probabilistic sampling scheme. From these trees, the position where the average Cd concentration could be expected could be determined. Then the rest of the stand can be sampled with a bulked sample taken at the inferred height assuming that these samples are representative for the average Cd concentration of all trees within the stand. When bulking care has to be taken to mix equal amounts of biomass from the different sampling positions.

The sampling strategy to describe the trend in the Cd concentration in tree leaves is optimised and validated in a subsequent chapter. As proposed by Goovaerts et al. (1997) the set of alternative realisations, i.e. 100, serves as an input to a transfer function, in this study the simulation of a sampling strategy. The resulting distribution of response values, i.e. the chance to find the true distribution pattern, provides a measure of the response uncertainty that can be used in subsequent decision-making.

Sampling aim II : risk assessment for pollution

If the study's objective is to decide whether a pollution problem exists anywhere in the leaves of a tree, one must first set a threshold Cd concentration that represents the risk and then evaluate the probability that a sample taken at a location exceeds the threshold. By sequential indicator simulation the conditional cumulative distribution function at every location was calculated. Based on this distribution function the chance that the threshold is exceeded was calculated at every location. The probability that a sampling unit exceeds the threshold of 6.0 mg.kg^{-1} DW ranged (averaged per layer of 0.3 m) from 0.3 at the top to 2.0 % at two-thirds of the tree height (Fig. 27). The observed average Cd concentration for the entire crown is 6.3 mg.kg^{-1} DW indicating that on average the risk to encounter a pollution problem is real. It can be concluded that, for the willow under study the current sampling procedure is not suitable to assess the risk of Cd pollution. A more favourable location for sampling the studied tree was between ground level and a height of 3 m. Within this height interval the average Cd concentration ranges from 7.0 to 7.7 mg.kg^{-1} DW with a single sampling unit having a probability between 89 and 94 % of exceeding the threshold of 6.0 mg Cd.kg^{-1} DW (Fig. 27).

Again these inferences could only been made because detailed information about the Cd distribution within the crown was collected. Several samples need to be taken to investigate whether a trend is present and if so, to describe it. The trend has to be studied in several trees to determine the height where sampling will result in a correct statement of the tree pollution. Once this height is located the rest of stand can be sampled at this height.

5.4.2 Cd concentration referenced to dry ash

5.4.2.1 Explorative data analysis

The null hypothesis that the samples came from a normal distribution was tested with the Kolmogorov-Smirnov test (Neter, 1996). Although the histogram (Fig. 28) shows a small negative skewness of -0.55 this null hypothesis was accepted with a probability of 0.18. The concentration ranges from 23.1 mg Cd kg^{-1} DA to 73.0 mg Cd kg^{-1} DA with an average of 51.2 mg Cd kg^{-1} DA. Both populations, the one expressing Cd concentration per unit dry weight (DW) and the one per unit dry ash (DA), are based on the same Cd analyses.

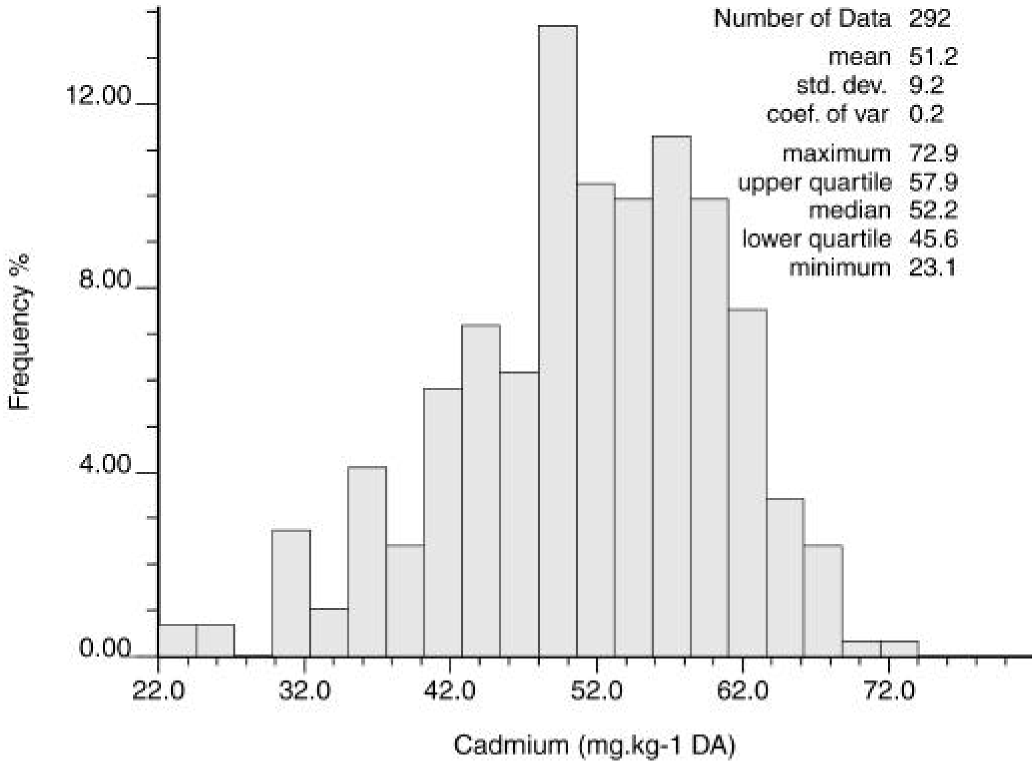


Fig. 28. Histogram of the 292 Cd concentrations expressed in mg.kg^{-1} per unit dry ash with some descriptive statistics

Using dry weight as a reference to express the Cd concentration results in a coefficient of variation (CV) of 26 % (see § 5.4.1.1) whereas using dry ash reduces the CV to 17 %. The reduction of the CV is in accordance with the observations of Claussen (1990).

Because the sampling locations for dry ash are the same as the locations for dry weight it was already shown that sampling was spatially representative for all possible locations within the crown (see § 5.4.1.1). Further, the lack of correlation between day of sampling and Cd concentration per unit dry ash was confirmed and similar to those shown in Fig. 13 for Cd concentration per unit dry weight.

The lack of correlation between the Cd concentration and the X-axis ($\rho = 0.05$) and the Y-axis ($\rho = -0.03$) and the absence of a distinct inner and outer crown (not shown) restricted the factors for detrending the Cd concentration to the Z-axis. The Z-axis, representing the height of the sample above the ground, revealed a strong significant correlation of -0.54 indicating high Cd values in the lower part of the tree. By analogy with detrending the Cd concentration per unit dry weight, a second order polynomial was used to represent the relationship between leaf Cd concentration per unit dry ash and height. The correlation coefficient was 0.38 for :

$$[3.9] \quad Cd = 52.46 + 3.5.H - 7.3610^{-3}.H^2$$

with Cd = Cadmium concentration (mg.kg^{-1} DA)
H = tree height above ground (m)

An interesting fact of the dry ash approach is that the ratio *kg DW* to *kg DA* (chapter 3, Eq. [3.1]) can be seen as a correction factor for dilution by growth. This is the ratio used to calculate the Cd concentration per unit dry ash from the Cd concentration per unit dry weight. For the determination of dry ash all carbon bounds are oxidised and lost as CO_2. Therefore the effect of a higher organic matter production caused by a higher nutrient supply and resulting in a lower Cd concentration in the leaves is repressed. Where the trend for Cd concentration per unit dry weight could be partly assigned to dilution (see § 5.4.1.1) the trend for Cd per unit dry ash cannot. The trend given by Eq. [3.9] is the result of a real difference in accumulation between the top and the bottom of the crown. A difference possibly caused by a combination of retranslocation from the top to the bottom, time dependent accumulation and a difference in physiological processes between leaves in the top and the bottom of the crown.

At this point expressing Cd concentration per unit dry ash seems to be preferable to the use of unit dry weight. Its CV is lower and it was shown by the lower correlation coefficient of Eq. [3.9] that the Cd concentration is less dependent from the sampling height. In addition, a threshold value referenced to dry weight should be time-dependent. Leaves that meet the threshold values for concentration referenced to dry weight at the onset of litter decay cannot do so later. The decay of the organic compounds led to a decrease in dry weight resulting in higher concentrations of heavy metals in the litter. This problem is avoided by the use of dry ash as a reference since ashing is the analytical equivalent of the biological breakdown of leaves to litter (Claussen, 1990).

Heavy metal concentration in leaves per unit ash can be seen as an important parameter in evaluating the long term effects of the metal pollution on the forest ecosystem because it can be used as a guideline for the expected metal concentration in litter. The presence of heavy metals in leaves slows down the litter decomposition (Tyler, 1975 b; Laskowski et al., 1994). Little & Martin (1972), Mayer (1983), Laskowski et al. (1995) and Dijkstra (1998) enunciate that the accumulation of metals in, and on the surfaces of tree leaves would result in a leaf litter rich in these metals. Rühling & Tyler (1973), Tyler (1974), (1975 a), Hutchinson & Whitby (1977), Coughtrey et al. (1979), Freedman and Hutchinson (1980), Grodzinski et al. (1990); Berg et al. (1991); Zwolinski (1994) and Cotrufo et al. (1995) provided evidence to support the theory that the woodland litter decomposition rate is clearly reduced by the heavy metal concentration in the litter. The litter layer represents 2-60 years of litter input (Friedland et al. 1984). It can as such represent a large proportion of the total capital of nutrients and metals involved in cycling within the woodland (Jackson & Watson, 1977; Strojan, 1978; Coughtrey et al., 1979; Løbersli & Steinnes, 1988; Huvenne et al., 1997). A decreasing decomposition rate causes a nutrient and base cation deficiency in the organic layer or O-horizon (Helmisaari et al., 1995; Derome & Lindroos, 1998).

5.4.2.2 Variograms

To use the variogram as a measure of spatial structure of the attribute it is important that the spatial behaviour of the variable is stationarity, i.e. free of a trend. A variable shown to behave stationair is the residual obtained by subtracting the predicted Cd concentration (Eq.[3.9]) from the observed concentration. The residuals could be considered to follow a normal distribution (p=0.43) with µ= 0.0 and s= 7.2 (Fig. 29). The correlation coefficient between height and these residuals was 0.00, so it was decided that the spatial behaviour of the residuals is stationary and therefore suitable for the usage of geostatistical tools.

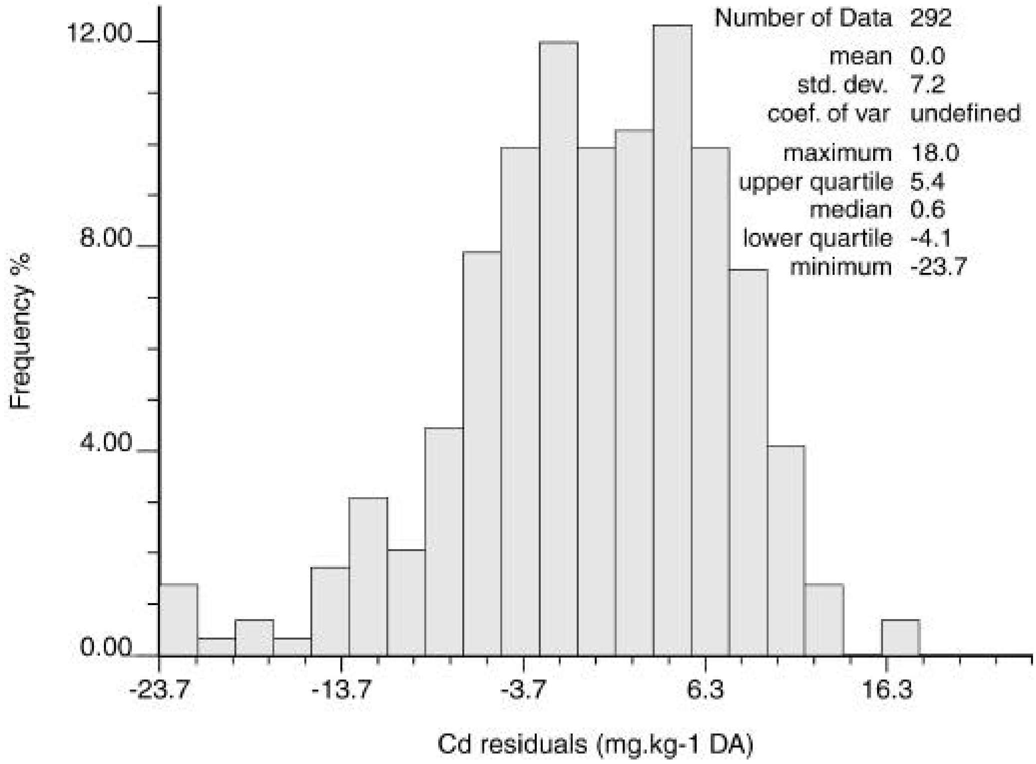


Fig. 29. Histogram of the Cd residuals in mg.kg^{-1} per unit dry ash with some descriptive statistics

Due to the isotropic behaviour of the spatial variance of the residuals a single omnidirectional variogram was calculated (Fig. 30). A nested model was fitted through the experimental values :

$$[3.10] \quad g(\mathbf{h}) = 0.2 + 42.1\left[1.5\frac{\mathbf{h}}{0.8} - 0.5\left(\frac{\mathbf{h}}{0.8}\right)^3\right] + 13.2\left[1 - \exp\left(-\frac{3\mathbf{h}}{3.6}\right)\right]$$

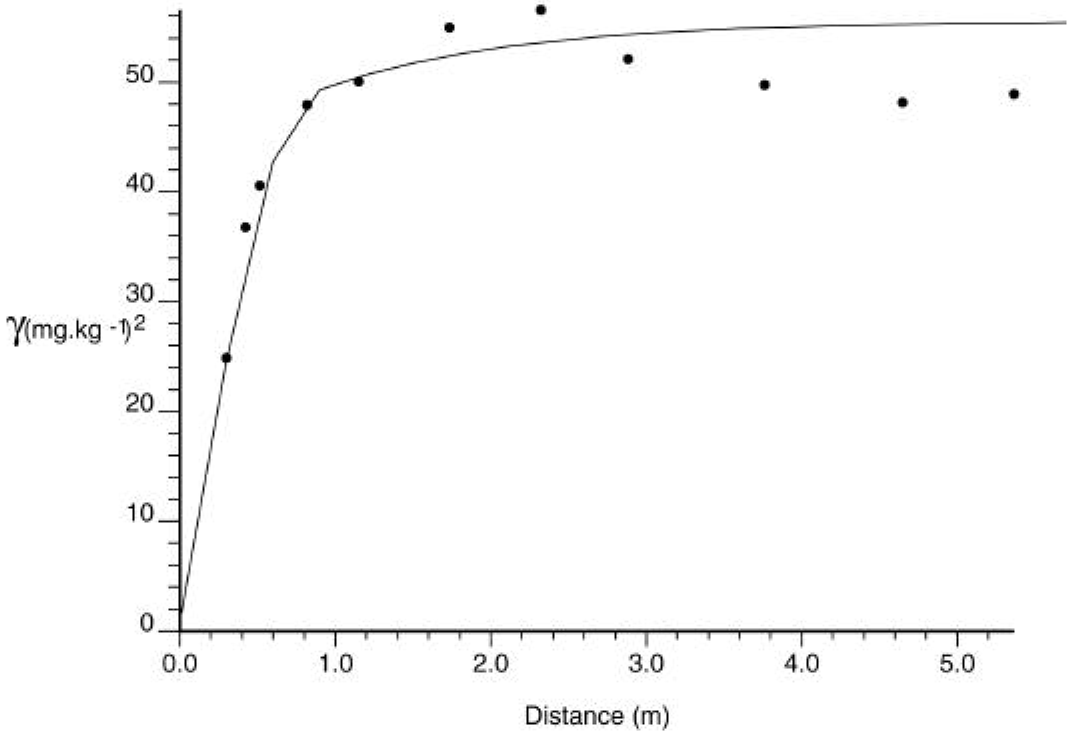


Fig. 30. Omnidirectional variogram for the Cd concentration residuals expressed per unit dry ash; the curve respresents the fitted model, the bullets are the experimental semivariances

The similarity between this variogram model and the model of the residuals obtained after detrending the Cd concentration per unit dry weight are remarkable. Spatial variance of the dry ash residuals was also described with a nested model, combining a nugget effect with an exponential and spherical model. The nugget is with 0.2 $(mg.kg^{-1})^2$ approximately the same as for dry weight residuals. Again 80 % of the variance is encountered at a lag distance of 0.8 m and the sill of both models was reached at a range of 3.6 m. Because both Equations [3.8] and [3.10] model the variance in the same way, both methods of expressing the Cd concentrations are of the same value for the spatial analysis. The similarity of the spatial structures can be explained by the fact that height and ash concentration are correlated (ρ = -0.64) with each other (Fig. 31). Therefore, in Eq. [3.8] the variable *H* partly accounts for the ash concentration of the leaves. So, in both cases the residuals were obtained after using height -twice explicit- and ash concentration -once explicit and once implicit- as dependent variables for predicting the Cd concentrations.

Fig. 31. Sampled ash concentration within the studied tree. Ash ranges linearly from 8 % (dark green) to 17 % (light green); the grey bar next to the color scale represents a height of 1 m (North-western view)

Based on the cumulative distribution function (cdf) of the Cd residuals, threshold values z_k were obtained at the 0.2, 0.4, 0.6 and 0.8 quantiles : -5.59, -1.64, 2.65 and 6.14 respectively. According to Eq. [3.5] every residual ($z(\mathbf{x}_\alpha)$) was transformed into 4 indicators $i(\mathbf{x}_\alpha\ ;\ z_k)$. Four omnidirectional indicator variograms were calculated (Eq.[3.6]). The variogram for the 0.2 quantile is given by :

$$g(\mathbf{h}) = 0.1 + 0.7\left[1.5\frac{\mathbf{h}}{0.7} - 0.5\left(\frac{\mathbf{h}}{0.7}\right)^3\right] + 0.2\left[1 - \exp\left(-\frac{3\mathbf{h}}{1.3}\right)\right];$$

the variogram for the 0.4 quantile by :

$$g(\mathbf{h}) = 0.1 + 0.9\left[1 - \exp\left(-\frac{3\mathbf{h}}{0.6}\right)\right];$$

the variogram for the 0.6 quantile by :

$$g(\mathbf{h}) = 0.1 + 0.6\left[1.5\frac{\mathbf{h}}{0.6} - 0.5\left(\frac{\mathbf{h}}{0.6}\right)^3\right] + 0.4\left[1 - \exp\left(-\frac{3\mathbf{h}}{0.8}\right)\right];$$

the variogram for the 0.8 quantile by :

$$g(\mathbf{h}) = 0.1 + 0.7\left[1.5\frac{\mathbf{h}}{0.7} - 0.5\left(\frac{\mathbf{h}}{0.7}\right)^3\right] + 0.4\left[1 - \exp\left(-\frac{3\mathbf{h}}{0.8}\right)\right]$$

The 0.2, 0.6 and 0.8 quantile variogram models show a similar spatial structure, again the 0.4 quantile was best described by a spherical model, justifying the conditional approach. The accuracy of the indicator variogram models was tested with a cross validation as in § 5.4.1.2. Again a close match between the predicted and true values was found (not shown) so the models were retained

5.4.2.3 Stochastic simulation

The indicator variograms described the class-specific patterns of spatial continuity in the sequential indicator simulation algorithm. The algorithm characteristics were identical to those for the Cd residuals referenced to dry weight. The simulation was repeated 100 times. On average 28 % order relation deviations were corrected. The expected value at every location was computed as the average of the 100 realisations showing dominantly low Cd concentrations in the top of the crown and high concentrations in the lower part of the crown (Fig. 32). In comparison with Fig. 24, Fig. 32 clearly shows the lower variation in the concentration and the weaker correlation between Cd concentration and sampling height.

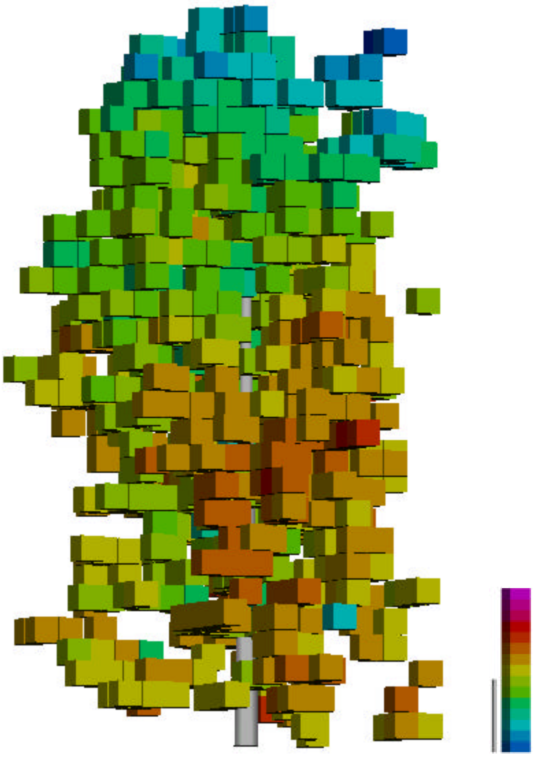


Fig. 32. Average Cd concentration (mg.kg-1 DA) predicted by the sequential indicator simulation and simulated annealing. 23 (blue) to 73 (purple) $mg.kg^{-1}$ DA; the grey bar next to the colour scale represents a height of 1 m (North-western view)

5.4.2.4 Evaluation of the current sampling strategy

To evaluate the current sampling strategy for (i) taking a representative sample and (ii) a risk assessment for pollution, target values are needed. In the arithmetic example for the first objective a sample will be considered as representative if its Cd concentration approximates the average of 51.0 ± 1.0 $mg.kg^{-1}$ DA (Fig. 28). In contrary with dry weight, no literature on threshold values nor legislation are available for metal concentrations referenced to dry ash (Claussen, 1990). Thus, an independent threshold value to evaluate the current sampling strategy for risk assessment was absent. By dividing the threshold of 6.0 $mg.kg^{-1}$ DW by the average ash concentration a threshold of 49.0 mg Cd kg^{-1} referenced to dry ash was obtained.

It has to be stressed that this threshold was not independent and therefore the results of the risk assessment for pollution are rather illustrative than conclusive.

A Q-Q plot comparing the quantiles of the average histogram of the 100 simulated realisations with the quantiles of the measured histogram is given in Fig. 33. Close matches to the bisector show that the algorithm simulated the observed histogram very well.

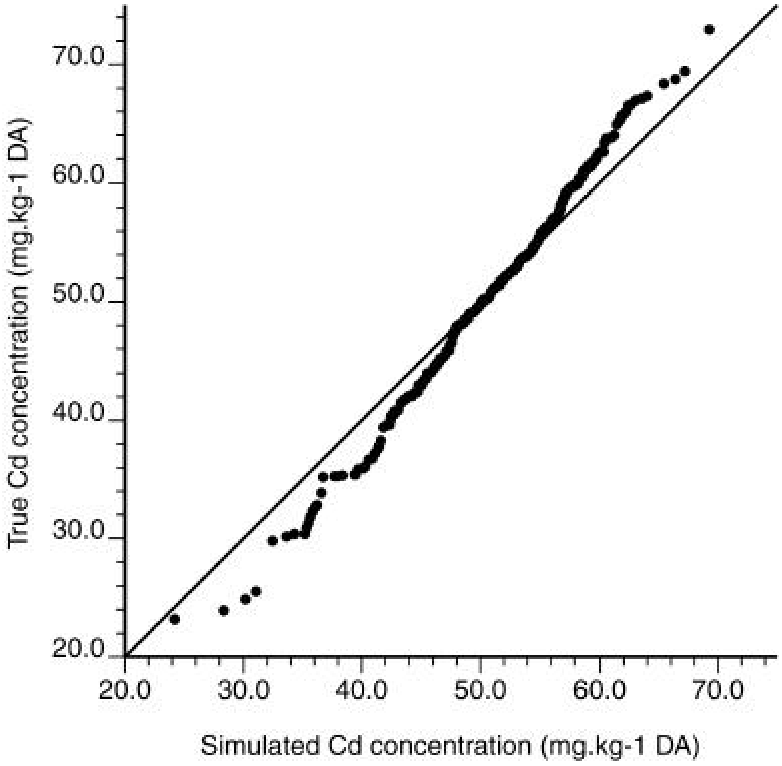


Fig. 33. Q-Q plot of the average of 100 simulated realisations of the Cd concentration (mg.kg^{-1} DA) versus observed Cd concentrations (mg.kg^{-1} DA)

Although the lower half of the distribution is over- and the upper half underestimated both histograms, the observed and simulated, represent a similar population : the observed average Cd concentration reached 51.2 mg.kg^{-1} DA, the simulated average 51.3 mg.kg^{-1} DA and the CV of the simulated distribution was only 2% lower than the CV of the measured distribution. Due to the lower variance of the observations it seemed that the histogram referenced to dry ash could be predicted at non-sampled locations by only using information from the observations. With DW as reference, it was concluded that such a prediction of the histogram was only possible by guessing the distribution outside the observed range.

Sampling aim I: taking a representative sample

Sampling Cd concentrations per unit dry ash at the conventional location led to similar conclusions as sampling these locations for Cd concentrations referenced to dry weight. This result might be influenced by the use of a threshold depending on the threshold value for DW.

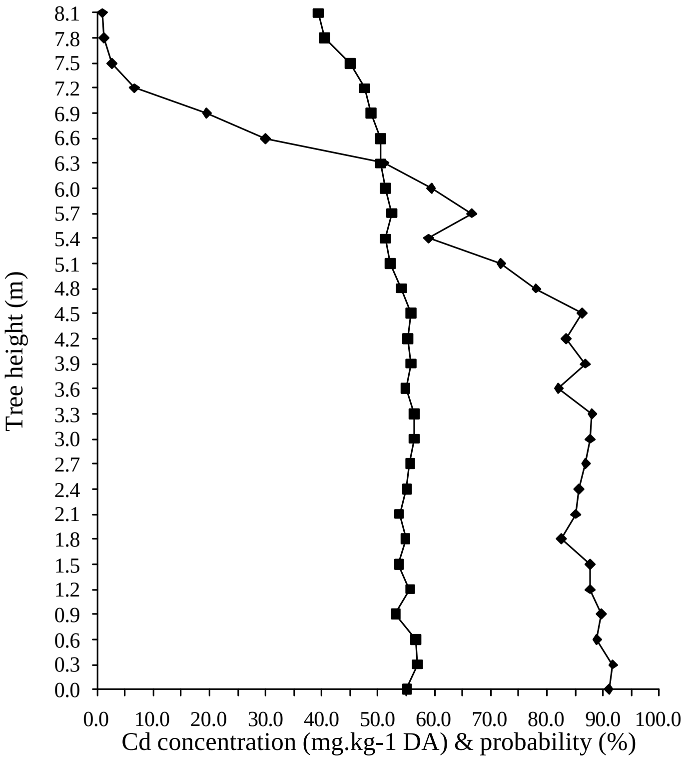


Fig. 34. Average Cd concentration in mg.kg^{-1} DA (-■-) and average probability of exceeding a 51 mg.kg^{-1} DA threshold in % (-◆-) versus tree height

Sampling the recommended locations, sun leaves of the upper third of the crown, on average returned concentrations of 48.0 to 39.0 mg Cd kg^{-1} DA (Fig. 34). Since the average Cd concentration was 51.0 mg Cd kg^{-1} DA, it was concluded that, for the studied tree, the recommended sampling procedure would return Cd concentrations which were not at all representative for the entire tree crown. Although, representative samples could be taken between 5.1 and 6.3 m above ground level (Fig. 34). Again the advantage of sampling a population with less variance became apparent, representative samples could be taken from an area twice as big as the area for sampling Cd concentrations per unit dry weight. But again, if the aim is to save analytical costs and sampling time by taking only one representative average leaf sample per tree, the paradox described in §5.4.1.4 comes to the fore.

Sampling aim II : risk assessment for pollution

For the studied tree, expressing the Cd concentration per unit dry ash is advantageous in risk assessment studies. Probabilities to exceed the threshold value of 49.0 mg Cd kg^{-1} DA at the conventional sampling locations range from 30 to 1 % whereas this probability was as low as 2 to 0.3 % for Cd concentrations per unit dry weight. Whenever a chance of 80 % is sufficient to encounter a pollution risk samples can be taken from ground level to 4.8 m above ground level (Fig. 34).

5.5 Conclusions

Sampling locations were spatially even distributed and the time span of sampling was found to have no effect on the Cd concentrations in the leaves. The Cd concentration in leaves of the willow ranged from 2.3 mg.kg^{-1} DW to 10.6 mg.kg^{-1} DW or 23.1 mg.kg^{-1} DA to 73.0 mg.kg^{-1} DA within the crown. Between Cd concentration, both referenced to dry weight (DW) and dry ash (DA), and aspect no correlation was found but a strong trend with the tree height was observed. The trend could not be refined by the introduction of the concept of an inner and outer crown. The prediction of Cd concentrations at non-sampled locations was improved when spatial connectivity of the residuals was added to the deterministic trend. Prediction of the Cd concentration referenced to DA favoured most.

For both methods of expression, but especially when dry weight was used as a reference, no evidence was found to support the generally used sampling strategy for tree leaves : taking a bulked sample of sun leaves within the upper third of the crown. These samples could result in a biased estimation of the average leaf Cd concentration or result in a wrong evaluation of the chance that an environmental threshold is exceeded.

The use of dry ash as a reference seems to be advantageous. The lower variance of the population results in better performing simulation algorithms and lower uncertainties on the sampling results. Further, the concentrations obtained by leaf analysis can be used right away in evaluating the effects of the pollution on the leaf litter. Although the differences between the two methods of expressions should not be neglected, they are not such that a consequent comparison of both methods will add to the fundamentals of the discussion. Therefore, in the continuation of this study only dry weight is used as a reference to express the Cd concentration. The lack of references to threshold values and the rarely application make it irrelevant to be used in this study.

6 Spatial variability in the crown of *Salix fragilis* : optimisation and verification of a sampling strategy

6.1 Abstract

A conceptual approach is given to optimise and verify a sampling strategy. The approach was illustrated with the optimisation of an alternative sampling strategy in favour of estimating the average Cd concentration in the crown. Performance of the generally used and the optimised alternative sampling strategy were verified. Optimisation and verification were performed on 100 simulated realisations of Cd in the leaves of a *Salix fragilis* L. The local simulations of the Cd concentration were resample by a Monte Carlo method and entered into a transfer function to evaluate the performance of the alternative sampling strategy. The conventional sampling strategy to investigate the presence of a trend i.e. sampling the lower, middle and upper crown was found to lack power. The chance to accurately describe the distribution pattern of Cd was as low as 12 %. Especially increasing the number of heights sampled raised the power of the conventional sampling strategy. After optimisation a chance of 70 to 80 % was found to accurately describe the Cd concentration trend. Using the optimised alternative sampling strategy to estimate the average Cd concentration of the crown yielded an slightly biased and more precise estimate compared to the generally used sampling strategy i.e. sampling the upper third of the crown.

6.2 Introduction

In the previous chapter the generally used sampling strategy for Cd in tree leaves, i.e. sampling sun leaves of the upper third of the tree crown, was evaluated. It was found that due to a vertical trend in the tree crown, only sampling the upper third could yield biased estimates of both the average Cd concentration and the risk to exceed a pollution threshold. Therefore an alternative sampling procedure was proposed (see § 5.4.1.4 and § 5.4.2.4). The alternative procedure uses the trend-description of Cd to determine the best sampling location for the aim of the study. A powerful strategy to describe the trend is needed.

In spite of using the same sampling strategy -sampling the lower, middle and upper crown-, to investigate the presence of a trend different authors found different distribution patterns of elements in tree leaves. Taking potassium (K) as an example it is illustrated that although, according to the methods and material sections, the same sampling strategy was used, no consistent distribution pattern was found. Wallihan (1944) found that the K concentration was distributed according to pattern I (Fig. 35) in *Acer saccharum* March., where in *Populus trichocarpa* Toor. & Gray ex Hook. McLennan (1990) described the distribution as pattern II. Previously Verry & Timmons (1976) concluded that K in *Populus tremuloides* Michx. showed a pattern III, Le tacon and Toutain (1973) found distribution IV in *Fagus sylvatica* L. whereas Ellis (1975) found that the distribution of K in *Prunus serotina* Ehrh. was best described by pattern V.

This chapter aims to provide a conceptual approach of a sampling strategy study characterising element concentrations in tree crowns. It was examined if the differences in Cd distribution patterns are due to differences in the true Cd distribution or caused by limitations of the sampling strategy. Second, the influences of the sampling parameters on the power of the sampling strategy were quantified. These allowed finalising the alternative sampling procedure to estimate the average Cd concentration in a tree crown by using the optimised strategy for trend description. Last, the finalised alternative sampling procedure was verified with the generally used sampling procedure (Leaf, 1973; van den Driessche, 1974; UN/ECE-EC, 1998).

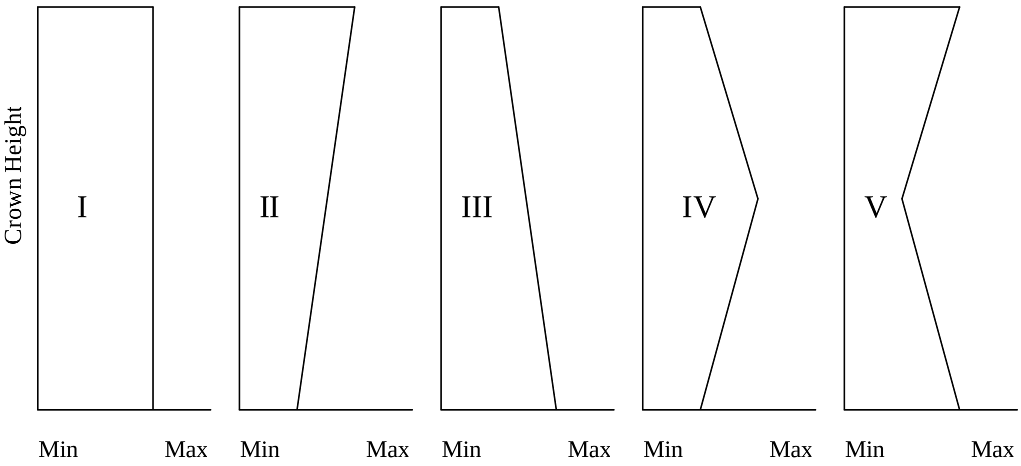


Fig. 35. Reported patterns of element distribution within tree crowns (after van den Driessche, 1974)

6.3 Data processing

6.3.1 Description of the transfer function

In the previous chapter the Cd concentration in the leaves of the non-sampled locations were simulated using the 292 observations of Cd concentration, the observed relationship between height and Cd concentration and the description of the spatial continuity of the residuals. These simulations were repeated 100 times using a conditional sequential indicator simulation. Because the incorporated information was not all-embracing several possible simulations of the Cd concentration were realised (Fig. 36). All these realisations honour the observed histogram and the vertical and the spatial trend. A computer program simulating sampling was applied as a transfer function (Goovaerts 1997) to this set of 100 realisations. The transfer function yielded a distribution of response values with Cd distribution patterns as those shown in Fig. 35.

The flow chart of the transfer function is given in Fig. 37. For *n* selected samples, a quadratic regression between height and Cd concentration was computed by calculating the least square estimators, *a*, *b* and *c* of the equation :

[1] $Cd = a + bX + cX^2$

with

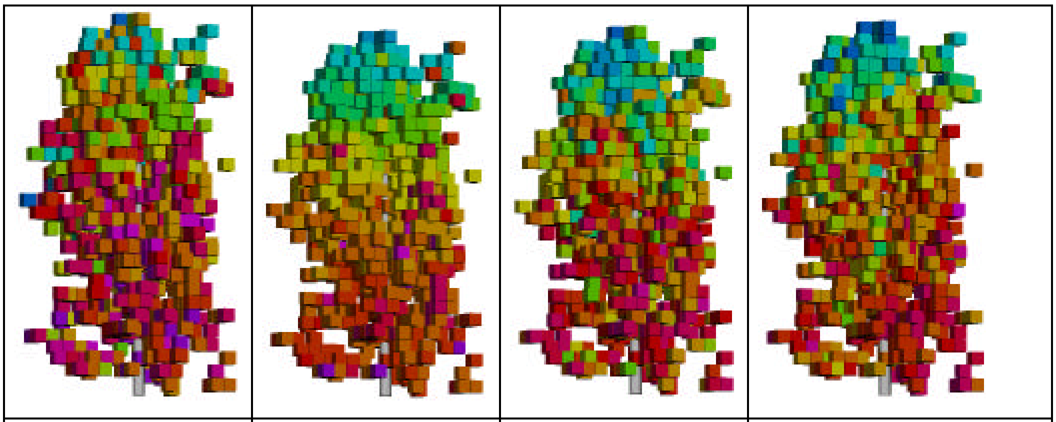
Cd = the Cd concentration ($mg.kg^{-1}$ DW)

X = the height within the crown (m)

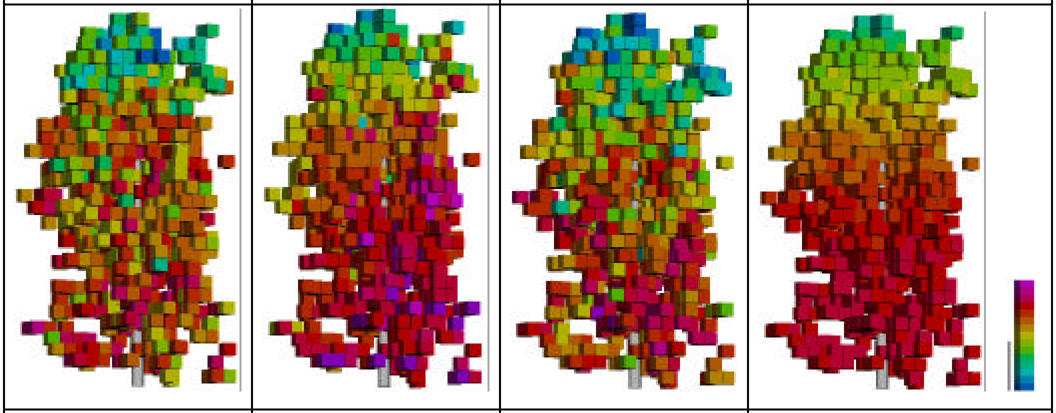
a, b, c = least square estimator

The regression sum of squares $SS_R\ (a,b,c)$ of the quadratic equation was calculated. The contribution of c to the regression was computed by assuming that the null hypothesis H_0: c=0 was true. Therefore the regression sum of squares $SS_R\ (a,b)$ of the reduced equation [1] given by [2] was calculated.

[2] $Cd = a + bX$



Realisation 1 | Realisation 15 | Realisation 23 | Realisation 56



Realisation 68 | Realisation 82 | Realisation 100 | Average

Fig. 36. Some of the 100 realisations and the average Cd concentration (mg.kg^{-1} DW) simulated by the sequential indicator algorithm. Cd scale ranges linearly from 2.3 to 3.8 (blue), 6.3 (green), 7.8 (orange), 9.3 (red) to 10.6 (purple) mg.kg^{-1} DW; the grey bar next to the colour scale represents a height of 1 m (North-western view)

$SS_R\,(a,b,c)$ and $SS_R\,(a,b)$ are needed to calculate the regression sum of squares due to c given that a and b are already present in the model $SS_R(c/a,b)$ (Eq.[3]). This sum of squares was calculated with one degree of freedom.

[3] $SS_R(c/a,b) = SS_R(a,b,c) - SS_R(a,b)$

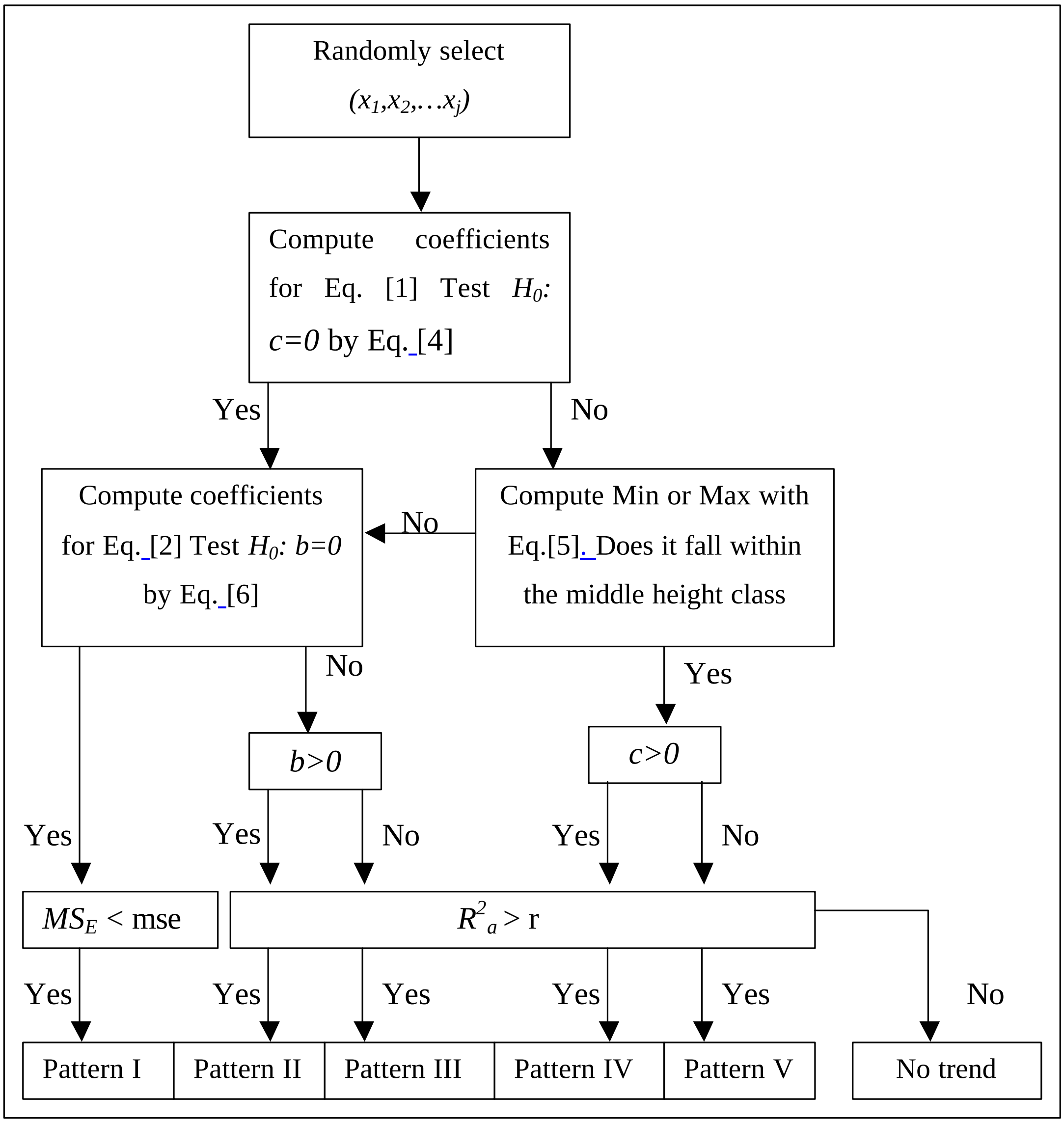


Fig. 37. Flow chart of the transfer function $g(x_1,x_2,\ldots,x_j)$ to compute the distribution patterns I to VI of the Cd concentration within a tree crown (see text for explanations)

with

$$SS_R(a,b,c) = \sum_{i=1}^{n} (\hat{y}_i - \bar{y})^2$$

with

$\hat{y}_i$ = estimated Cd concentration by [1]

$\bar{y}$ = mean of the estimated Cd concentrations by [1]

$$SS_R(a,b) = \sum_{i=1}^{n} (\hat{y}_i - \overline{y})^2$$

with

$\hat{y}_i$ = estimated Cd concentration by [2]

$\overline{y}$ = mean of the estimated Cd concentrations by [2]

Because SS_R *(c/a,b)* is independent of SS_E, the null hypothesis c=0 was tested by the statistic

$$[4] \quad F_0 = \frac{SS_R(c/a,b)}{SS_E/(n-2-1)}$$

with

$$SS_E = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$$

y_i = observed Cd concentration

$\hat{y}_i$ = estimated Cd concentration by [1]

With $F_0 < F_{0.05,1,n-3}$ it was concluded that c=0 and so a linear model (Eq.[2]) was adopted. Else, if $F_0 > F_{0.05,1,n-3}$ the H_0 that c=0 was rejected and the quadratic model (Eq.[1]) was retained when the additional conditions specified below were satisfied.

A function reaches its minimum, or maximum, at :

$$[5] \quad \frac{d(Cd)}{dX} = 0$$

Differentiation of Eq. [1] gives $b + 2cX = 0$. Thus the exact height where the quadratic function reaches its minimum, or maximum, can be determined. When this value is situated in the middle of the crown it was concluded that the Cd concentration followed the distribution of pattern IV (c>0) or of pattern V (c<0). Else, the distribution of the Cd concentration followed a distribution best described by pattern III or pattern II. These two distributions can be described by equation [2] and the H_0: b=0 was tested by the statistic:

[6] $$F_0 = \frac{SS_R(a,b)}{SS_E/(n-1)}$$

If it was found that $F_0 > F_{0.05,1,n-2}$ the regressor X was considered as an influential factor and included in the regression now described by Eq.[2]. Whenever b>0 a pattern II distribution was adopted, with b<0 the Cd concentration followed a pattern III distribution. The regressor X was deleted from the regression if $F_0 < F_{0.05,1,n-2}$. This reduced the equation to its basic form :

[7] $$Cd = a$$

For the distribution patterns II to V the adjusted coefficient of determination R_a^2 was calculated and tested with a user defined threshold correlation coefficient. If the correlation coefficient was bigger than the threshold value the regression was accepted.

A R_a^2 that equals zero can be the result of a constant Cd-concentration along the tree height or the result of a non-constant Cd concentration lacking correlation with the tree height. In the transfer function pattern I was interpreted as constant Cd concentrations along the tree height. So, there was a need to reject the models with $R_a^2 = 0$ and non-constant Cd concentrations. Therefore an additional quality measure to R_a^2 had to be used. The MS_E ([8]) was calculated as :

[8] $$MS_E = \frac{SS_E}{(n-1)}$$

This MS_E was tested with a threshold value calculated from the MS_E of the accepted models of pattern II to V. Models of pattern I were accepted if their MS_E was lower than the maximum calculated MS_E for models of pattern II to V.

The transfer function can be summarised as follows:

1. a specified number of samples is selected;
2. the trend revealed by these samples is calculated according to a set of parameters: (i) the threshold correlation coefficient to accept a model, (ii) the width of the middle of the crown, and (iii) the sampling precision parameterised by an approximations of the sampling heights;
3. the trend is classified as one of the five possible distribution patterns for element concentrations within the crown of a tree reported by van den Driessche (1974; Fig. 35). If no similarities with one of the five described distribution patterns was found, the distribution was classified as *no trend* in class VI*;*
4. the output of the function is the assigned distribution class (I to VI).

6.3.2 Hit and Miss Monte Carlo method

Because it is analytically impossible to determine the probability that, given a Cd distribution in the crown of a tree, a certain sampling strategy would reveal a certain distribution pattern (e.g. pattern I to V), this probability was simulated by the "Hit or Miss" Monte Carlo method (Rubinstein, 1981). The method description is due to Rubinstein (1981) and was adapted for the *multi*-dimensional character of this simulation problem. Assume that the transfer function to describe the distribution of Cd concentration within a tree crown can be written as $g(x_1,x_2,...,x_j)$ with j the number of height classes and x_j the local Cd concentration randomly selected from height class j within the constrains of the sampling strategy. Further, assume that : $0 \le g(x_1,x_2,...,x_j) \le c$, $a_j \le x_j \le b_j$ with a_j the lower limit and b_j the upper limit of height class j. Let Ω denote the j-dimensional regular subspace as $\Omega = \left\{ \left(x_1, x_2,..., x_j, y \right) : a_1 \le x_1 \le b_1, a_2 \le x_2 \le b_2, ...a_j \le x_j \le b_j, 0 \le y \le c \right\}$ and let $(X_1, X_2,..., X_j, Y)$ be a random vector uniformly distributed over the subspace Ω with a probability density function given by $f_{XY}\left(x_1, x_2,...x_j\right)$.

$$f_{XY}\left(x_1, x_2,...x_j\right) = \frac{1}{c(b_1 - a_1)(b_2 - a_2)...(b_j - a_j)} \qquad \text{if } \left(x_1, x_2,...x_j, y\right) \in \Omega$$

$$f_{XY}\left(x_1, x_2, ...x_j\right) = 0$$

otherwise.

With all definitions set, what is the probability p that the random vector $(X_1, X_2, \ldots, X_j, Y)$ falls within the area under the j-dimensional surface? Let us assume that N independent random vectors $(X_{11}, X_{21}, \ldots, X_{j1}, Y_1)$, $(X_{12}, X_{22}, \ldots, X_{j2}, Y_2), \ldots, (X_{1N}, X_{2N}, \ldots, X_{jN}, Y_N)$ are generated. The parameter p can be estimated by

$$[9] \quad \bar{p} = \frac{N_H}{N}$$

Where N_H is the number of occasions on which $g(X_{1l}, X_{2l}, ..., X_{jl}) \geq Y_l, l = 1,2,...N$, that is the number of hits, and N-N_H is the number of misses.

Application flowchart of the Monte Carlo simulation is given in Fig. 38. The input of the transfer function, being the n local simulations of the Cd concentration, was selected randomly from the entire crown. This random selection was guided by a set of the following sampling parameters: (i) the number of height classes to distinguish, ranging from 3 to 10, (ii) the number of samples which have to be taken at each level, ranging from 1 to 10. Although the sample selection is constrained, different sets of n Cd values could be selected. Each selected set was fed into the transfer function. A *hit* was a simulation where the transfer function classified the Cd concentrations as distribution pattern III (see further). A *miss* was a simulation generating another distribution pattern. The ratio of the *hits* to the total number of simulations gives the probability to reveal the true distribution pattern (Eq. [9]).

Fig. 38. Flowchart of the computer program to apply the Hit and Miss Monte Carlo simulation. The flowchart is concretised with a step by step example

Flowchart of a Hit an Miss Monte Carlo simulation	Example
- Read the sampling strategy	- Read the R_a^2 threshold, width of the middle of the crown, measurement error, number of

	heights and number of repeats
- Do $k = 2$ to K	- Repeat for each realisation (R) $n = 1$ to 100 (see Fig. 36)
- Read data of R_n	- Read simulated Cd concentrations of R_n
- Do $l = 1$ to L	- Repeat for number of simulations within a run $l = 1$ to 10,000
- Select *n* at random observations within the constrains of the sampling strategy	- Make a table (T_1) with the *n* Cd concentrations and their height in the crown selected from R_n
- Calculate the transfer function	- Feed T_1 in Fig. 37
- Classify as a *hit* or *miss*	- Make a table (T_2) with the distribution pattern (I to V and *no trend*) as value
- End do	
- Calculate $\overline{p}$	- Calculate from the 10,000 values in T_2 the chance that the sample strategy will reveal the true distribution
- Save $\overline{p}$	- Make a table T_3 with as value $\overline{p}$
- Plot this data point on graph	- Create a graph as Fig. 39 and Fig. 42
- End do	
- Calculate mean $\overline{p}$	- Calculate from T_3 the mean chance that the sample strategy will reveal the true distribution

6.3.3 Random selection

As all Monte Carlo methods the Hit and Miss method is driven by a random data input in the transfer function that describes the process under study. For each run in the Monte Carlo method, the unit's height class labelled the Cd concentration of each unit. Within each height class an equal amount of samples were chosen randomly. Pseudorandom numbers were generated by combining two Minimum Standard

Generators as proposed by L'Ecluyse in 1988 (Press et al., 1992). The two different sequences with different periods were combined to obtain a new sequence whose period is the least-common multiple of the two periods. Both random number generators are given by the algorithm :

[10] $$I_{j+1} = aI_j \pmod m$$

with

$a = 40014$ in the first and $a = 40692$ in the second generator

$m = 2147483563$ in the first and $m = 2147483399$ in the second generator

$I_j =$ initial seed number

This algorithm has a period of $\approx 2.1 \times 10^{18}$ meaning that generating additional random numbers would result in correlated numbers. Therefore within a run, the amount of random numbers generated was counted and reported to avoid the use of correlated numbers.

6.4 Results and discussion

6.4.1 Defining the true distribution pattern

As described in the previous chapter, a trend was found with high values in the lower parts of the crown and low Cd concentrations in the top. All 100 realisations were built on this trend and the spatial structure of the residuals using the sequential indicator simulation. A quadratic polynomial was fitted through the trend. Because the polynomial reached its minimum out of the middle of the crown, the trend was best described as distribution pattern III. Therefore, pattern III was considered as the true distribution pattern of all realisations and a Hit was defined as a Monte Carlo simulation of the transfer function resulting in the assignment of pattern III.

6.4.2 Defining the number of simulations within a run

The number of simulations depends on three considerations. First, the number of simulations should allow evaluating the frequency of a rare event. Second, this number should be large enough to give an trustworthy estimation of the chance that the sampling strategy reveals a certain distribution and last, this number should be as small as possible to keep the simulation time to a minimum. Because only two events were defined, a Hit and a Miss, an event was considered rare if it occurred once out of 100. Fig. 39 shows that to obtain a trustworthy estimation of the probability that a hit occurs at least 10,000 simulations are required. Consequently, the number of simulations of the sampling strategy within one run was set to 10,000.

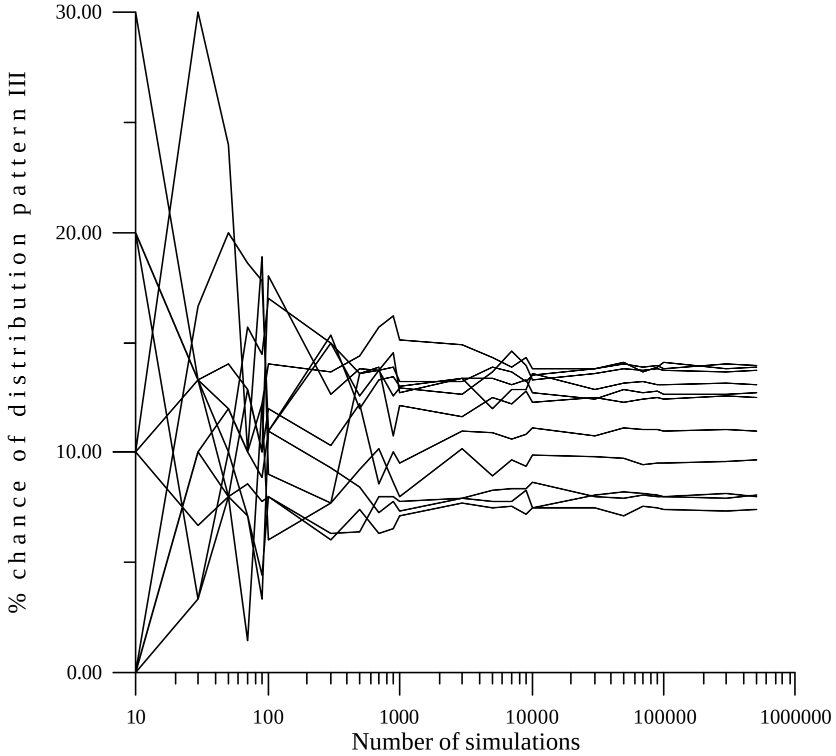


Fig. 39. Percent chance to find distribution pattern III versus the number of simulations, shown for 10 realisations

6.4.3 Power of the conventional sampling strategy

The first aim of this study was to examine if reported differences in element distribution patterns are due to differences in the true element distribution or could be caused by the limited power of the conventional sampling strategies to study a trend. Based on the methods and materials section of international literature, six sampling strategies were compiled. The amount of leaves bulked in one sample varied from 7 g DW, being the average mass of a unit in this study, over 15 g DW (Ellis, 1975; or 30 to 50 g fresh weight e.g. Morrison, 1975; Erdmann et al., 1988; McLennan, 1990) till 100 g DW (200 g fresh weight e.g. Keller, 1994). Combining these 3 sample sizes with two methods of measuring the height location i.e. the exact (e.g. this study) and average height location of each sample (e.g. Morrison, 1974; Ellis, 1975; Verry & Timmons, 1976; Morrison, 1985; Erdmann, 1988; McLennan, 1990), resulted in six different and realistic sampling strategies. Within each of these six sampling strategies 12 ways of computing are given. The definition of the width of the middle of the crown was varied in 3 steps (0.8, 1.6 and 2.4 m) and the correlation coefficient needed for a fit to get accepted was varied in 4 steps (0.6, 0.5, 0.4 and 0.3). The results of the simulations are given in ? .

- Percent chance to find distribution pattern III when sampling the lower, middle and upper crown of a tree. Minimum and maximum chances given for varying sample mass, width of the middle of the crown, critical R_a^2 and way of calculating the pattern (exact or average location)

	Mass of a sample		7 g			15 g			100 g		
	R_a^2	Width of middle (m)	0.8	1.6	2.4	0.8	1.6	2.4	0.8	1.6	2.4
Average height location	0.6		5 15	5 15	5 15	1 100	2 100	2 100	0 100	0 100	0 100
	0.5		5 15	5 15	5 15	5 100	5 100	5 100	1 100	1 100	1 100
	0.4		5 15	5 15	5 15	12 100	12 100	12 100	4 100	5 100	4 100
	0.3		6 16	5 16	6 15	21 100	21 100	16 100	17 100	17 100	17 100
Exact height location	0.6		6 19	6 19	6 18	3 100	3 100	3 100	0 100	0 100	0 100
	0.5		6 19	6 18	6 18	8 100	8 100	8 100	1 100	2 100	2 100
	0.4		6 19	6 18	6 18	17 100	17 100	18 100	9 100	9 100	8 100
	0.3		6 19	6 19	6 18	28 100	28 100	26 100	26 100	26 100	26 100

Sampling the lower, middle and upper crown by a 7 g sample, measuring the exact sampling location, it was found that, although there is only one true distribution pattern in the sampled willow (pattern III), four distribution patterns were possible. On average, pattern I occurred in 86%, pattern II in 1%, pattern III in 12% and pattern IV in 1% of the data selected by this strategy. ? shows no clear trend. At the first sight similar results had a completely different interpretation. Sampling 15 g could result in a 1 % chance to find the correct distribution pattern and 99 % of the fitted parameters followed distribution pattern I meaning that a wrong pattern was attributed to the Cd concentration in the crown. Sampling 100 g could result in a 100 % change to find *no trend* meaning that no distribution pattern was attributed to the tree. It was concluded that the power of these strategies was low and that trees with the same Cd distribution in their crown were described by different distribution patterns.

6.4.4 Influence of the sampling strategy parameters

Quantifying the influence of the individual sampling parameters on the power of the sampling strategy, it was aimed to propose a strategy with an increased discriminating power. Two types of sampling strategy parameters were distinguished. The first group are the data processing parameters included in the transfer function : the threshold correlation coefficient to accept a model, the width of the middle of the crown and the measurement error on the sampling location. The second group are the sample selection parameters included in the Monte Carlo method : number of height classes to distinguish and number of samples to take at each level. In the following paragraphs the influence of each of these parameters is investigated.

6.4.4.1 Data processing parameters

Fig. 40 shows the chance to find the true distribution pattern versus the total number of samples for the same 10 realisations at two levels of R_a^2. Most striking in the $R_a^2 = 0.5$ sequence is the lack of correlation. In practice this means that increasing the number of samples will not necessarily result in higher chances to find the correct distribution pattern.

This apparent inconsistency can be explained by the role of R_a^2 in the transfer function (Fig. 37). Setting the threshold for R_a^2 higher than the correlation present in the data, i.e. 0.47 (see chapter 5) samples which could reveal the real distribution pattern are rejected because their correlation is lower than the threshold. By lowering the threshold for R_a^2 to 0.4 this inconsistency disappeared. Simulations with this choice of R_a^2 confirm that sampling the tree more intensively increases the chance to find the correct distribution pattern. R_a^2 Is a sensitive parameter in a small range i.e. 0.5 to 0.4. A R_a^2 threshold for the transfer function, which is a little higher than the observed correlation between the data, lowers the chances to find the correct distribution considerably. In addition, raising the number of samples is ineffective it lowers the changes even more. Therefore the R_a^2 threshold to accept a distribution pattern should be chosen very carefully in accordance with the observed correlation between element concentration and sampling height.

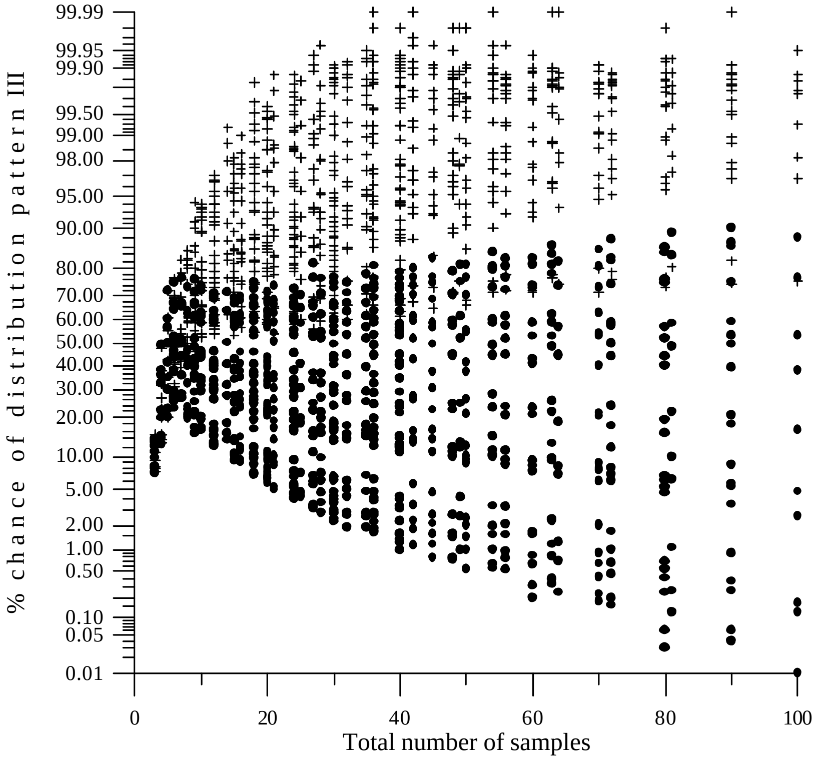


Fig. 40. Percent chance to find distribution pattern III versus the total number of samples; (•) shows for 10 realisations the chance to find distribution pattern III when $R_a^2 = 0.5$, (+) shows this chance for 10 realisations when $R_a^2 = 0.4$

The width of the middle of the crown was defined as the difference between the upper and lower bound symmetrically spread around the middle of the crown. For example, with an upper bound of 4.5 m and a lower bound of 3.7 m the width of the middle of the crown is 0.8 m. From Fig. 37 it was expected that when the middle of the crown was defined wider, the position where the minimum or maximum was reached became less restricting. So, a part of the data would be classified wrongly as distribution pattern IV or V. At the other hand, limiting the width of the middle of the crown could result in near to zero chances to find a distribution pattern IV or V. The chance would be low even when the true distribution of the Cd concentration is IV or V. Based on the set of 100 realisations no influence of the width of the crown on the identification of the pattern could be shown. This leads to the conclusion that within the conditions studied, i.e. a clear trend of pattern III, the width of the middle crown is a robust parameter and thus of a minor influence.

In field sampling an exact positioning of the sampling locations is impossible. Therefore we investigated the influence of deviations from the aimed sampling height. To simulate measurement errors the transfer function was calculated with an approximation of the sampling height. A random generated, normal distributed error $N(0,s^2)$ was added to the exact height. In Fig. 41 an error of 0.2 m corresponded with the 95% confidence interval on the exact height location plus or minus 0.2 m.

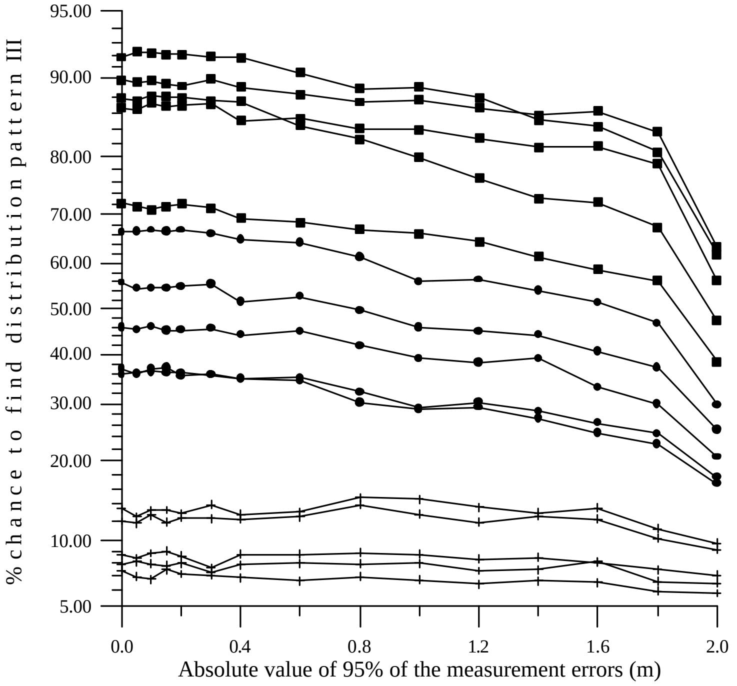


Fig. 41. Percent chance to find distribution pattern III versus the absolute value of 95 % of the measurement errors; (+) shows this chance for sampling 3 heights, (•) sampling 3 repeats at 3 heights and (■) sampling 9 heights; for each series 5 realisations are shown

As could be expected the chance on a correct distribution pattern decreased continuously with increasing magnitude of the measurement errors. Limiting 95 % of the errors to 0.3 m, which seems reasonable for field sampling, reduced the chance to find the correct distribution pattern with only 3%. Comparison of the series for sampling three heights with the series for sampling at nine heights showed that the magnitude of the influence depended on the total number of samples. By comparing the two series which sampled in total nine locations the influence of the division of the samples between heights and number of repeats per height can be seen (Fig. 41).

6.4.4.2 Sample selection parameters

The total number of samples is the product of the number of heights with the number of repeats at each height. As observed earlier, a larger number of samples relate with a higher chance to find the correct distribution. The relation between the number of heights and the chance to find pattern III is presented in Fig. 42.

When taking three repeats at each height, raising the number of heights from three to four results in an up to 15 % increase of the chance to find the correct distribution pattern. Every additional height class distinguished raised the chances by a few percentages. The gain of increasing the number of repeats at every height sampled is shown in Fig. 42. Sampling four heights and raising the number of repeats from one to two at every height results in a gain reaching 40 %. The addition of an extra sample, so three repeats at every height, generates a gain of another 15 %. There was a limited effect on the chance to find distribution pattern III by taking additional repeats at each height.

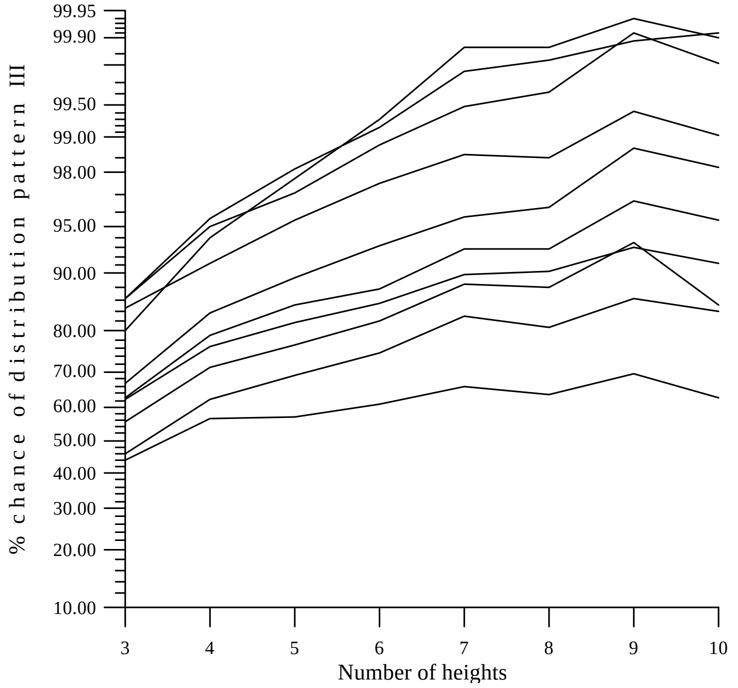


Fig. 42. Percent change to find the distribution pattern III versus the number of sampled heights. Shown for 10 realisations sampled with 2 repeats at each height

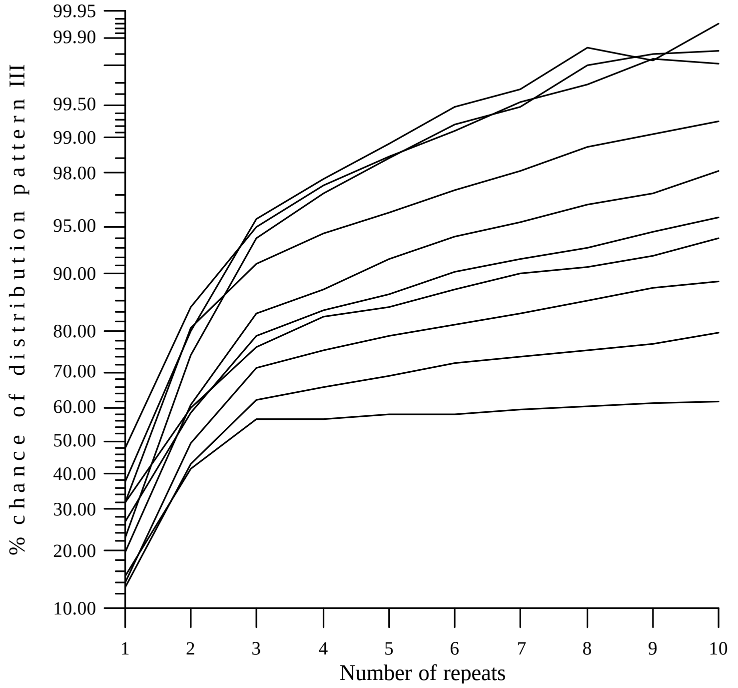


Fig. 43. Percent chance to find distribution pattern III versus the number of repeats. Shown for 10 realisations sampled at 4 heights

The relative gain of an additional repeat is larger than the relative gain of an extra sampling height, due to the higher absolute value of the latter it is advisable to increase the number of heights. As an example, a total number of nine samples are used. A first strategy could be to sample three repeats at three heights resulting in a 67 % chance to find the correct distribution. As concluded from above, a preferred alternative is to take one sample at nine heights, which results in a 76 % chance.

6.4.5 Verification of the alternative sampling procedure

In chapter 5 the current sampling strategy for Cd in tree leaves, i.e. sampling sun leaves of the upper third of the tree crown was evaluated. It was found that due to a vertical trend in the tree crown, solely sampling the upper part could yield biased estimates of both the average Cd concentration and the risk to exceed a pollution threshold. Therefore an alternative sampling procedure, accounting for the presence of a trend was proposed. This sampling procedure starts with investigating in a selected number of trees whether a trend is present and if so, it is described. Then the height where sampling will result in a representative estimation of the Cd concentration is deduced and the rest of the stand can be sampled at this height. In the previous paragraphs the current method to describe the trend of Cd concentrations was evaluated and improved in favour of its power. This paragraph aims to verify the alternative sampling procedure.

A representative sample was defined as a sample that returns the average Cd concentration of the whole tree. Therefore, the stand of the 100 realisations was sampled at nine heights, one sample per height and the position of the samples was localised with a precision of 0.3 m. The correlation coefficient threshold was set at 0.4, the middle of the crown was defined as all locations between three and five meter above ground level. As a result the average chance to describe the Cd distribution by the true distribution pattern was 76 %. The average Cd concentration for each tree was calculated from the nine samples. The samples were also used to model the trend. Then the height where sampling will result in a correct statement was derived from the model of the trend. This was repeated 500 times for each of the

100 realisations resulting in a distribution of the best sampling height. The height distribution ranged from 3.9 to 4.5 m (Fig. 44).

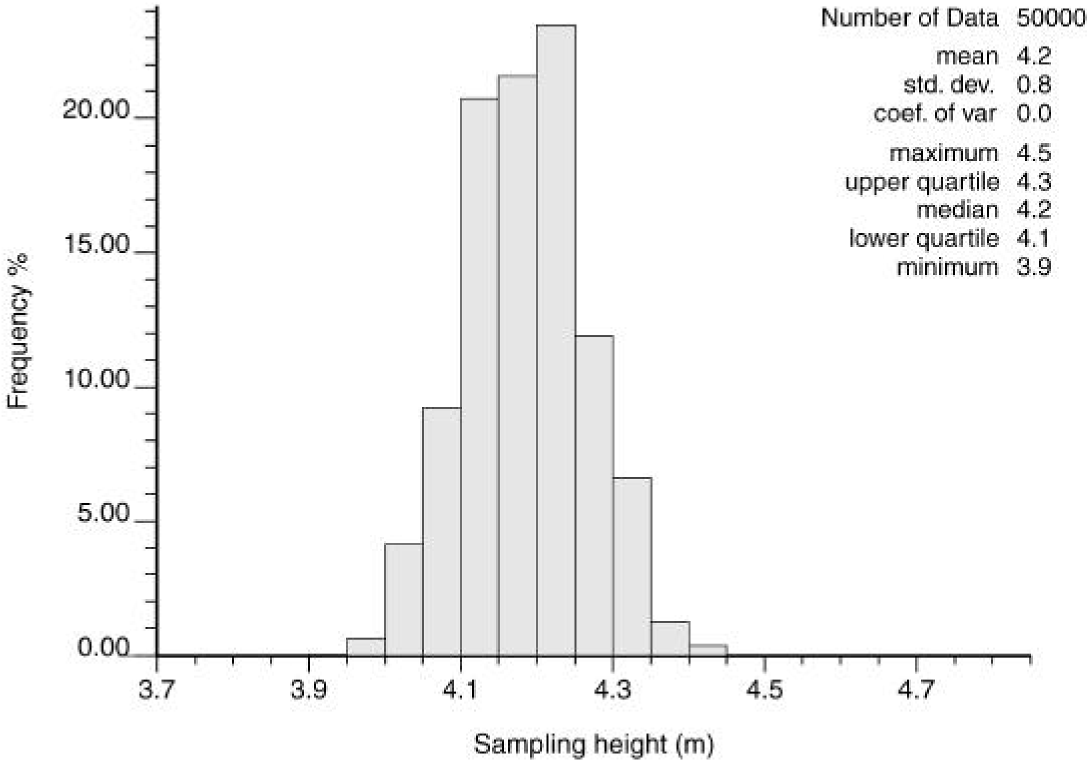


Fig. 44. Histogram of heights to sample to extract a sample representative for the average Cd concentration of the entire crown

According to the alternative sampling strategy all trees are sampled at a height of 4.2 m and the returned Cd value is expected to represent the average Cd concentration of the tree. The comparison of the true average Cd concentration and the average Cd concentration obtained by sampling one location at a height of 4.2 m is shown in Fig. 45. The bisector on the figure shows that the estimation of the average by the alternative sampling strategy is slightly biased whereas the generally used sampling procedure i.e. sampling the upper third of the crown, resulted in a severe underestimation of the mean Cd concentration for all realisations. Furthermore the 25 and 75 percentiles for the alternative sampling strategy are smaller than they are when sampling the upper third of the crown indicating that it provides more precise estimations.

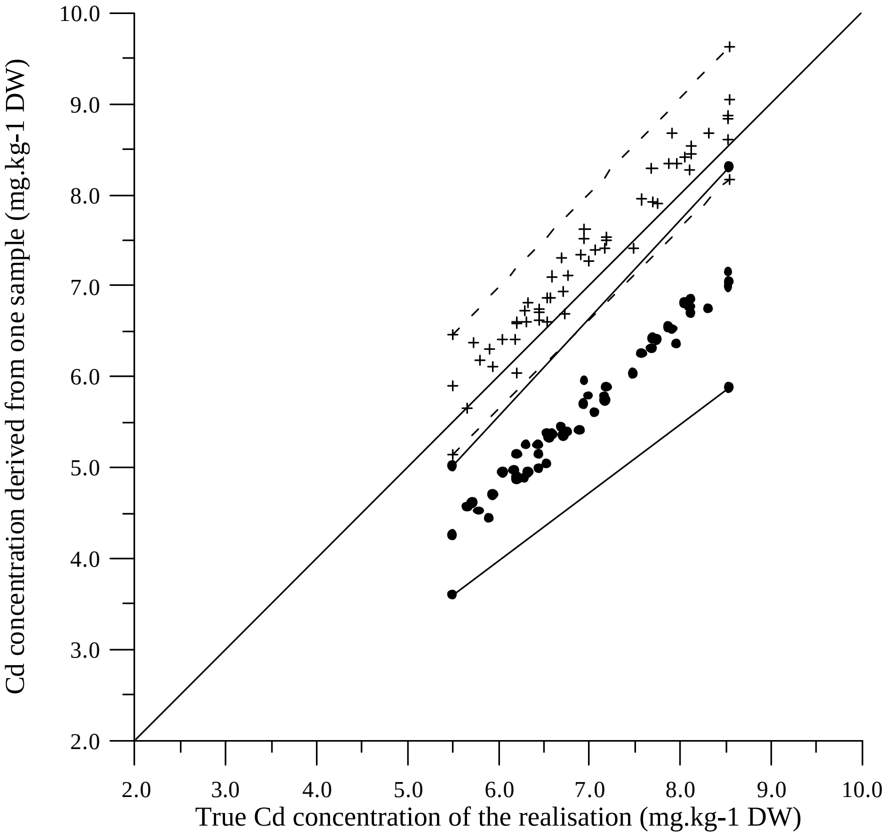


Fig. 45. Average Cd concentration derived from sampling at one height with the 25 and 75 percentile of the Cd concentration at that height; (•) shows these values when the upper third of the crown is sampled, (+) shows these values when sampling at 4.2 m height. Figure is based on the results of 50 realisations

6.5 Conclusion

The conventional sampling strategy to study the presence of a trend was shown to lack power to describe the distribution of Cd in the crown of a willow. Although just one true distribution pattern of Cd existed in the crown, i.e. pattern III, applying the conventional sampling strategy could lead to four different conclusions concerning the distribution pattern, i.e. pattern I, II, III and IV. Therefore, it was concluded that a more powerful sampling strategy was needed. The power could be increased and thus the strategy optimised by increasing the number of sampling locations. This was found to be only effective if the threshold value of R_a^2 above which trend-models were accepted, was chosen after careful deliberation of the data. In addition the samples had to be taken at different heights instead of taking repeats at a more limited number of heights. The effect of imprecise measuring of sampling locations was found to be small if locations were measured with a precision of more than 0.3 m.

The alternative sampling strategy to estimate the average Cd concentration in the crown of a willow, proposed in the previous chapter, was found to result in a slightly biased estimate of the average. The generally used sampling procedure i.e. sampling the upper third of the crown, resulted in a severe underestimation of the mean Cd concentration. Furthermore, the alternative sampling procedure estimated the Cd concentration with a higher precision than the generally used strategy.

7 Spatial variability in the crown of *Salix fragilis* : representativity within the stand

7.1 Abstract

To be valuable the results of the previous chapters should apply to the majority of the trees in the stand. The Cd concentration within the stand was found to range from 1.1 to 14.3 mg.kg^{-1} DW. The data of the single tree should be representative for the majority of the trees in the stand. Using a bootstrap approach two possible parameters of representativiness were validated: the average Cd concentration and the Cd distribution pattern within the crown. An *ideal* simulated stand was compared with a *true* observed stand. It was found that sampling 5 heights resulted in an unbiased estimate of the average Cd concentration with a precision of plus or minus 1.0 mg Cd kg^{-1} DW in 95 % of the simulations. Further, 45 % of the sampled trees were best described by high Cd concentration in the lower part and low Cd concentrations in the upper part of the crown. Because this value was above the average of the *ideal* stand it was concluded that it was likely that the *true* stand was an *ideal* stand. This means that the vertical and spatial trend found in the single tree were likely to apply to the whole stand. Overall, it was concluded that the Cd distribution and concentration in the single tree were common in the stand and that the results of the previous chapters were not affected by the use of a single tree.

7.2 Introduction

The representative elementary unit of a stand is an individual tree. A decision has effects on the whole tree e.g. the whole tree will be removed when the average Cd concentration of the crown is above a given critical threshold even if the concentration in a part of the crown is below that threshold. Decisions on the stand level will be based on the results of individual trees. To be of any value the proposed sampling strategy for an individual tree should apply to the majority of trees in the stand. This condition is fulfilled provided the analysed tree was not an exception compared to the majority of the trees in the stand.

This chapter aims to validate that (i) the Cd concentrations found in the single tree were representative for the stand's average Cd concentration and (ii) the distribution pattern found in the single tree was representative for the majority of the trees in the stand. The elaboration of these aims completes the study on the spatial variability of cadmium in the crown of a *Salix fragilis* L. and its implications for leaf sampling.

7.3 Data processing

The resampling approach known as the bootstrap is explained in § 4.3.2. To use the bootstrap to solve the validation problem a simulated *ideal* and an observed *true* stand were used.

An *ideal* stand was simulated. In such a stand the Cd concentration of each individual tree can be described by an identical vertical relationship between Cd concentration and tree height and an identical spatial structure of the residuals. Suppose that the vertical relationship is the second order polynomial given in § 5.4.1.1 by equation [5.3] and the spatial structure by the variogram given in § 5.4.1.2 by equation [5.4]. On these assumptions the 100 simulated realisations (see § 6.3.1) represent 100 possible trees from the *ideal* stand. For each of these realisations the parameter of interest was calculated. All 100 realisations were, as in the real stand, sampled at five heights (1.5, 3.0, 4.5, 6.0 and 7.5 m). Based on this sampling the distribution of the estimated parameter of interest was calculated. This technique, using an empirical distribution to infer the distribution of a parameter of interest, is known as the bootstrap (Hjorth, 1994).

The *true* stand was measured. As explained in chapter 3, all 20 trees were sampled at five heights i.e. 1.5, 3.0, 4.5, 6.0 and 7.5 m above ground. The Cd distribution pattern was determined by the transfer function given in Fig 6.3. The percentage trees in the *true* stand best described by pattern III can be estimated by (Rubenstein, 1981):

$$[3.11] \quad \overline{p} = \frac{N_{patternIII}}{N_{total}}$$

the standard deviation of an estimated chance ($\overline{p}$) is estimated as:

$$[3.12] \quad s_{\overline{p}} = \sqrt{\frac{1}{N_{totaal}} \overline{p}\left(1 - \overline{p}\right)}$$

The percentage trees in the *true* stand, best described by pattern III, were bootstrapped (Efron & Tibshirani, 1993; Neter, 1996). From the 20 measured trees

20 trees were selected with replacement and the percentage trees best described by pattern III calculated. This was repeated 5000 times and so the distribution was built on 5000 bootstrap replicates. The distribution of the estimated average Cd concentration and the estimated distribution pattern of the *ideal* stand were compared with the Cd concentration and distribution pattern of the *true* stand observed in the field.

7.4 Results and discussion

7.4.1 Explorative data analysis

In Fig. 46 the sampled locations in the *Salix fragilis* L. stand are shown. From the selected sampling locations 93 contained leaf biomass.

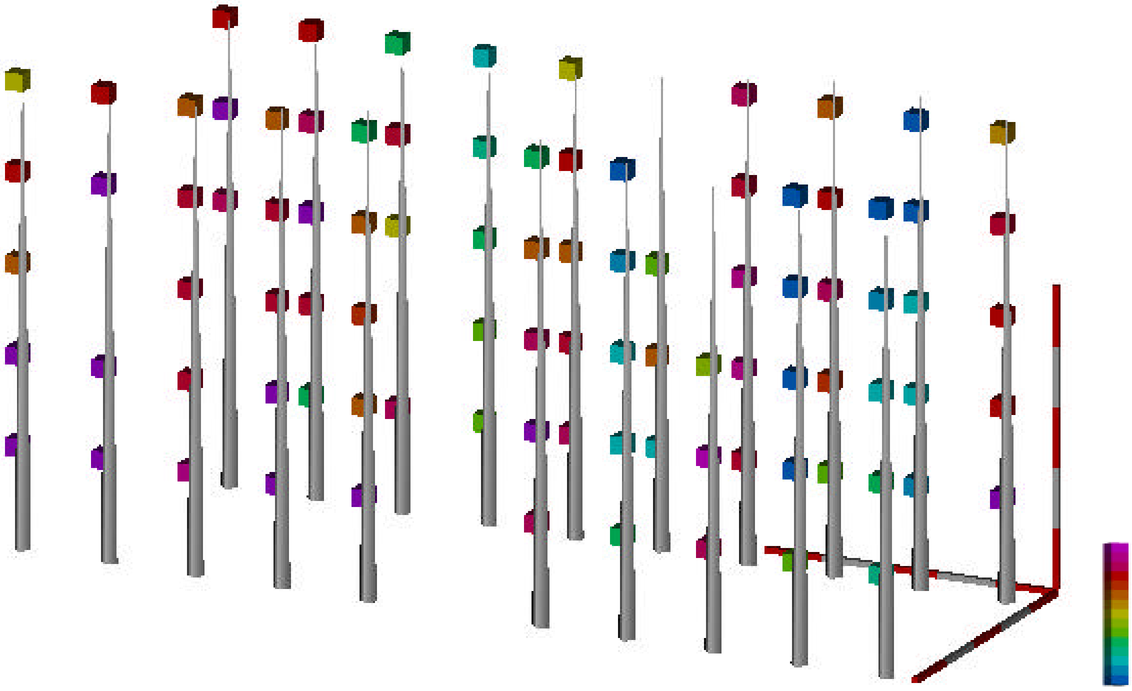


Fig. 46. Cd concentrations at the sampled locations within the studied stand. Cd scale ranges linearly from 1.1 (blue) to 14.3 mg.kg^{-1} DW (purple); the markers show the origin of the longitudinal axis and represent 5 m, a single unit 1 m. Stand is shown from a North-eastern view.

The Kolmogorov-Smirnov test for normality (Neter, 1996) was accepted with a probability of 0.53 and corroborated what can be seen in Fig. 48 in specific that the data came from a normal distribution. The distribution was characterised by a mean Cd concentration of 6.9 mg.kg^{-1} DW and a standard deviation of 3.2 mg.kg^{-1} DW. As a result the coefficient of variation approached 0.5, which indicated a high variation.

Based on the samples from the *true* stand Fig. 47 was constructed. On average the Cd concentration of a single tree measured 6.9 mg.kg^{-1} DW. The standard deviation calculated on the average Cd concentrations of the individual trees was 2.7 mg.kg^{-1} DW. The standard deviation of the stand amounted to 3.2 mg.kg^{-1} DW. Due to the limited number of repeats (number of trees in the stand = 20) the standard deviation was considered as directive rather than descriptive.

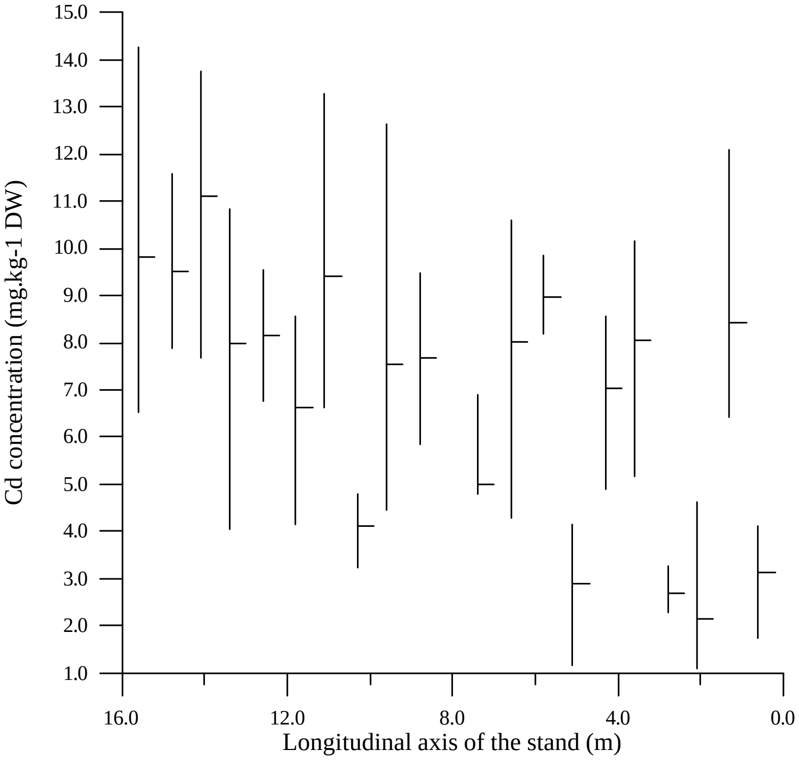


Fig. 47. Minimum, maximum and average Cd concentration of each tree in the stand

The Cd concentration increased along the longitudinal axis of the stand. The relation between distance on the longitudinal axis and Cd concentration in the crown could be described with a Pearson correlation coefficient of 0.53 (p=0.00). On the other hand the NH_4-EDTA extractable fraction of Cd in the soil was determined on 12 locations at a depth of 0.3 meter. A not significant (p=0.18) relation between distance on the longitudinal axis of the stand and Cd concentration in the soil was found ($\rho = 0.41$). Because soil and leaf samples were taken at different locations in the stand it was not possible to formulate the direct correlation between the soil and leaf concentrations. Both relations along the longitudinal axis suggest that the Cd concentration in the soil is one of the factors affecting the Cd concentration in the leaves.

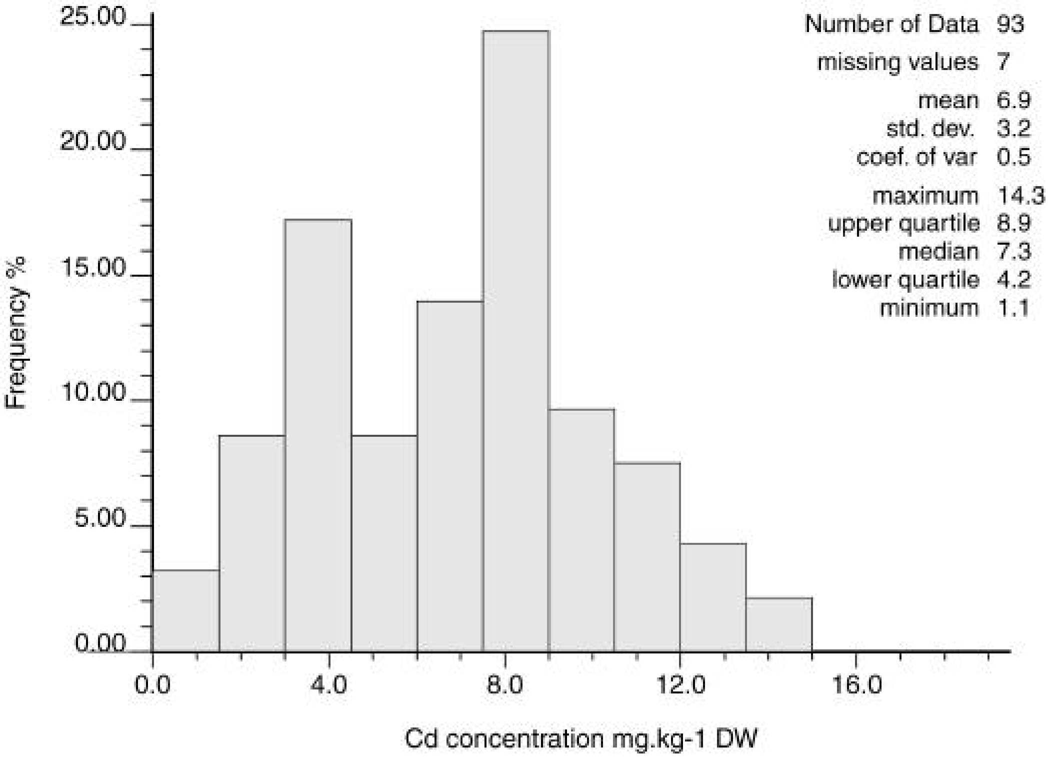


Fig. 48. Histogram of the Cd concentration in the 20 crowns of a Salix fragilis L. stand with some descriptive statistics

The lack of correlation stressed the idea that presence of metal in foliage does not imply that the foliar levels of metal are a direct result of concentrations present in the soil (Hogan & Wotton, 1984). Biogeochemical and physiological processes not well understood determine the uptake and distribution within the plant. Uptake is known to be both active and passive (Hagemeyer et al., 1986). Metabolic thus active transport seems to be prevailing at low concentrations, whereas passive transport becomes more important at toxic levels (Godbold, 1991). Most investigators showed that the uptake of heavy metals into roots increases with increasing supply in the medium (Kahle, 1993; Alloway 1995).

From the roots the metals are distributed all over the tree. But many researchers have noted the marked accumulation of soilborne heavy metals in root systems (Jarvis et al., 1976; Garcia Sánchez et al., 1999), only small amounts are transported to the shoots (Sanità di Toppi & Gabbrielli, 1999). Heavy metal movement is regulated by vascular tissues. The transfer of Cd across the endodermis in the roots to the vascular system in the stele is not well understood. Translocation of Cd to the tops would not only be related to water flow (Hughes et al. 1980; Prasad, 1995) but also to Cd uptake by the root system (Hardiman et al., 1984; Prasad, 1995). Seasonal changes in organic content of xylem sap (Hughes et al. 1980), plant organ, and age of the plant (Alloway, 1995) would also be important. The processes which result in the final foliar element concentrations were divided by Keller et al. (1994) in (i) xylem transport, leaf-internal processes e.g. translocation and dilution, and leaching from leaves (ii) leaf-external processes e.g. deposits entering the tissue, epidermal contamination and wash off. The final heavy metal distribution in tree crowns is the result of an interaction of these processes. If the physiological processes will be better understood it would be possible to better explain the observed Cd concentration in tree leaves.

The complex relation between soil and plant processes was illustrated by the weak correlation between soil and leaf concentration. Due to this complex of processes a leaf sample contains additional information to a soil sample.

7.4.2 Validation of the use of the single tree

7.4.2.1 Validation of the average Cd concentration

The hypothesis will be tested that the Cd concentrations found in the single tree are representative for the stand. If so, this means that the conclusions based on the Cd concentration of the sampled tree are related to a common situation in the stand and are therefore likely to apply to all trees within the stand.

Suppose an *ideal* stand as described in § 7.3. On these assumptions the 100 simulated realisations (see § 6.3.1) represent 100 possible trees from the *ideal* stand. For each of these realisations the *exact* average Cd concentration was calculated. All 100 realisations were, as in the *true* stand, sampled at five heights (1.5, 3.0, 4.5, 6.0 and 7.5 m). Based on this sampling the *estimated* average Cd concentration was calculated. For each simulation the difference between its *exact* and *estimated* average concentration was determined. Because the sampling campaign was simulated 50 times at 100 realisations a data set of 5000 differences could be used. It was found that sampling 5 heights resulted in an unbiased estimate of the average Cd concentration with a precision of plus or minus 1.0 mg Cd kg^{-1} DW in 95 % of the simulations (Fig. 49).

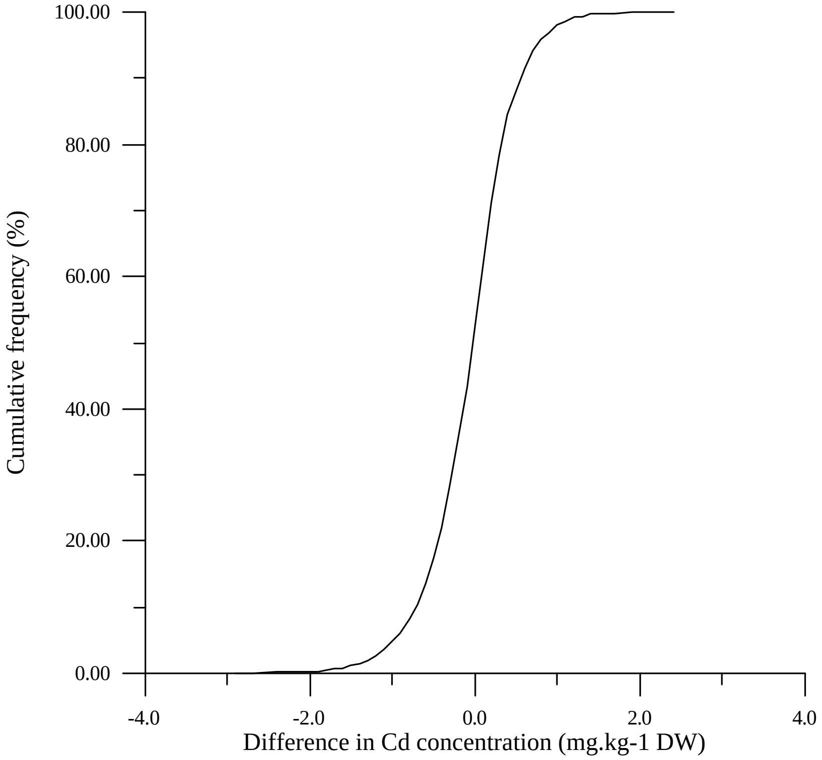


Fig. 49. Difference between the *exact* and *estimated* average Cd concentration (mg.kg^{-1} DW)

The estimated average Cd concentration of the correct distribution class (III) was found to differ significantly (p=0.000) with an effect size of 0.02 mg.kg^{-1} DW from the average Cd concentration of the miss classified. Due to the high number of samples i.e. 5000 this difference was found to be significant. Johnson (1995) warns for overemphasis on statistical testing, a statistical significance should not be confused with a biological significance which implies importance in some sense. In earlier chapters importance was defined in two ways :

- (i) In § 3.3 importance was defined in a pure analytical sense by using the measured relative standard deviation on the laboratory control sample. An effect size of 0.1 mg.kg^{-1} DW was then considered as relevant. Smaller deviations were interpreted as noise. As a consequence all Cd concentrations are given with one significant decimal figure.

- (ii) In § 4.4.2 an effect size was considered important if two samples differed more than 0.5 mg.kg^{-1} DW. The magnitude of the effect size relates to considerations as explained in § 4.3.1 and § 4.4.2.

In both definitions effect sizes are bigger than the reported difference of 0.02 mg.kg^{-1} DW between the average Cd concentration in crowns with the correct and a miss classified distribution pattern. So, the effect size was considered as unimportant and therefore it was concluded that sampling 5 heights was suitable to infer the crown's average Cd concentration, even if the assigned distribution pattern was wrong.

From the above conclusions it was inferred that for each individual tree, given in Fig. 47 the mean Cd concentration was correctly estimated. Due to the sampling strategy a 95 % confidence interval of 1.0 mg.kg^{-1} DW was applied. By averaging the mean values for all trees within the stand the stand average was correctly calculated. Due to the sampling strategy, averaging widened the 95 % confidence interval of the stand's mean Cd concentration to 1.7 mg.kg^{-1} DW. A comparison of the stand's mean Cd concentration i.e. 6.9 mg.kg^{-1} DW with the tree's mean Cd concentration i.e. 6.3 mg.kg^{-1} DW reveals a difference smaller than the uncertainty caused by the sampling strategy. Therefrom it was concluded that the single tree was an average tree in the stand and therefore common in the stand.

7.4.2.2 Validation of the distribution pattern of Cd

The discussions and conclusions of chapters 5 and 6 are partly based on the distribution pattern of Cd measured in a single tree crown. This far, the use of a single tree limited the impact of the conclusions to that single tree. The hypothesis will be tested that the distribution pattern found in the single tree was valid for the majority of the trees in the stand. If so, this would mean that the conclusions of chapters 5 and 6 could be generalised to all trees in the stand.

The data of a single willow showed that the Cd concentration was lower in the top than in the bottom of the crown (§ 5.4.1.1). A similar trend was found, 17 out of 20 trees had a lower Cd concentration in the top sample, taken at 7.5 m compared to the bottom sample, taken at 1.5 m above ground level. Based on the field sampling for each tree in the *true* stand the distribution pattern was calculated according to the transfer function shown in Fig. 6.3. Using equation [3.11] it was calculated that the Cd distribution in 45% of the trees in the stand was best described by pattern III which means low concentrations in the top and high concentrations in the bottom of the crown. The standard deviation was 11% estimated by equation [3.12]. Similar results were found with the bootstrap: a mean chance of 41 % with a standard deviation of 12 %, the cumulative histogram is given in Fig. 50.

Suppose an *ideal* stand as explained in § 7.3. On these assumptions the 100 simulated realisations (see § 6.3.1) represent 100 possible trees from the *ideal* stand. From these 100 realisations, as in the real stand, five samples from five heights (1.5, 3.0, 4.5, 6.0 and 7.5 m) were selected. This sampling campaign was simulated 50 times. For each of these 50 simulations the percentage trees with distribution pattern III was computed, the cumulative histogram is given in Fig. 50. The figure shows that sampling an *ideal* stand would on average result in 35 % of the trees described by pattern III. Because in the *true* stand it was found that 45 % of the sampled trees were best described by pattern III, it was concluded that it was likely that the *true* stand was an *ideal* stand. This means that the vertical and spatial trend found in the single tree were likely to apply to the whole stand. Therefore the conclusions of chapters 5 and 6 could be generalised for all trees within the stand. This favourable result might be explained partly by the fact that willow stands are often planted with a single clone. The monoclonal origin of the stand could not be proven by Fig. 47 or by the records of the stand.

Comparing both distributions with a t-test at the 0.05 significance level it was found that the distributions does differ and that the percentage trees with distribution pattern III in the *true* stand was higher than in the *ideal* stand. Because all trees in the ideal stand were built on a distribution pattern III the following explanations were withdrawn :

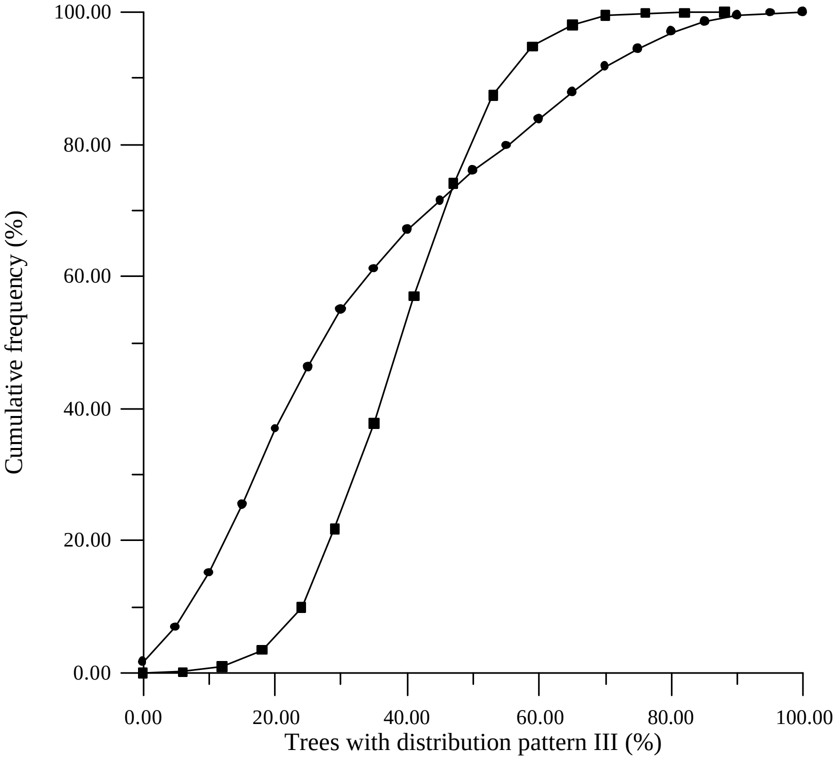


Fig. 50. Cumulative histogram of the percentage of trees best described by distribution pattern III; (●) represents sampling 100 realisations at 5 heights; (■) represents the bootstrapped distribution of sampling 20 realisations at 5 heights .

(i) Due to the high number of replicates a statistical significance was found. Johnson (1995) warns for overemphasis on statistical testing. A statistical significance should not be confused with a biological significance. Biological significance implies importance in some sense (e.g. in § 7.4.2.1). It was not clear if the 6 to 10 % differences between the means corresponded with a biological significance.

(ii) Evidence was found in the *true* stand's data where six out of the nine trees with distribution pattern III showed a steeper trend than the one used to built the ideal stand. Using a limited number of samples a steep trend is easier to recognise than a weak one resulting in a higher number of trees crowns best described by Cd distribution pattern III.

(iii) Although the variation within the 20 trees, calculated on five samples per tree, does not support this explanation the variation introduced by the sequential indicator based algorithm might overestimate the true variation. If the *true* stand shows a lower variation it would be easier to recognise a trend which will result in a higher number of trees best described by distribution pattern III.

The above considerations do not change the conclusion that the vertical and spatial trend found in the single tree were likely to apply to the whole stand.

7.5 Conclusions

The stand showed an average Cd concentration of 6.9 mg.kg^{-1} DW with a standard deviation of 3.2 mg.kg^{-1} DW. Sampling 5 heights resulted in an unbiased estimate of the average Cd concentration even if the assigned distribution pattern was wrong. Comparison of the stand's with the single tree's average Cd concentration led to the conclusion that the average Cd concentration in the single tree was representative for the average Cd concentration in the stand. Because the number of trees in the *true* stand, best described by pattern III, was higher than the average number of trees in the *ideal* stand, best described by distribution pattern III, it was concluded that it was likely that the *true* stand was an *ideal* stand. The distribution pattern of the single tree was representative for the majority of the trees in the stand. Overall, it was concluded that the results of the previous chapters, based on a single tree were valid for the whole stand. Although not known whether the explanation applies to the studied stand, the favourable results might be explained by the presumed monoclonal origin of the stand.

8 General conclusions

Literature supplied evidence that beside a sensitive tool in pollution studies leaf analysis can be used in nutrient status and forest health studies. Leaf sampling was found to be complicated by several factors causing a spatial and temporal variation. Because studies can have different objectives calling for different information this variation is thought to be the opportunity to design different sampling strategies to answer the aims of the study. To explore this opportunity the generally used and recommended procedure for foliar analysis was questioned and tested. It was thought to raise a biased estimate of the average Cd concentration and of the risk that the foliage of *Salix fragilis* L. is polluted.

Due to the observed variability between leaves, leaves had to be bulked in representative elementary sampling units. Different approaches i.e. the analytical, the resampling and the geostatistical were found to cause the number of leaves ranging from 32 to 56 within a representative elementary sampling unit. Most of the reported sample sizes were bigger than the calculated ones. Therefore, objectifying the determination of the size of a representative elementary sampling unit was found to be an improvement irrespective of the mathematical approach used. Violating the assumption on which the methods are based could lose improvements made by objectifying the determination of the size of a representative elementary sampling unit. It was concluded that a sampling unit with dimensions of 0.30 m by 0.30 m by 0.30 m did not smooth the large-scale variance and was therefore suitable to be used as a representative elementary sampling unit in the continuation of the study.

Sampling the whole crown of a willow, based on this sampling unit was found to be spatially even distributed and unaffected by the 15-day span of sampling. Cadmium concentrations of sampling units from the crown of the willow tree varied between 2.3 $mg.kg^{-1}$ DW and 10.6 $mg.kg^{-1}$ DW or 23.1 $mg.kg^{-1}$ DA to 73.0 $mg.kg^{-1}$ DA. Between Cd concentration, referenced to dry weight (DW) and dry ash (DA), and aspect no correlation was found but a strong trend with the low concentrations in the top and the high concentrations in the bottom of the crown was observed. Compared to the deterministic approach, the use of the sequential indicator simulation, a geostatistical tool, improved the estimation of the Cd concentration at non-sampled locations. The lower variance of the Cd concentration referenced to dry ash resulted in better performing simulation algorithms and lower uncertainties on the predicted Cd concentrations. Unless the advantages, the lack of threshold values and the rareness of its application made that instead of dry ash, dry weight was used as a reference to express the Cd concentration. For both methods of expression, but especially when dry weight was used as reference, no evidence was found to support the general sampling strategy for tree leaves. The samples could result in a biased estimate of the average leaf Cd concentration or result in a wrong evaluation that an environmental threshold is exceeded. Therefore an alternative sampling procedure which derived the sampling location based on the description of the trend in element concentration was proposed.

The developed conceptual approach to evaluate a sampling strategy led to the conclusion that the sampling strategy applied by several authors to describe a concentration-trend lacked power. By raising the number of heights to sample, the sampling strategy was optimised in favour of its power. Using this optimised strategy as part of the alternative sampling procedure it was concluded that the alternative sampling procedure resulted in an slightly biased and more precise estimate of the mean Cd concentration in willow than the generally used sampling procedure.

It was shown that the vertical and spatial trend found in the single tree were likely to apply to the whole stand and that the average Cd concentration of the crown of the single tree was representative for the average Cd concentration of the whole stand. Overall, it was concluded that the single tree was a common tree in the stand and that the results apply to the whole stand. The favourable result that the single tree was likely to represent the whole stand might be caused by the presumed monoclonal origin of the stand.

Summary

Many authors who studied and monitored pollution in forests generally agreed on a leaf sampling procedure for nutrients i.e. sampling fully developed leaves from the upper third of the crown of a dominant or co-dominant tree before the very beginning of senescence. Pollution studies and monitoring programs can have different objectives, each objective requiring different information and possibly calling for a different sampling strategy. Due to the variation in element concentration in the tree crown there is an opportunity to develop different sampling strategies in compliance with the aims of the study. To show the need of different sampling strategies the central hypothesis was introduced that the generally used and recommended technique for foliar analysis yields a biased estimate of the average Cd concentration and of risk that the willow leaves are polluted.

The size of the representative elementary sampling unit is a measure to control practical sampling and the processes that can be studied. The latter are related to the study's objectives. Based on the variability between single leaves the size of a representative elementary sampling unit to describe Cd in the crown of *Salix fragilis* L. was determined to be 0.3 m by 0.3 m by 0.3 m. An analytical, an independent bootstrap, a dependent bootstrap and a geostatistical approach were compared. Important differences were found between calculated and reported sample sizes. It was concluded that objectifying the determination of the sample size in accordance with the aim of the study was an improvement. Violating the assumptions on which the mathematical methods are based can lose part of this improvement. The dimension of the representative elementary sampling unit determined by the geostatistical approach was used in the continuation of the study. The study proceeded with sampling a whole crown of *Salix fragilis* L.. To allow the use of geostatistical tools 292 samples were analysed on their Cd concentration. Cadmium concentration was expressed referenced to dry weight and dry ash. For both expressions a clear trend with high Cd concentration in the bottom and low concentrations in the top of the crown was found. The residuals after removal of the trend showed a clear spatial structure. The residuals for both methods of expression could be modelled by a similar variogram. Trend and variogram were used in a sequential indicator simulation to predict the Cd concentration at non-sampled

locations. The average simulated Cd concentration in the crown enabled the evaluation of the current sampling strategy. It was found that the current sampling strategy could yield biased estimates of the average Cd concentration. Referenced to DW the Cd concentration was underestimated with 1.3 to 3.3 mg Cd.kg^{-1}. When referenced to DA the underestimation amount 3.0 to 12.0 mg Cd.kg^{-1}. The chance that a 6 mg Cd.kg^{-1} DW threshold or its equivalent in DA value was exceeded was underestimated with 70.0 to 99.8%. Therefore an alternative sampling procedure was proposed. This procedure investigates whether a trend is present. Once the height where sampling results in a correct statement of the tree's pollution is located, the rest of the stand is sampled at this height. A conceptual approach to optimise and validate a sampling strategy was developed and tested on the alternative sampling procedure. Its performance was compared with the performance of the general sampling strategy. The alternative sampling strategy was found to be an improvement to estimate the average Cd concentration compared to the general strategy. The alternative sampling strategy was found to underestimate the average Cd concentration with less than 0.5 mg Cd.kg^{-1} DW. The study on the spatial variability of Cd in the crown of *Salix fragilis* and its implications for leaf sampling was completed with the justification of the use of a single tree. An *ideal* simulated stand was compared with a *true* observed stand. It was found that the Cd distribution and concentration in the single tree were common in the stand. The results of the study were not affected by the use of a single tree and apply to the majority of the trees within the stand.

The study strongly recommended to abandon the practice of generally used standardised sampling in pollution studies. Instead the sampling strategy should be optimised based on the study’s objectives. The variation is thought to be the opportunity to design a sampling strategy in compliance with the objectives of the study. Sampling strategy and configuration then depends on the aimed precision and the accepted chance to wrongly reject the tested hypothesis. As a consequence this approach needs to characterise the variation and therefore more data, resulting in higher costs, are required than in the generally used procedures. The higher costs are often justified by the objectives of the study especially when aiming decision-making which involves high financial repercussions e.g. sanitation of polluted sites, scientific research, etc.

The results of a share of leaf sampling programmes are used in decision-making with less clear financial repercussions e.g. monitoring of the health state of forests, detection of time and spatial patterns, etc. In this context generally used standardised methods were developed e.g. the programme on assessment and monitoring of air pollution effects on forests developed the sampling strategy referred to in this work as UN/ECE-EC (1998). This sampling strategy is advantageous to attain a high degree of standardisation and to minimise the sampling costs. At the other hand important information on the variability of the element concentration is lost. The sampling strategy to detect the time and spatial patterns of heavy metals in deciduous trees, in specific Cd in *Salix fragilis* L. by a large scale monitoring program could be improved by the following measures :

- The use of dry ash as reference to express the metal concentration in leaves.
- The use of a pilot study to gain insight in the variability between leaves and consequentially in the size requirements of a representative elementary sampling unit. This would ensure that the size of the sampling unit is in compliance with the aims of the monitoring program.
- The use of an aimed precision to infer the number of samples needed to describe the element status of a tree.
- The use of the description of the element concentration trend to derive the sampling location.

The scientific insights can then be used as a base to develop an accurate and precise standardised method. The study gives an impetus to continue methodological research on leaf sampling. The gained insights should be extended for all common heavy metals in common broad-leaved tree species. By studying several sources of pollution the results could bring a better understanding of how to sample and of the pollution itself in deciduous trees. Often, management decisions are made on the stand level. So, besides extending the knowledge on leaf analysis to sample on a tree level, a sound sampling methodology for the stand level should be developed. This study started from the idea that because leaves from within a crown show an important variation, a single tree is best described by several samples with a representative elementary volume. To decide whether a stand is contaminated based on these input data an interpolation and increase of support known as upscaling are involved. Upscaling from a representative elementary volume to characterise the Cd concentration of the stand is expected to be data intensive. Future research, aiming to underpin management decisions on the stand level could ignore the existence of trees and consider the stand as an individual. Elementary sampling units are then selected so they are representative to describe the element concentration in the stand. It is conceivable that this straightforward approach describes the element concentration of the stand with the same precision as the upscaling method but requires less input data. As a drawback of this approach it is mentioned that information on the tree level will not be available. This approach needs to be proven valid.

Samenvatting

De algemene bladbemonsteringsstrategie voor nutriëntenconcentraties in kronen nl. het bemonsteren van ontwikkelde bladeren uit de bovenste derde van de kroon van een dominante of co-dominante boom voor senescentie, wordt frequent gebruikt voor het bestuderen of monitoren van verontreinig in bossen. Echter, studie en monitoring van verontreiniging kunnen andere doelstellingen hebben dan de studie van de nutriëntenconcentraties. Verschillende doelstellingen vereisen andere informatie en bijhorend een andere bemonsteringsstrategie om gerealiseerd te kunnen worden. Doordat de elementconcentratie in de kroon een variatie vertoont ontstaat de mogelijkheid om verschillende bemonsteringstrategieën, op maat van de doelstellingen uit te werken. Om de noodzaak van verschillende bemonsteringsstrategieën aan te tonen werd uitgegaan van de hypothese dat de algemeen gebruikte bemonsteringsstrategie de gemiddelde Cd-concentratie in bladeren en de kans op een overschrijding van een drempelwaarde voor Cd in de bladeren vertekend schat.

De grootte van een representatieve bemonsteringseenheid is een manier om de bemonsteringspraktijk en de processen die bestudeerd kunnen worden te beïnvloeden. Deze processen zijn nauw verbonden met de doelstellingen van de studie. Uitgaande van de variatie tussen individuele bladeren werd de grootte van een representatieve bemonsteringseenheid bepaald op 0.3 m bij 0.3 m bij 0.3 m. Een analytische, een onafhankelijke bootstrap, een afhankelijke bootstrap en een geostatistische methode werden vergeleken. Tussen de in deze studie berekende en in andere studies gerapporteerde grootte werden er belangrijke verschillen gevonden. Er werd besloten dat het objectiveren van de bepaling van de grootte van een bemonsteringseenheid in overeenstemming met de doelstellingen van de studie een verbetering is. Een deel van de verbetering kan tenietgedaan worden door het niet respecteren van de basis veronderstellingen van de methode. De dimensies, bepaald door de geostatistische methode, werden gebruikt in het verder verloop van de studie. Vervolgens werd een volledige kroon van een *Salix fragilis* L. bemonsterd. De Cd-concentratie werd voor 292 stalen geanalyseerd zodat op deze gegevens een geostatistische analyse kon uitgevoerd worden. De Cd-concentratie werd zowel uitgedrukt per eenheid droog gewicht als per eenheid droge as. Voor beide

benaderingen werd een duidelijke trend met de hoogste concentraties onderaan en de laagste concentraties boven in de kroon vastgesteld. Na het verwijderen van deze trend kon door middel van een variogram, voor beide benaderingen een duidelijke en gelijkvormige ruimtelijke structuur in de residuen beschreven worden. De trend en het variogram werden gebruikt in een sequentiële indicator simulatie om de Cd-concentraties op niet geanalyseerde locaties in de kroon te voorspellen. De voorspelling van de gemiddelde Cd-concentratie voor elke locatie in de kroon maakt het mogelijk om de bruikbaarheid van de algemene bemonsteringstrategie te beoordelen. Er werd vastgesteld dat de algemene bemonsteringsstrategie kan leiden tot een vertekende schatting van de gemiddelde Cd-concentratie. Uitgedrukt op het gehalte DW werden onderschatting van 1.3 tot 3.3 mg Cd.kg^{-1} gerapporteerd. Voor het gehalte DA beliepen de onderschattingen 3.0 tot 12.0 mg Cd.kg^{-1}. De kans op een overschrijding van een drempelwaarde van 6.0 mg Cd.kg^{-1} werd 70.0 tot 99.8 % te laag ingeschat. Om hieraan te verhelpen werd een alternatieve bemonsterings-strategie voorgesteld. Deze strategie onderzoekt of er een trend aanwezig is. Indien aanwezig wordt de hoogte waarop een juiste waarde voor de verontreiniging van de boom bekomen wordt bepaald. De rest van het bestand wordt op deze hoogte bemonsterd. Een conceptuele benadering om een bemonsteringsstrategie te optimaliseren en te valideren werd ontwikkeld en uitgetest op deze alternatieve bemonsteringswijze. Op die manier konden de resultaten van deze bemonsterings-strategie vergeleken worden met de resultaten van de algemene bemonsteringsstrategie. Er werd aangetoond dat de alternatieve strategie een duidelijke verbetering was ten aanzien van de algemene strategie. De alternatieve strategie onderschatte de gemiddelde Cd-concentratie met slecht 0.5 mg.kg^{-1} DW. De studie van de ruimtelijke variabiliteit van Cd in de kroon van *Salix fragilis* en de implicaties voor bladbemonstering werd afgesloten met een verantwoording voor het gebruik van een enkele boom. Een *ideaal* bestand werd gesimuleerd en vergeleken met een *echt* gemeten bestand. Hieruit bleek dat de Cd-concentratie en verdeling in de enkele boom representatief was voor het volledige bestand. De resultaten van de studie werden niet beïnvloed door het gebruik van een enkele boom en zijn van toepassing op de meerderheid van de bomen in het bestand.

De studie bevat sterke aanwijzingen om het gebruik van gestandaardiseerde algemene bemonsteringsstrategieën te verlaten voor het bemonsteren van verontreiniging in bomen. In de plaats daarvan zouden strategieën die gebaseerd zijn op de doelstellingen van de studie moeten aangewend worden. De variatie wordt beschouwd als de mogelijkheid om bemonsteringsstrategieën te ontwerpen op maat van de doelstellingen van de studie. De bemonsteringsstrategie wordt dan bepaald door de nagestreefde precisie en de aanvaarde kans om ten onrechte de te testen hypothese te verwerpen. Als gevolg van deze benadering dient de variatie in de kroon beschreven te worden. Dit vergt meer gegevens en dus meer middelen dan de algemeen gebruikte bemonsteringsstrategieën. Deze kosten worden vaak verantwoord door het doel van de studie. Hierbij wordt gedacht aan studies die moeten resulteren in een beslissing met grote financiële gevolgen zoals sanering van historisch verontreinigde bossen, wetenschappelijk onderzoek, enz.

Een deel van de bladanalyses worden aangewend in studies met minder duidelijke financiële gevolgen vb. monitoring van de gezondheidstoestand van bossen, het beschrijven van temporele en ruimtelijke patronen, enz. Het is dan ook in deze context dat de algemeen gebruikte gestandaardiseerde methodes ontwikkeld werden vb. *the programme on assessment and monitoring of air pollution effects on forests* ontwikkelde de bemonsteringsstrategie waarnaar in dit werk verwezen wordt als UN/ECE-EC (1998). Deze strategie is voordelig om een hoge graad van standaardisatie te bekomen met minimale bemonsteringskosten. Hiertegenover staat dat een belangrijk deel van de informatie over de variatie van de elementconcentratie verloren gaat. De bemonsteringsstrategie om temporele en ruimtelijke patronen van zware metalen in loofbomen, meer in bijzonder Cd in *Salix fragilis* L., te bemonsteren binnen een monitoringprogramma kan door middel van de volgende maatregelen verbeterd worden :

- Het gebruik van droge as als referentie om de metaalconcentraties in de bladeren uit te drukken.
- Het gebruik van een pilootstudie om inzicht te verwerven in de grootte van een representatieve bemonsteringeenheid. Dit zou de garantie bieden dat de bemonsteringseenheid in overeenstemming is met de doelstellingen van het monitoringprogramma.
- Het gebruik van de na te streven precisie om het aantal stalen af te leiden dat noodzakelijk is om de elementconcentratie van een boom te beschrijven.

- Het gebruik van de trend in elementconcentratie om de bemonsteringslocatie binnen de kroon af te leiden.

De hierdoor verworven inzichten kunnen dan gebruikt worden om een accurate en precieze gestandaardiseerde methode te ontwikkelen. De studie geeft een aanzet tot verder methodologisch onderzoek over bladbemonstering. De verworven inzichten dienen uitgebreid te worden voor alle veel voorkomende zware metalen in veel voorkomende loofboomsoorten. Het betrekken van verschillende bronnen van verontreiniging in de studie kan leiden tot een beter begrip van de bemonsteringstrategie voor en van de verontreiniging in loofbomen. Veelal worden beheersbeslissingen op bestandsniveau genomen. Behalve een uitbreiding van de kennis over bladbemonstering om een boom te beschrijven dient er ook een bemonsteringsstrategie ontwikkeld te worden om een bestand te beschrijven. Deze studie ging uit van het idee dat, aangezien de bladeren binnen een kroon variatie vertonen, een individuele boom het best beschreven wordt door verschillende bemonsteringseenheden. Deze gegevens dienen opgeschaald te worden om te beslissen of een bestand verontreinigd is. Meer bepaald dienen de gegevens geïnterpoleerd te worden en de *support* vergroot. Deze opschaling van een elementaire bemonsteringseenheid tot bestandsniveau is gegevensintensief. Toekomstig onderzoek, dat dient om beslissingen op bestandsniveau te ondersteunen, kan het bestaan van bomen negeren en het bestand beschouwen als een individu. Bemonsteringseenheden worden dan dusdanig gekozen dat ze representatief zijn om de elementconcentraties van het bestand te beschrijven. Het valt te verwachten dat deze benadering de element concentraties van het bestand kan beschrijven met dezelfde precisie als de opschalingsmethode, echter met minder gegevens. Als nadeel van deze benadering dient vermeld te worden dat informatie over het boomniveau niet beschikbaar zal zijn. De juistheid van deze benadering dient nog aangetoond te worden.

References

Alban, D. H. 1985. Seasonal changes in nutrient concentration and content of Aspen suckers in Minnesota. Forest Science **31**: 785-794.

Alfani, A., Baldantoni, D., Maisto, G., Bartoli, G., and De Santo, A. V. 2000. Temporal and spatial variation in C, N, S and trace element contents in the leaves of *Quercus ilex* within the urban area of Naples. Environmantal Pollution **109**: 119-129.

Alfani, A., Bartoli, G., Rutigliano, F. A., Maisto, G., and Virzo de Santo, A. 1996a. Trace metal biomonitoring in the soil and the leaves of *Quercus ilex* in the urban area of Naples. Biological Trace Element Research **51**: 117-131.

Alfani, A., Maisto, G., Iovieno, P., Rutigliano, F. A., and Bartoli, G. 1996b. Leaf contamination by atmospheric pollutants as assessed by elemental analysis of leaf tissue, leaf surface deposit and soil. Journal of Plant Physiology **148**: 243-248.

Alloway, B. J. 1995. Heavy metals in soils. Blackie Academic & Professional, London. pp. 386.

Anderson, S., Chappelka, A. H., Flynn, K. M., and Odom, J. W. 2000. Lead accumulation in *Quercus nigra* and *Q. velutina* near smelting facilities in Alabama, USA. Water, Air and Soil Pollution **118**: 1-11.

Auchmoody, L. R. and Hammack, K. P. 1975. Foliar nutrient variation in four species of upland oak. U.S. Forest Service Research Paper. NE-331. *Source* : Erdmann, G. G., Crow, T. R., and Rauscher, H. M. 1988. Foliar nutrient variation and sampling intensity for *Acer rubrum* trees. Canadian Journal of Forest Research **18**: 134-139.

Austin, M. P. 1987. Models for the analysis of species response to environmental gradient. Vegetatio **69**: 35-45.

Bache, C. A., Elfving, D. C., and Lisk, D. J. 1992. Cadmium and lead concentration in foliage near a municipal refuse incinerator. Chemosphere **24**: 475-481.

Baker, A. J. M., Brooks, R. R., and Reeves, R. 1988. Growing for gold...and copper...and zinc. New Scientist **10** : 44-48.

Baker, A. J. M., McGrath, S. P., Sidoli, C. M. D., and Reeves R.D. 1994. The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resources, Conservation and Recycling **11**: 41-49.

Balsberg Påhlsson, A.-M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air, and Soil Pollution **47**: 287-319.

Beckett, P. H. T. and Davis, R. D. 1977. Upper critical levels of toxic elements in plants. New Phytologist **79**: 95-106.

Berg, B., Ekbohm, G., Soderstrom, B., and Staaf, H. 1991. Reduction of decomposition rates of Scots pine needle litter due to heavy-metal pollution. Water, Air and Soil Pollution **59**: 165-177.

Bergkvist, B., Folkeson, L., and Berggren, D. 1989. Fluxes of Cu, Zn, Pb, Cd, Cr, and Ni in temperate forest ecosystems. Water, Air and Soil Pollution **47**: 217-286.

Berglund, J. V., Leaf, A. L., and Leonard, R. E. 1976. Red pine foliage variation and field sampling intensity. Canadian Journal of Forest Research **6**: 268-280.

Bernier, B. and Brazeau, M. 1988. Foliar nutrient status in relation to sugar maple dieback and decline in the Quebec Appalachians. Canadian Journal of Forest Research **18**: 754-761.

Biondini, M. E., Mielke, P. W., and Berry, K. J. 1988. Data-dependent permutation techniques for the analysis of ecological data. Vegetatio **75**: 161-168.

Böhm, P., Wolterbeek, H., Verburg, T., and Musilek, L. 1998. The use of tree bark for environmental pollution monitoring in the Czech Republic. Environmental Pollution **102**: 243-250.

Borgegård, S.-O. and Rydin, H. 1989. Biomass, root penetration and heavy-metal uptake in birch in a soil cover over copper tailings. Journal of Applied Ecology **26**: 585-595.

Boruvka, L., Kozak, J., and Kristoufkova, S. 1997. Distribution of cadmium, lead and zinc in plants grown on heavily polluted soils. Rostlinna Vyroba **43**: 249-256.

Bowersox, T. W. and Ward, W. W. 1977. Seasonal variation in foliar nutrient concentrations of black cherry. Forest Science **23**: 429-432.

Breckle, S. W. and Kahle, H. 1992. Effects of toxic heavy-metals (Cd, Pb) on growth and mineral nutrition of beech (*Fagus sylvatica* L.). Vegetatio **101**: 43-53.

Brieger, G., Wells, J. R., and Hunter, R. D. 1992. Plant and animal species composition and heavy-metal content in fly ash ecosystems. Water Air and Soil Pollution **63**: 87-103.

Browne, R. H. 1995. On the use of a pilot sample for sample size determination. Statistics in Medicine **14**: 1933-1940.

Brundin, N. H., Ek, J. I., and Selinus, O. C. 1987. Biogeochemical studies of plants from stream banks in northern Sweden. Journal of Geochemical Exploration **27**: 157-188.

Buchauer, M. J. 1973. Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, copper and lead. Environmental Science and Technology **7**: 131-135.

Burton, K. W., Morgan, E., and Roig, A. 1983. The influence of heavy metals upon the growth of sitka spruce in South Wales forests I. Upper critical and foliar concentrations. Plant and Soil **73**: 327-336.

Burton, K. W., Morgan, E., and Roig, A. 1984. The influence of heavy metals upon the growth of sitka spruce in South Wales forests II. Greenhouse experiments. Plant and Soil **78**: 271-282.

Capelli, M., Manfredi, V. R., Moretti, G. F., and Trenta, A. 1989. Seasonal behavior of lead concentration in poplar leaves. Toxicological and Environmental Chemistry **18**: 257-268.

Chang, A. C., Granato, T. C., and Page, A. L. 1992. A methodology for establishing phytotoxicity criteria for chromium, copper, nickel and zinc in agricultural land application of municipal sewage sludges. Journal of Environmental Quality **21**: 521-536.

Chapin, F. S. and Kedrowski, R. A. 1983. Seasonal changes in nitrogen and phosphorous fractions and autumn retranslocation in evergreen and deciduous taiga trees. Ecology **64**: 376-391.

Chapman, H. D. 1966. Diagnostic criteria for plants and soils. University of California, Division of Agricultural Sciences. *Source :* Ellis, R. C. 1975. Sampling deciduous broadleaved trees for the determination of leaf weight and foliar elemental concentrations. Canadian Journal of Forest Research **5**: 310-317.

Chen, H. Y. H., Klinka, K., and Kabzems, R. D. 1998. Site index, site quality, and foliar nutrients of trembling aspen: relationships and predictions. Canadian Journal of Forest Research **28**: 1743-1755.

Claussen T. 1990. Dry ash, a better reference base than dry matter for heavy metals and other persistent pollutants. Plant and Soil **127**: 91-95.

Cohen, J. 1977. Statistical power analysis for the behavioural science . Academic Press, New York. pp. 474.

Combes, R. 1926. Emigration des substances azotées des feuilles vers tiges et les racines des arbes, au cours du jaunissement automal. Revue Générale de Botanique **38**: 430-448, 510-517, 632-645.

Cotrufo, M. F., Desanto, A. V., Alfani, A., Bartoli, G., and Decristofaro, A. 1995. Effects of urban heavy-metal pollution on organic-matter decomposition in *Quercus ilex* L. woods. Environmental Pollution **89**: 81-87.

Coughtrey, P., Jones, C., and Shales, S. 1979. Litter accumulation in woodlands contaminated by Pb, Zn, Cd and Cu. Oecologia **39** : 51-60.

Dane, J. H., Reed, R. B., and Hopmans, J. W. 1986. Estimating soil parameters and sample size by bootstrapping. Soil Science Society of America Journal **50**: 283-287.

Dawson, J. O. and Funk, D. T. 1981. Seasonal change in foliar nitrogen concentration of *Alnus glutinosa*. Forest Science **27**: 329-243.

DeBell, D. S. and Rawdan, M. A. 1984. Foliar chemical concentrations in red alder stands of various age. Plant and Soil **77**: 391-394.

Dell, B., Jones, S., and Wilson, S. A. 1987. Phosphorus nutrition of jarrah (*Eucalyptus marginata*) seedlings use of bark for diagnosing phosphorus deficiency. Plant and Soil **97**: 369-379.

Derome, J. and Lindroos, A. J. 1998. Effects of heavy-metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni smelter, SW Finland. Environmental Pollution **99**: 225-232.

Deutsch, C. V. and Journel, A. G. 1998. GSLIB - Geostatistical software library and user's guide. Oxford University Press, New York. pp. 369.

Dickinson, N. M. 2000. Strategies for sustainable woodland on contaminated soils. Chemosphere **41**: 259-263.

Dickinson, N. M., Turner, A. P, and Lepp, N. W. 1991a. How do trees and other long-lived plants survive in polluted environments? Functional Ecology **5**: 5-11.

Dickinson, N. M., Turner, A. P., and Lepp, N. W. 1991b. Survival of trees in a metal-contaminated environment. Water , Air, and Soil Pollution **57-58**: 627-633.

Dijkstra, E. F. 1998. A micromorphological study on the development of humus profiles in heavy-metal polluted and non-polluted forest under Scots pine. Geoderma **82**: 341-358.

Dinelli, E. and Lombini, A 1996. Metal distributions in plants growing on copper mine spoils in Northern Apennines, Italy: the evaluation of seasonal variations. Applied Geochemistry **11**: 375-385.

Djingova, R., Wagner, G., and Kuleff, I. 1999. Screening of heavy metal pollution in Bulgaria using *Populus nigra 'Italica'*. Science of the Total Environment **234**: 175-184.

Djingova, R., Wagner, G., Kuleff, I., and Peshev, D. 1996. Investigations on the time-dependant variations in metal concentration in leaves of *Populus nigra 'Italica'*. Science of the Total Environment **184**: 197-202.

Dmuchowski, W. and Bytnerowicz, A. 1995. Monitoring environmental pollution in Poland by chemical analysis of Scots pine (*Pinus sylvestris* L.) needles. Environmental Pollution **87**: 87-104.

Duncan, H. J., McGregor, S. D., Pulford, I. D., and Wheeler, C. T. 1995. The phytoremediation of heavy metal contamination using coppice woodland. *In* Contaminated soil '95. *Edited by* W.J. van den Brink, R. Bosman, and F. Arendt. Kluwer Academic Publishers, pp. 1187-1188.

Duquesnay, A., Dupouey, J. L., Clement, A., Ulrich, E., and Le Tacon, F. 2000. Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in northeastern France. Tree Physiology **20**: 13-22.

Dutilleul, P. and Legendre, P. 1993. Spatial heterogeneity against heteroscedasticity: an ecological paradigm versus a statistical concept. Oikos **66**: 152-168.

Efron, B. and Tibshirani, R. 1993. An introduction to the bootstrap. Chapman and Hall, New York. pp. 436.

Eklund, M. 1995. Cadmium and lead deposition around a Swedish battery plant as recorded in oak tree rings. Journal of Environmental Quality **24**: 126-131.

Ellis, R. C. 1975. Sampling deciduous broadleaved trees for the determination of leaf weight and foliar elemental concentrations. Canadian Journal of Forest Research **5**: 310-317.

Eltrop, L., Brown, G., Joachim, O., and Brinkmann, K. 1991. Lead tolerance of *Betula* and *Salix* in the mining area of Mechernich / Germany. Plant and Soil **131**: 275-285.

Erdmann, G. G., Crow, T. R., and Rauscher, H. M. 1988. Foliar nutrient variation and sampling intensity for *Acer rubrum* trees. Canadian Journal of Forest Research **18**: 134-139.

Ericsson, A., Hellkvist, C., Långström, B., Larsson, S., and Tenow, O. 1985. Effects on growth of simulated and induced shoot pruning by *Tomicus piniperda* as related to carbohydrate and nitrogen dynamics in Scots pine. Journal of Applied Ecology **22**:105-124.

Ernst, W. H. O. 1996. Bioavailability of heavy metals and decontamination of soils by plants. Applied Geochemistry **11**: 163-167.

Ettala, M. O. 1988. Short-rotation tree plantations at sanitary landfills. Waste Management and Research **6**: 291-302.

Everard, J. 1973. Foliar Analysis sampling methods interpretation and application of the results. Quarterly Journal of Forestry **76**: 51-66.

Farago, M. E. 1994. Plants as indicators of mineralisation and pollution. *In* Plants and the chemical elements biochemistry, uptake, tolerance and toxicity. *Edited by* M.E. Farago. VCH, Weinheim. pp. 221-240.

Faucherre, J. and Dutot, A. 1976. Zinc and cadmium in tree ring as chronological tracers of pollution of a river. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences **Serie D 282**: 187-190.

Ferm, A. and Markkola, A. 1985. Nutritional variation of leaves, twigs and buds in *Betula pubescens* stands during the growing season. Folia Forestalia **613**: 1-28.

Fleck, J. A., Grigal, D. F., and Nater, E. A. 1999. Mercury uptake by trees: An observational experiment. Water, Air and Soil Pollution **115**: 513-523.

Folkeson, L. and Andersson-Bringmark, E. 1988. Impoverishment of vegetation in a coniferous forest polluted by copper and zinc. Canadian Journal of Botany **66**: 417-428.

Ford, B. J. 1986. Even plants excrete. Nature **323**: 763.

Freedman, B. and Hutchinson, T. C. 1980. Effects of smelter pollutants on forest leaf litter decomposition near a nickel-copper smelter at Sudbury, Ontario. Canadian Journal of Botany **58**: 1722-1736.

Freedman, B. and Hutchinson, T. C. 1981. Sources of metal and elemental contamination of terrestrial environments. *In* Effects of Heavy Metal Pollution on Plants, Vol. 2. *Edited by* N.W. Lepp. Applied Science Publishers, London and New Jersey. pp. 35-94.

Freese, F. 1962. Elementary Forest Sampling. U.S. Department of Agriculture, Forest Service, Agricultural Handbook No. 232. pp. 87.

Friedland, A. J., Johnson, A. H., and Siccama, T. G. 1984. Trace metal content of the forest floor in the Green Mountains of Vermont: spatial and temporal patterns. Water, Air and Soil Pollution **21**: 161-170.

Gagnon, J. D. 1964. Relationship between site index and foliage nitrogen at two crown levels for mature black spruce. Forestry Chronicle **40**: 169-175.

Garcia Sánchez, A., Moyano, A., and Munez, C. 1999. Forms of cadmium, lead, and zinc in polluted mining soils and uptake by plants (Soria Province, Spain) . Communications in Soil Science and Plant Analysis **30**: 1358-1402.

Glimmerveen, I. 1996a. Heavy metals and trees. ICF, Edinburgh. pp. 206.

Glimmerveen, I. 1996b. Should trees now be more actively used in the rehabilitation of heavy metal contaminated sites? Aspects of Applied Biology **44**: 357-361.

Godbold, D. L. 1991. Cadmium uptake in Norway spruce (*Picea abies*[L.] Karst.) seedlings. Tree Physiology **9**: 349-357.

Goovaerts, P. 1997. Geostatistics for natural resources evaluation. Oxford University Press, New York. pp. 483.

Goovaerts, P., Webster, R., and Dubois, J.-P 1997. Assessing the risk of soil contamination in the Swiss Jura using indicator geostatistics. Environmental and Ecological Statistics **4**: 31-48.

Grace 1983. Plant-atmosphere relationships. Chapman and Hall, London. pp. 92.

Granier, L. and Chevreuil, M. 1992. On the use of tree leaves as bioindicators of the contamination of air by organochlorines in France. Water , Air and Soil pollution **64**: 575-584.

Greger, M. and Landberg, T. 1997. Use of willow clones with high Cd accumulating properties in phytoremediation of agricultural soils with elevated Cd levels. *In* Contaminated soils. *Edited by* INRA. Les Colloques, no 85, Paris. pp. 505-511.

Grigal, D. F., Ohmann, L. F., and Brander, R. B. 1976. Seasonal dynamics of tall shrubs in northeastern Minnesota: biomass and nutrient element changes. Forest Science **22**: 195-208.

Grodzinski, W., Greszta, J., Laskowski, R., Maryanski, M., and Rozen, A. 1990. Effect of the chemical composition of industrial dust on forest floor organic-matter accumulation. Water, Air and Soil Pollution **53**: 169-178.

Guha, M. M. and Mitchell, R. L. 1965. The trace and major element composition of the leaves of some deciduous trees I. Sampling techniques. Plant and Soil **23**: 323-338.

Guha, M. M. and Mitchell, R. L. 1966. The trace and major element composition of the leaves of some deciduous trees. II. Seasonal changes. Plant and Soil **24**: 90-112.

Gutenmann, W. H., Rutzke, M., Elfving, D. C., and Lisk, D. J. 1992. Analysis of heavy metals in foliage near a modern refuse incinerator. Chemosphere **24**: 1905-1910.

Gworek, B. 1992. Lead inactivation in soils by zeolites. Plant and Soil **143**: 71-74.

Gy, P. M. 1992. Sampling of heterogeneous and dynamic material systems. Theories of heterogeneity, sampling and homogenizing. Elsevier, Amsterdam. pp. 653.

Haase, D. L. and Rose, R. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. Forest Science **41**: 54-66.

Hagemeyer, J. 1995. Radial distributions of Cd in stems of oak trees (*Quercus robur* L.) re-analysed after 10 years. Trees Structure and Function **9**: 200-203.

Hagemeyer, J., Kahle, H., Breckle, S.-W., and Waisel, Y. 1986. Cadmium in *Fagus sylvatica* L. trees and seedlings: leaching, uptake and interconnection with transpiration. Water, Air and Soil Pollution **29**: 347-359.

Hagemeyer, J. and Lohrie, K. 1995. Distribution of Cd and Zn in annual xylem rings of young spruce trees (*Picea abies* (L.) Karst) grown in contaminated soil. Trees Structure and Function **9**: 195-199.

Hagemeyer, J., Lülfsmann, A., Perk, M., and Breckle, S.-W. 1992. Are there seasonal variations of trace elements concentrations (Cd, Pb, Zn) in wood of *Fagus* trees in Germany. Vegetatio **101**: 55-63.

Hagemeyer, J. and Schäfer, H. 1995. Seasonal-variations in concentrations and radial distribution patterns of Cd, Pb and Zn in stem wood of beech trees (*Fagus sylvatica* L.). Science of the Total Environment **166**: 77-87.

Hagemeyer, J., Schäfer, H., and Breckle, S. W. 1994. Seasonal variations of nickel concentrations in annual xylem rings of beech trees (*Fagus sylvatica* L.). Science of the Total Environment **145**: 111-118.

Hager, H. and Kazda, M. 1985. The influence of stand density and canopy position on sulfur content in needles of Norway spruce (*Picea abies* L. Karst.). Water, Air and Soil Pollution **25**: 321-329.

Hall, C. 1977. A study of certain heavy metals in woody plants. Ph.D. Thesis. Liverpool polytechnic.

Hall, C., Hughes, M. K., Lepp, N. W., and Dollard, G. J. 1975. Cycling of heavy metals in woodland ecosystems. *In* Proceedings of the Proceeding of the International Conference on Heavy Metals in the Environment Vol II, Toronto, Canada. pp. 227-246.

Hampel, F. R., Ronchetti, E. M., and Rousseeuw, P. J. 1986. Robust statistics: the approach based on influence functions. Wiley, New York. pp. 502.

Hardiman, R. T., Jacoby, B., and Banin, A. 1984. Factors affecting the distribution of cadmium, copper and lead and their effect upon yield and zinc content in bush beans (*Phaseolus vulgaris* L.). Plant and Soil **81**: 17-27.

Harold, G., Halbwachs, G., Edelbauer, A., and Wimmer, R. 1993. Black alder as a bioindicator - Determination of heavy-metal emissions from an aluminum plant . Staub Reinhaltung der Luft **53**: 27-29.

Hasselgren, K. 1998. Use of municipal waste products in energy forestry: highlights from 15 years of experience. Biomass and Bioenergy **15**: 71-74.

Heilman, P. E. 1985. Sampling and genetic variation of foliar nitrogen in black cottonwood and its hybrids in short rotation. Canadian Journal of Forest Research **15**: 1137-1141.

Heinrichs, H. and Mayer, R. 1980. The role of forest vegetation in the biogeochemical cycle of heavy metals. Journal of Environmental Quality **9**: 111-118.

Heliövaara, K. and Väisänen, R 1990. Air pollution levels and abundance of forest insects. *In* Acidification in Finland. *Edited by* P. Kauppi, P. Antilla, and K. Kenttämies. Springer-Verlag, Berlin. pp. 447-467.

Hellriegel, R. 1869. Landwirtsch. Vers Sta. **11**: 136-140. *Source* : Smith, P. F. 1962. Mineral analysis of plant tissue. Annual Review of Plant Physiology **13**: 81-108.

Helmisaari, H. S. 1992. Nutrient retranslocation in three *Pinus sylvestris* stands. Forest Ecology and Management **51**: 347-367.

Helmisaari, H. S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M., and VanhaMajamaa, I. 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air and Soil Pollution **85**: 1727-1732.

Henderson, P. J., McMartin, I., Hall, G. E., Percival, J. B., and Walker, D. A. 1998. The chemical and physical characteristics of heavy metals in humus and till in the vicinity of the base metal smelter at Flin Flon, Manitoba, Canada. Environmental Geology **34**: 39-58.

Hippeli, P. and Branse, C. 1992. Veränderungen der Nährelementkonzentration in den Nadeln mittelalter Kiefernbestände auf pleistozänen Sandstandorten Brandeburgs in den Jahren 1964 bis 1988. Forstwissenschaftliches Centralblatt **111**: 44-60.

Hjorth, J. S. U. 1994. Computer intensive statistical methods : validation, model selection and bootstrap. Chapman and Hall, London. pp. 263.

Hogan, G. D. and Wotton, D. L. 1984. Pollutant distribution and effects in forests adjacent to smelters. Journal of Environmental Quality **13**: 377-382.

Hosker, R. P. and Lindberg, S. E. 1982. Review - Atmospheric deposition and plant assimilation of gases and particles. Atmospheric Environment **16**: 889-910.

Houle, D., Ouimet, R., Paquin, R., and Laflamme, J. G. 1999. Determination of sample size for estimating ion throughfall deposition under a mixed hardwood forest at the Lake Clair Watershed (Duchesnay, Quebec). Canadian Journal of Forest Research **29**: 1935-1943.

Hoyle, M. C. 1965. Variation in foliage composition and diameter growth of yellow birch with season, soil, and tree size. Soil Science of America Proceedings **29**: 475-480.

Huber, P. J. 1964. Robust estimation of a location parameter. Annals of Mathematical Statistics **35**: 73-107.

Huber, P. J. 1973. Robust regression: asymptotics, conjectures and Monte Carlo. Annals of Statistics **1**: 799-821.

Hughes, K, Lepp, N., and Phipps, D. 1980. Aerial heavy-metal pollution and terrestrial ecosystems. Advances in Ecological Research **11**: 217-327.

Huhn, G., Schulz, H., Stärk, H.-J., Tolle, R., and Schuurmann, G. 1995. Evaluation of regional heavy-metal deposition by multivariate-analysis of element contents in pine tree barks. Water, Air and Soil Pollution **84**: 367-383.

Hutchinson, T. C. and Whitby, L. M. 1977. The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the Sudbury smelting region of Canada. Water, Air and Soil Pollution **7**: 421-438.

Huvenne P., Lust N., Speleers L., and De Vos B. 1997. Boisements des dépôts de boues draguées. Revue Forestière Française **2**: 115-130.

Innes, J. L. 1993. Foliar Analysis. *In* Forest health : its assessment and status. *Edited by* J.L. Innes. CABI publishing, Wallingford, UK. pp. 656.

Insley, H., Boswell, R. C., and Gardiner, J. B. H. 1981a. Foliar macronutrients (N,P,K,Ca and Mg) in lime (*Tilia spp*). I sampling techniques. Plant and Soil **61**: 377-389.

Insley, H., Boswell, R. C., and Gardiner, J. B. H. 1981b. Foliar macronutrients (N,P,K,Ca and Mg) in lime (*Tilia spp*). II Seasonal variation. Plant and Soil **61**: 391-401.

Isaaks, E. H. and Srivastava, R. M. 1989. An introduction to applied geostatistics. Applied geostatistics series. Oxford University Press, New York, Oxford. pp. 561.

Jackson, D. and Watson, P. 1977. Disruption of nutrient pools and transport of heavy metals in a forested watershed near a lead smelter. Journal of Environmental Quality **6**: 331-338.

Jarvis, J. C., Jones, L. H. P., and Hopper, M. J. 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant and Soil **44**: 179-191.

Johnson, C. E., Johnson, A. H., and Huntington, T. G. 1990. Sample size requirements for the determination of changes in soil nutrient pools. Soil Science **150**: 637-644.

Johnson, D. H. 1995. Statistical sirens: the allure of nonparametrics. Ecology **76**: 1998-2000.

Jonsson, A., Eklund, M., and Hakansson, K. 1997. Heavy metals of the 20th century recorded in oak tree rings. Journal of Environmental Quality **26**: 1638-1643.

Jordan, M. J. 1975. Effects of zinc smelter emission and fire on a chestnut-oak woodland. Ecology **56**: 78-91.

Jung, M. C. and Thornton, I. 1996. Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. Applied Geochemistry **11**: 53-59.

Kabata-Pendias, A. and Pendias, H. 1992. Trace elements in soils and plants. CRC press, Boca Raton Florida. pp. 365.

Kahle, H. 1993. Response of roots of trees to heavy metals. Environmental and Experimental Botany **1**: 99-119.

Kardell, L. and Larsson, J. 1978. Lead and cadmium in oak tree rings (*Quercus robur* L.). Ambio **7**: 117-121.

Kayahara, G. J., Carter, R. E., and Klinka K. 1995. Site index of western hemlock (*Tsuga heterophylla*) in relation to soil nutrient and foliar chemical measures. Forest Ecology and Management **74**: 161-169.

Keller, Th., Matyssek, R., and Günthardt-Goerg, M. S. 1994. Beech foliage as a bioindicator of pollution near a waste incinerator. Environmental Pollution **85**: 185-189.

Kim, N. D. and Fergusson, J. E. 1994. Seasonal variations in the concentrations of cadmium, copper, lead and zinc in leaves of the horse chestnut (*Aesculus hippocatanum* L.). Environmental Pollution **86**: 89-97.

Klumpp, A., Klumpp, G., and Domingos, M. 1994. Plants as bioindicators of air-pollution at the Serra-Do-Mar near the indistrial complex of Cubatao, Brazil. Environmental Pollution **85**: 109-116.

Koricheva, J. and Haukioja, E. 1995. Variations in chemical composition of birch foliage under air pollution stress and their consequences for *Eriocrania* Miners. Environmental Pollution **88**: 41-50.

Kozlov, M. V., Haukioja, E., Bakhtiarov, A. V., and Stroganov, D. N. 1995. Heavy metals in birch leaves around a nickel-copper smelter at Monchegorsk, northwestern Russia. Environmental Pollution **90**: 291-299.

Kuik, P. and Wolterbeek, H. 1994. Factor analysis of atmospheric trace-element data from tree-bark samples in The Netherlands. Environmental Monitoring and Assessment **32**: 207-226.

Kupper, L. L. and Hafner, K. B. 1989. How appropriate are popular sample size formulas? American Statistical Association **43**: 101-105.

Kurczynska, E. U., Dmuchowski, W., Wloch, W., and Byttnerowicz, A. 1997. The influence of air pollutants on needles and stems of Scots pine (*Pinus sylvestris* L.) trees. Environmental Pollution **98**: 325-334.

l'Ecuyer, P. 1988. Communications of the ACM **31** : 742-774. *Source* : Press, W. H., Teukolsky, S. A., Vetterling, W. T., and Flannery, B. P. 1992. Numerical Recipes in Fortran 77. Cambridge University of Press, New York. pp. 933.

Labrecque, M., Teodorescu, T., and Daigle, S. 1995. Effects of wastewater sludge on growth and heavy metal bioaccumulation of two *Salix* species. Plant and Soil **171**: 303-316.

Lamé, F. P. J. and Defize, P. R. 1993. Sampling of contaminated soil: sampling error in relation to sample size and segregation. Environmental Science and Technology **27**: 2035-2044.

Lamersdorf, N. P., Godbold, D. L., and Knoche, D. 1991. Risk assessment of some heavy metals for the growth of Norway spruce. Water, Air and Soil Pollution **57**: 535-543.

Landberg, T. and Greger, M. 1994. Can heavy metal tolerant clones of *Salix* be used as vegetation filters on heavy metal contaminated land? *In* Willow vegetation filters for municipal wastewaters and sludges; A biological purification system. *Edited by* P. Aronsson and K. Perttu. Swedish University of Agricultural Sciences, Uppsala. pp. 133-144.

Landberg, T. and Greger, M. 1996. Differences in uptake and tolerance to heavy metals in Salix from unpolluted and polluted areas. Applied Geochemistry **11**: 175-180.

Landolt, W., Guecheva, M., and Bucher, J. B. 1989. The spatial distribution of different elements in and on the foliage of Norway spruce growing in Switzerland. Environmental Pollution **56**: 155-167.

Laskowski, R., Maryanski, M., and Niklinska, M. 1994. Effect of heavy metals and mineral nutrients on forest litter respiration rate. Environmental Pollution **84**: 97-102.

Laskowski, R., Niklinska, M., and Maryanski, M. 1995. The dynamics of chemical elements in forest litter. Ecology **76**: 1393-1406.

Latimer, S. D., Devall, M. S., Thomas, C., Ellgaard, E. G., Kumar, S. D., and Thien, L. B. 1996. Dendrochronology and heavy-metal deposition in tree rings of baldcypress. Journal of Environmental Quality **25**: 1411-1419.

Lavender, D. P. 1970. Foliar analysis and how it is used. A review. Oregon State University Research Note **52**: 1-8.

Le Tacon, F. and Toutain, F. 1973. Variations saisonnières et stationelles de la teneur en éléments minéraux des feuilles de hêtre (*Fagus sylvatica*) dans l'est de la France. Annales des sciences Forestières **30**: 1-29.

Lea, R., Tierson, W. C., Bickelhaupt, D. H., and Leaf, A. L. 1979a. Stand treatment and sampling time of hardwood foliage. I. Macro-element analysis. Plant and soil **51**: 515-533.

Lea, R., Tierson, W. C., Bickelhaupt, D. H., and Leaf, A. L. 1979b. Stand treatment and sampling time of hardwood foliage. II. Micro-element analysis. Plant and soil **51**: 533-550.

Leaf, A. L. 1970. America's oldest intensive forest fertilization experiments. Some lessons learned. Advancing Frontiers of Plant Sciences **24**: 1-39.

Leaf, A. L. 1973. Plant Analysis as an Aid in Fertilizing Forests. *In* Soil Testing and Plant Analysis. *Edited by* L.M. Walsh and J.D. Beaton. Soil Science of America, Inc., Madinson, Wisconsin USA. pp. 427-454.

Ledin, S. and Willebrand E. 1996. Handbook on how to grow short rotation forests. Department of Short Rotation Forestry. Swedish University of Agricultural Sciences. pp. 180.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology **74**: 1659-1673.

Legrand, I. and Asta, J. 1993. Forest decline observed in the department Isere (France) on silver fir trees (*Abies alba* Mill) and Norway pruce trees (*Picea abies* (L) Karsten) - Relationship with the physicochemical characteristics of the barks. Annales des Sciences Forestières **50**: 235-246.

Lemoine B., Ranger J., and Gelpe J. 1990. Interprétation et modélisation de la distribution des éléments nutritifs dans les différents compartiments de la biomasse d'un jeune peuplement de pin maritime. Annales des Sciences Forestières **47**: 101-115.

Lepp, N. W. 1975. The potential of tree-ring analysis for monitoring heavy metal pollution patterns. Environmental Pollution **Serie B 9**: 49-63.

Leroy 1968. Variations saisonnières des teneurs en eau et éléments minéraux des feuilles de chêne (*Quercus pendunculata*). Annales des Sciences Forestières **25**: 83-117.

Leyton, L. 1956. The relationship between the growth and mineral composition of the foliage of Japanese larch (*Larix leptolepis* Murr.). Plant and Soil **7**: 167-177.

Leyton, L. and Armson, K. A. 1955. Mineral composition of foliage in relation to the growth of Scots pine. Forest Science **1**: 210-211.

Lim, M. T. and Cousens, J. E. 1986. The internal transfer of nutrients in Scots pine stands. 2. The pattern of transfer and effects of nitrogen availability. Forestry **59**: 17-27.

Linzon, S. N. 1986. Sugar maple dieback studies in Canada. *In* Proceedings of the Proceedings of the IUFRO 18th world congress, 7-21 September, Ljubljana, Yugoslavia. *Edited by* Division 2, Vol. I. pp. 389-398.

Lippo, H., Poikolainen, J., and Kubin, E. 1995. The use of moss, lichen and pine bark in the nationwide monitoring of atmospheric heavy metal deposition in Finland. Water, Air, and Soil Pollution **85**: 2241-2246.

Little, P. 1973. A study of heavy metal contamination of leaf surfaces. Environmental Pollution **5**: 159-172.

Little, P. 1977. Deposition of 2.75, 5.0 and 8.5 µm particles to plant and soil surfaces. Environmental Pollution **12**: 293-305.

Little, P. and Martin, M. H. 1972. A survey of zinc, lead and cadmium in soil and natural vegetation around a smelter complex. Environmental Pollution **3**: 241-254.

Little, P. and Martin, M. H. 1974. Biological monitoring of heavy metals pollution. Environmental Pollution **6**: 1-20.

Little, P. and Wiffen, R. D. 1977. Emission and deposition of petrol exhaust Pb. I. Deposition of exhaust Pb to plant and soil surfaces. Atmospheric Environment **11**: 437-447.

Løbersli, E. M. and Steinnes, E. 1988. Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water, Air and Soil Pollution **37**: 25-39.

Loppi, S., Cenni, E., Bussotti, F., and Ferretti, M. 1998. Biomonitoring of geothermal air pollution by epiphytic lichens and forest trees. Chemosphere **36**: 1079-1082.

Lowry, G. L. and Avard, P. M. 1965. Nutrient content of black spruce needles. I. Variation due to crown position and needle age. Pulp Paper Research Institute . Technical report 425. pp. 21.

Lowry, G. L. and Avard, P. M. 1968. Nutrient content of black spruce needles. II. Variations with crown class and relationship to growth and yield. Pulp Paper Research Institute. Woodland papers 3. pp. 20.

Luyssaert, S., Mertens, J., Vervaeke, P., De Vos, B., and Lust, N. 2001. Preliminary results of afforestation of brackish sludge mounds. Ecological Engineering **16**:567-572.

Mader, D. L. and Thompson, B. W. 1969. Foliar and soil nutrients in relation to sugar maple decline. Soil Science Society of America Proceedings **33**: 794-800.

Mankovska, B. 1980. The natural content of F, As, Pb, and Cd in the forest trees. Biologia Bratislava **35**: 267-274.

Marion, G. M., Berglund, J. V., and Leaf, A. L. 1968. Morphological and chemical analysis of red pine (*Pinus resinosa* Ait.) buds: their relationship to tree growth on a K-deficient to nondeficient site continuum. Plant and Soil **28**: 313-324.

Markert, B. 1995. Sample preparation (cleaning, drying, homogenization) for trace element analysis in plant matrices. The Science of the Total Environment **176**: 45-61.

Marshall, P. L. and Jahraus, K. 1987. Sample size for foliar analysis of coastal Douglas-fir. Canadian Journal of Forest Research **17**: 1240-1245.

Marshall, P. L., LeMay, V. M., and Nussbaum, A. 1992. Sample size adjustment to reduce the probability of exceeding a specified confidence interval width. The Forestry Chronicle **68**: 747-751.

Martin, M. H. and Coughtrey, P. 1981. Impact of heavy metals on ecosystem function and productivity. *In* The effects of heavy-metal pollution on plants, Vol. 2. *Edited by* N. Lepp. Applied Science Publishers, London and New York. pp. 119-158.

Martin, M. H., Duncan, E., and Coughtrey, P. 1982. The distribution of heavy metals in a cadmium contaminated woodland ecosystem. Environmental Pollution **Series B 3**: 147-157.

Matzka, J. and Maher, B. A. 1999. Magnetic biomonitoring of roadside tree leaves: identification of spatial and temporal variations in vehicle-derived particulates. Atmospheric Environment **33**: 4565-4569.

Mayer, H. 1976. Waldbau. Gustav Fisher Verlag, Stuttgart. pp. 483.

Mayer, R. 1983. Interaction of forest canopies with atmospheric constituents: aluminum and heavy metals. *In* Effects of accumulation of air pollutants in forest ecosystems. *Edited by* B. Ulrich and Pankrath J. Reidel Publishing Company, Dordrecht. pp. 47-55.

McBratney, A. B. and Webster, R. 1986. Choosing functions for semi-variograms of soil properties and fitting them to sampling estimates. Journal of Soil Science **36**: 617-639.

McColl, J. 1980. Seasonal nutrient variation in trembling aspen. Plant and Soil **54**: 323-328.

McLennan, D. S. 1990. Spatial variation in black cottonwood (*Populus trichocarpa*) foliar nutrient concentrations at seven alluvial sites in coastal British Columbia. Canadian Journal of Forest Research **20**: 1089-1097.

McVickar, J. S. 1949. Composition of white oak leaves in Illinois as influenced by soil type and soil composition. Soil Science **2**: 317-328.

Meier, C. E., Grier, C. C., and Cole, D. W. 1985. Below and aboveground N and P use by *Abies amabilis* stands. Ecology **66**: 1928-1942.

Memon, A. R., Itô, S., and Yatazawa, M. 1980. Distribution of zinc and cadmium in the temperate forest taxa of central Japan. Soil Science and Plant Nutrition **26**: 281-290.

Mench, M. J., Didier, V. L., Loeffer, M., Gomez, A., and Masson, P. 1994. A mimicked in-situ remediation study of metal-contaminated soils with emphasis on cadmium and lead. Journal of Environmental Quality **23**: 58-63.

Mench, M., Vangronsveld, J., and Didier, V. 1986. Evaluation of metal mobility, plant availability and immobilization by chemical agents in a limed-silty soil. Environmental Pollution **86**: 279-286.

Meneses, M., Llobet, J. M., Granero, S., Schumacher, M., and Domingo, J. L. 1999. Monitoring metals in the vicinity of a municipal waste incinerator: temporal variation in soils and vegetation. The Science of the Total Environment **226**: 157-164.

Mengel, K. and Kirkby, E. A. 1982. Principles of plant nutrition. International Potash Institute, Worblaufen-Bern. pp. 569-570.

Mertens, J., Luyssaert, S., Verbeeren, S., Vervaeke, P., and Lust, N. 2001. Cd and Zn concentrations in small mammals and willow leaves on disposal facilities for dredged material. Environmental Pollution Accepted.

Miller, H. G. 1984. Dynamics of nutrient cycling in plantation ecosystems. *In* Nutrition of plantation forests. *Edited by* G.D. Bowen and E.K.S. Nambiar. Academic Press, London. pp. 53-78.

Miller, H. G. 1986. Carbon x nutrient interactions - the limitations to productivity. Tree Physiology **2**: 373-385.

Miller, W. F. 1966. Annual changes in foliar nitrogen, phosphorus, and potassium levels of loblolly pine (*Pinus taeda L.*) with site and weather factors. Plant and Soil **24**: 369-378.

Mitchell, H. L. 1934. Pot culture tests of forest soil fertility. Black rock Forest Bulletin **5**: 1-138.

Mitchell, H. L. 1936. Trends in nitrogen, phosphorus, potassium and calcium content of the leaves of some forest trees during the growing season. Black Rock Forest Paper **1**: 30-44.

Mitchell, H. L. 1939. The growth and nutrition of white pine (*Pinus strobus* L.) seedlings in culture with varying nitrogen, phosphorus, potassium and calcium. Black Rock Forest Bulletin **9**: 1-135.

Monaci, F., Moni, F., Lanciotti, E., Grechi, D., and Bargagli, R. 2000. Biomonitoring of airborne metals in urban environments: new tracers of vehicle emission, in place of lead. Environmental Pollution **107**: 321-327.

Monether, N. 1998. The uptake of heavy metals by willow. Thesis. University of Derby.

Morrison, I. K. 1972. Variation with crown position and leaf age in content of seven elements in leaves of *Pinus banksiana* Lamb. Canadian Journal of Forest Research **2**: 89-94.

Morrison, I. K. 1974. Within tree variation in mineral content of leaves of young balsam fir. Forest Science **20**: 276-278.

Morrison, I. K. 1985. Effects of crown position on foliar concentrations of 11 elements in *Acer saccharum* and *Betula alleyhaniensis* trees on a till soil. Canadian Journal of Forest Research **15**: 179-183.

Nabais, C., Freitas, H., and Hagemeyer, J. 1999. Dendroanalysis: a tool for biomonitoring environmental pollution ? Science of the Total Environment **232**: 33-37.

Nelson, L. E., Switzer, G. L., and Smith, W. H. 1970. Dry matter and nutrient accumulation in young loblolly pine (*Pinus taeda* L.). *In* Tree growth and forest soils. *Edited by* C.T. Youngberg and C.B. Davey. Oregon State University. Press, Corvallis. pp. 261-274.

Neter, J., Kutner, M. H., Nachtsheim, C. J., and Wasserman, W. 1996. Applied Linear Statistical Models. WCB McGraw-Hill, Boston, Massachusetts. pp. 1408.

Nieminen, T. and Helmisaari, H. S. 1996. Nutrient retranslocation in the foliage of *Pinus sylvestris* L. growing along a heavy metal pollution gradient. Tree Physiology **16**: 825-831.

Nilsson, L. O. and Ericsson, T. 1986. Influence of shoot age on growth and nutrient uptake patterns in a willow plantation. Canadian Journal of Forest Research **16**: 185-190.

Nuorteva, H. and Kurkela, T. 1993. Effects of crown reduction on needle nutrient status of scleroderris-canker-diseased and green-pruned Scots pine. Canadian Journal of Forest Research **23**: 1169-1178.

Östman, G. 1994. Cadmium in *Salix* - a study of the capacity of *Salix* to remove cadmium from arable soils. *In* Willow vegetation filters for muncipal wastewaters and sludges; A biological purification system. *Edited by* P. Aronsson and K. Perttu. Swedish University of Agricultural Science, Uppsala. pp. 153-156.

Ovington, J. D. and Madgwick, H. A. I. 1958. The sodium, potassium and phosphorus content of tree species grown in close stands. The New Phytologist **57**: 273-284.

Ovington, J. D. and Madgwick, H. A. I. 1959a. Distribution of organic matter and plant nutrients in a plantation of Scots pine. Forest Science **5**: 344-355.

Ovington, J. D. and Madgwick, H. A. I. 1959b. The growth and composition of natural stands of Birch, 2. The uptake of mineral nutrients. Plant and Soil **10**: 389-400.

Parker, G. R., McFee, W. W., and Kelly, J. M. 1978. Metal distribution in forested ecosystems in urban and rural northwestern Indiana. Journal of Environmental Quality **7**: 337-342.

Perttu, K. L. 1993. Biomass production and nutrient removal from municipal wastes using willow vegetation filters. Journal of Sustainable Forestry **1**: 57-70.

Perttu, K. L. and Kowalik, P. J. 1997. Salix vegetation filters for purification of water and soils. Biomass and Bioenergy **12**: 9-19.

Piene, H. 1980. Effects of insect defoliation on growth and foliar nutrients of young balsam fir. Forest Science **26**: 665-673.

Piene, H. and Percy, K. E. 1984. Changes in needle morphology, anatomy, and mineral content during the recovery of protected balsam fir trees initially defoliated by the spruce budworm. Canadian Journal of Forest Research **14**: 238-245.

Pilgrim, W. and Hughes, R. N. 1994. Lead, cadmium, arsenic and zinc in the ecosystem surrounding a lead smelter. Environmental Monitoring and Assessment **32**: 1-20.

Poikolainen, J. 1997. Sulphur and heavy metal concentrations in Scots pine bark in northern Finland and the Kola Peninsula. Water, Air and Soil Pollution **93**: 395-408.

Ponder, F., Phares, R. E., and Schlesinger, R. C. 1979. Seasonal variation in foliar composition of black walnut trees. Communication in Soil Science and Plant Analysis **10**: 1513-1521.

Prasad, M. N. V. 1995. Cadmium toxicity and tolerance in vascular plants. Environmental and Experimental Botany **33**: 525-545.

Prégent, G. and Camiré, C. 1985. Biomass production by alders on four abandoned agricultural soils in Québec. Plant and Soil **87**: 185-193.

Press, W. H., Teukolsky, S. A., Vetterling, W. T., and Flannery, B. P. 1992. Numerical Recipes in Fortran 77. Cambridge University of Press, New York. pp. 933.

Punshon, T. 1996. Heavy metal resistance in *Salix*. Ph.D. Thesis. School of Biological and Earth Sciences, John Moores University, Liverpool.

Punshon, T. and Dickinson, N. M. 1997. Mobilisation of heavy metals using short rotation coppice. Aspects of Applied Biology **49** : 285-292.

Raitio, H. 1993. Chemical needle analysis as a diagnostic and monitoring method. *In* Proceedings of the Nutrient uptake and cycling in forest ecosystems, 7-10 June, Halmstad, Sweden. *Edited by* L.O. Nilsson, R.F. Hüttl, U.T. Johansson and P. Mathy, pp. 197-206.

Raitio, H. 1995. Influence of sample washing on the foliar chemical composition. A review. *In* Proceedings of the 3rd Meeting of the foliar expert panel, International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests - ICP Forests, 6-8 November, Vienna, Austria. pp. 8.

Raitio, H. and Merilä, P. 1998. Seasonal variation in the size and chemical composition of Scots pine and Norway spruce needles in different weather conditions. The Finnish Forest Research Institute, Parkano Research Station. Pilot Study, Thechnical Report. pp. 44.

Raitio, H. and Sarjala, T. 2000. Effects of provenance on free amino acid and chemical composition of Scots pine needles. Plant and Soil **221**: 231-238.

Rautio, P., Huttunen, S., and Lamppu, J. 1998. Seasonal foliar chemistry of northern Scots pines under sulphur and heavy metal pollution. Chemosphere **37**: 271-287.

Rice, R. C. and Bowman, R. S. 1988. Effect of sample size on parameter estimates in solute transport experiments. Soil Science **146**: 108-112.

Ricklefs, R. E. and Matthew, K. K. 1982. Chemical characteristics of the foliage of some deciduous trees in southeastern Ontario. Canadian Journal of Botany **60**: 2037-2045.

Riddell-Black, D. 1994. Heavy metal uptake by fast growing willow species. *In* Willow vegetation filters for municipal wastewaters and sludges; A biological purification system. *Edited by* P. Aronsson and K. Perttu. Swedish University of Agricultural Sciences, Uppsala. pp. 145-153.

Riddell-Black, D., Pulford, I. D., and Stewart, C. 1997. Clonal variation in heavy metal uptake by willow. Aspects of Applied Biology **49**: 327-336.

Robert, B., Bertoni, G., Sayag, D., and Masson, P. 1996. Assessment of mineral nutrition of cork oak through foliar analysis. Communications in Soil Science and Plant Analysis **27**: 2091-2109.

Rosner, B. 2000. Fundamentals of Biostatistics. Duxbury, Australia. pp. 229-236.

Ross, S. M. 1994. Toxic metals in soil-plant systems. John Wiley & Sons, Chichester. pp. 451.

Rubinstein, R. Y. 1981. Simulation and the Monte Carlo method. John Wiley & Sons, New York. pp. 278.

Rühling, A. and Tyler, G. 1973. Heavy-metal pollution and decomposition of spruce needle litter. Oikos **24**: 402-416.

Rytter, L. and Ericsson, T. 1993. Leaf nutrient analysis in *Salix viminalis* (L.) energy forest stands growing on agricultural land. Zeitschrift für Pflanzenernährung und Bodenkunde **156**: 349-356.

Saarinen, M. 1996. Effects of the removal of shelterwood on the foliar nutrient concentrations of Norway spruce (*Picea abies* (L.) Karst.) on drained peatlands. Suoseura **47**: 95-102.

Saarinen, M. 1999. Assessment of the potassium status of peatlands drained for forestry in connection with forest management planning. Suoseura **48**: 14-25.

Saarinen, M and Sarjala, T. 1999. Effects of fertilization and removal of overstorey on foliar nutrient status and chlorophyll fluorescence of Norway spruce (*Picea abies* (L.) Karst.) underwood in drained peatlands. International Peat Journal **9**: 83-93.

Sander, M.-L. and Ericsson, T. 1998. Vertical distribution of plant nutrients and heavy metals in *Salix viminalis* stems and their implications for sampling. Biomass and Bioenergy **14**: 57-66.

Sanità di Toppi, L. and Gabbrielli, R. 1999. Response to cadmium in higher plants. Environmental and Experimental Botany **41**: 105-130.

Santa Regina, I., Rico, M., Rapp, M., and Gallego, H. A. 1997. Seasonal variation in nutrient concentration in leaves and branches of *Quercus pyrenaica*. Journal of Vegetation Science **8**: 651-654.

Santamariá, J. M. and Martin, A. 1998. Influence of air pollution on the nutritional status of Navarra's forests, Spain. Chemosphere **36**: 943-948.

Scharenberg, W. and Ebeling, E. 1996. Distribution of heavy metals in a woodland food web. Bulletin of Environmental Contamination Toxicology **56**: 389-395.

Schebek, L., Lieser, K. H., and Höllwarth, M. 1984. Die Birke (*Betula pendula* Roth.) als Bioindikator für Schwermetallimmissionen. Journal of Applied Botany **58**: 475-482.

Schulz, H., Popp, P., Huhn, G., Stärk, H.-J., and Shürmann, G. 1999. Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks. I. Temporal and spatial variations. The Science of the Total Environment **232**: 49-58.

Sehmel, G. A. 1980. Particle and gas dry deposition **-** A review. Atmospheric environment **14**: 983-1011.

Shao, J. and Dongsheng, T. 1995. The Jackknife and Bootstrap. Springer, New York. pp. 516.

Sieghardt, H. 1988. Schwermetall- und Nährelementgehalt von Raum und Bodenproben schwermetallhaltiger Halden im Raum Bleiberg in Kärnten (Österreich). II. Holzpflanzen. Zeitschrift für Pflanzenernährung und Bodenkunde **151**: 21-26.

Smith, G. C. and Brennan, E. 1984. Response of silver maple seedlings to an acute dose of root applied cadmium. Forest Science **30**: 582-586.

Smith, P. F. 1962. Mineral analysis of plant tissue. Annual Review of Plant Physiology **13**: 81-108.

Smith, W. H. 1973. Metal contamination of urban woody plants. Environmental Science and Technology **7**: 631-636.

Smith, W. H., Switzer, G. L., and Nelson, L. E. 1970. Development of shoot system of young loblolly pine: 1. Apical growth and nitrogen concentration. Forest Science **16**: 483-490.

Smodis, B. and Parr, R. M. 1999. Biomonitoring of air pollution as exemplified by recent IAEA programs. Biological Trace Element Research **71**: 257-266.

Starr, J. L., Parkin, T. B., and Meisinger, J. J. 1992. Sample size consideration in the determination of soil nitrate. Soil Science Society of America Journal **56**: 1824-1830.

Stauffer, H. B. 1982. A sample size table for forest sampling. Forest Science **28**: 777-784.

Stewart, C., Norton, D. A., and Fergusson, J. E. 1991. Historical monitoring of heavy metals in kahikatea ring wood in Christchurch, New-Zealand. Science of the Total Environment **105**: 171-190.

Stjernquist, I., Nihlgärd, B., Filiptchouk, A. N., and Strakhov, V. V. 1998. Soil and forest vitality as affected by air pollutants on the Kola Peninsula. Chemosphere **36**: 1119-1124.

Stoeppler, M. 1991. Sampling and Storage. *In* Analytical Chemistry of Metal Compounds. *Edited by* M. Stoeppler. Springer, New York. pp. 110-119.

Strojan, C. L. 1978. Forest leaf litter decomposition in the vicinity of a zinc smelter. Oecelogia **32**: 203-212.

Sutherland, E. and Martin, B. 1990. Growth response of *Pseudotsuga menziesii* to air pollution from copper smelting. Canadian Journal of Forest Research **20**: 1020-1030.

Suzuki, T. 1975. The ring width and contents of Cd, Zn and Pb in wood of annual rings of the sugi tree growing in an area contaminated by Cd from a zinc refinery at Annaka, Gunma. Journal of Japanese Forest Society **57**: 45-52.

Switzer, G. L. and Nelson, L. E. 1972. Nutrient accumulation and cycling in loblolly pine (*Pinus taeda* L.) plantation ecosystems: the first 20 years. Soil Scienes of America Proceedings **36**: 143-147.

Tamm, C. O. 1951. Seasonal variation in composition of birch leaves. Physiologia Plantarium **4**: 461-469.

Tanaka, I. and Ichikuni, M. 1982. Monitoring of heavy metals in airborne particulates by using bark samples of Japanese cedar collected from the metropolitan region of Japan. Atmospheric Environment **16**: 2015-2018.

Thomas, W., Ruhling, A., and Simon, H. 1984. Accumulation of airborne pollutants (PAH, chlorinated hydrocarbons, heavy metals) in various plant species and humus. Environmental Pollution **36**: 295-310.

Timmer, V. R. 1991. Interpretation of seedling analysis and visual symptoms. *In* Mineral nutrition of conifer seedlings. *Edited by* R. van den Driessche. CRC Press, Boca Raton. pp. 113-134.

Tordoff, G. M., Baker, A. J. M., and Willis, A. J. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere **41**: 219-228.

Tremolada, P., Burnett, V., Calamari, D., and Jones, K. C. 1996. Spatial distribution of PAHs in the UK atmosphere using pine needles. Environmental Science and Technology **30**: 3570-3577.

Trlica, M., Child, R. D., and Bauerle, B. A. 1985. Leaf injury and elemental concentrations in vegetation near a coal-fired power plant. Water, Air and Soil Pollution **24**: 375-396.

Trüby, P. 1995. Distribution patterns of heavy metals in forest trees on contaminated sites in Germany. Journal of Applied Botany **69**: 135-139.

Turner, A. P. 1991. The tolerance of trees to metal pollution. Ph.D. Thesis. School of Natural Sciences, Polytechnic.

Turner, A. P. and Dickinson, N. M. 1993. Survival of *Acer pseudoplatanus* L. (sycamore) seedlings on metalliferous soils. New Phytologist **123**: 509-521.

Tyler, G. 1974. Heavy metal pollution and soil enzymatic activity. Plant and Soil **41**: 303-311.

Tyler, G. 1975a. Effect of heavy metal pollution on decomposition and mineralization rates in forest soils. *In* International conference on heavy metals in the environment, Symposium Proceedings, Vol.2, part 1. *Edited by* T. Hutchinson. pp. 217-226.

Tyler, G. 1975b. Heavy-metal pollution and mineralization of nitrogen in forest soils. Nature **255**: (5511) 701-702.

UN/ECE-EC 1998. Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. PCC-BFH. International co-operative programme on assessment and monitoring of air pollution effects on forests. Part IV, pp. 1-30.

Van den Burg, J. 1985. Foliar analysis for determination of tree nutrient status - a compilation of literature data. Rijksinstituut voor onderzoek in de bos- en landschapsbouw "De Dorschkamp". Rapport nr. 414. pp. 615.

van den Driessche, R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. The Botanical Review **40**: 347-394.

Van Den Eede 1997. Betreft: Uw vraag van doctoraatsonderzoek. Letter, personal communication.

Van Grieken, R 1996. Verspreiding van zware metalen. *In* Milieu- en natuurrapport Vlaanderen 1996 Leren om te Keren. *Edited by* A. Verbruggen. Vlaamse Milieumaatschappij, Garant, Leuven, Apeldoorn. pp. 345-352.

van Hook, R. I. Harris W. F. and Henderson, G. S. 1977. Cadmium, lead and zinc distribution and cycling in a mixed deciduous forest. Ambio **6**: 281-286.

Vangronsveld, J., Colpaert, J., and Tichelen, Van K. 1995a. Reclamation of a bare industrial area contaminated by non-ferrous metals : *in situ* metal immobilization and revegetation. Environmental Pollution **87**: 51-59.

Vangronsveld, J., Sterckx, J., Van Assche, F., and Clijsters, H. 1995b. Rehabilitation studies on an old non-ferrous waste dumping ground: effects of revegetation and metal immobilization by beringite. Journal of Geochemical Exploration **52**: 221-229.

Verry, E. and Timmons, D. R. 1976. Elements in leaves of a trembling aspen clone by crown position and season. Canadian Journal of Forest Research **6**: 436-440.

Vervaeke, P, Luyssaert, S., Mertens, J., De Vos, B., Speleers, L., and Lust, N. 2001. Dredged sediment as a substrate for biomass production of willow trees established using the SALIMAT technique. Biomass and Bioenergy Accepted.

VLAREA 1998. Besluit van de Vlaamse regering tot vaststelling van het Vlaams reglement inzake afvalvoorkoming en -beheer. Bestuur van het Belgische staatsblad, Brussel.

Wagner, G. 1995. Basic approaches and methods for quality assurance and quality control in sample collection and storage for environmental monitoring. The Science of the Total Environment **176**: 63-71.

Walkenhorst, A. and Hagemeyer, J. 1997. Distribution of Cd, Pb, and Zn in above ground parts of a 55-year old spruce tree (*Picea abies* L. Karst.). Journal of Applied Botany **71**: 164-167.

Wallihan, E. F. 1944. Chemical composition of leaves in different parts of sugar maple trees. Journal of Forestry **42**: 486.

Wang, G. G. 1995. White spruce site index in relation to soil, understory vegetation, and foliar nutrients. Canadian Journal of Forest Research **25**: 29-38.

Wang, G. G. and Klinka, K. 1997. White spruce foliar nutrient concentrations in relation to tree growth and soil nutrient amounts. Forest Ecology and Management **98**: 89-99.

Ward, N. I., Brooks, R. R., and Reeves, R. D. 1974. Effect of lead from motor-vehicle exhausts on trees along a major thoroughfare in Palmerston North, New Zealand. Environmental Pollution **6**: 149-158.

Watmough, S. A. 1994. Adaptation to pollution stress in trees: metal tolerance traits. Ph.D. Thesis. School of Biological and Earth Sciences, John Moores University, Liverpool.

Watmough, S. A. 1999. Monitoring historical changes in soil and atmospheric trace metal levels by dendrochemical analysis. Environmental Pollution **106**: 391-403.

Watmough, S. A. and Dickinson, N. M. 1995. Dispersal and mobility of heavy metals in relation to tree survival in an aerally contaminated woodland soil. Environmental Pollution **90**: 135-142.

Watmough, S. A. and Hutchinson, T. C. 1996. Analysis of tree rings using inductively coupled plasma mass spectrometry to record fluctuations in a metal pollution episode. Environmental Pollution **93**: 93-102.

Watterson, K. G., Leaf, A. L., and Engelken, J. H. 1963. Effect of N, P, and K fertilization on yield and sugar content of sap of sugar maple trees. Soil Science Society of America Proceedings **27**: 236-238.

Webster, R. and Oliver, M. A. 1992. Sample adequately to estimate variograms of soil properties. Journal of Soil Sciences **43**: 177-192.

Weeda, E. J., Westra, R., Westra, Ch., and Westra, T. 1994. Nederlandse oecologische FLORA wilde planten en hun relaties 5. De Lange/Van Leer, Deventer. pp. 400.

Weinhold, A. 1862. Landwirtsch. Vers Sta. **4**: 188-193. Smith, P. F. 1962. Mineral analysis of plant tissue. Annual Review of Plant Physiology **13**: 81-108.

Wells, C. G. and Metz, L. J. 1963. Variation in nutrient content of loblolly pine needles with season, age, soil and position on the crown. Soil Science Society of America Proceedings **27**: 90-93.

White, D. P. 1954. Variation in the nitrogen, phosphorus, and potassium contents of pine needles with season, crown position, and sample treatment. Soil Science Society of America Proceedings **18**: 326-330.

Wolnik, K. A., Fricke, F. L., Capar, S. G., Braude, G. L., Meyer, M. W., Satzger, D., and Bonnin, E. 1983. Elements in major raw agricultural crops in the United States. 1. Cadmium and lead in lettuce, peanuts, potatoes, soybeans, sweet corn and wheat. Journal of Agricultural and Food Chemistry **31**: 1240-1244.

Wolnik, K. A., Fricke, F. L., Capar, S. G., Meyer, M. W., Satzger, D., Bonnin, E., and Gaston, C. M. 1985. Elements in major raw agricultural crops in the United States. 3. Cadmium, lead, and eleven other elements in carrots, field corn, onions, rice, spinach, and tomatoes. Journal of Agricultural and Food Chemistry **33**: 807-811.

Wolterbeek, H. T. and Bode, P. 1995. Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution. Science of the Total Environment **176**: 33-43.

Wolterbeek, H. T., Kuik, P., Verburg, T. G., Wamelink, G. W. W., and Van Dobben, H. 1996. Relations between sulphate, ammonia, nitrate, acidity and trace element concentrations in tree bark in the Netherlands. Environmental Monitoring and Assessment **40**: 185-201.

Woodwell, G. M. 1974. Variation in the nutrient content of leaves of *Quercus alba*, *Quercus coccinea*, and *Pinus rigida* in the Brookhaven Forest from bud-break to abscission. American Journal of Botany **61**: 749-753.

Wright, T. W. and Will, G. M. 1958. The nutrient content of Scots and Corsican pines growing on sand dunes. Forestry **31**: 13-25.

Wyttenbach, A., Bajo, S., Tobler, L., and Keller, Th. 1985. Major and trace element concentrations in needles of *Picea abies*: levels, distribution functions, correlations and environmental influences. Plant and Soil **85**: 313-325.

Zayed, J., Loranger, S., and Kennedy, G. 1992. Variations of trace-element concentrations in red spruce tree rings. Water, Air and Soil Pollution **65**: 281-291.

Zedaker, S. m., Gregoire, T. G., and Miller, J. H. 1993. Sample-size needs for forestry herbicide trails. Canadian Journal of Forest Research **23**: 2153-2158.

Zhang, R., Warrick, A. W., and Myers, D. E. 1990. Variance as a function of sample support size. Mathematical Geology **22**: 107-121.

Zinke, P. J. 1968. Tree analysis to determine nutrient status. *In* Proceedings of the Californian forest soil fertilising conference, soil improvement committee. Proceedings, Redding, USA. pp. 30-44.

Zwolinski, J. 1994. Rates of organic matter decomposition in forests polluted with heavy metals. Ecological Engineering **3**: 17-26.

---

[1] Little & Martin, 1972; Buchauer, 1973; Hutchinson & Whitby, 1977; Parker et al., 1978; Heinrichs & Mayer, 1980; Martin & Coughtrey, 1981; Martin et al., 1982; Schebek et al., 1984; Thomas et al., 1984; Alban, 1985; Trlica et al., 1985; Løbersli & Steinnes, 1988; Capelli et al., 1989; Claussen, 1990; Granier & Chevreuil, 1992; Gutenmann et al., 1992; Harold et al., 1993; Keller et al., 1994; Kim & Fergusson, 1994; Klumpp et al., 1994; Koricheva & Haukioja, 1995; Kozlov et al., 1995; Raitio, 1995; Trüby, 1995; Alfani et al., 1996a; Alfani et al., 1996b; Djingova et al., 1996; Loppi et al., 1998; Santamaria & Martin, 1998; Stjernquist et al., 1998; Djingova et al., 1999; Matzka & Maher, 1999; Smodis & Parr, 1999; Alfani et al., 2000; Anderson et al., 2000; Duquesnay et al., 2000; Monaci et al., 2000

[2] Jordan, 1975; van Hook et al., 1977; Mankovska, 1980; Borgegård & Rydin, 1989; Turner, 1991; Eltrop, 1991; Brieger et al., 1992; Bache et al., 1992; Turner & Dickinson, 1993; Riddell-Black, 1994; Duncan et al., 1995; Trüby, 1995; Dinelli & Lombini, 1996; Punshon, 1996; Boruvka et al., 1997; Punshon & Dickinson, 1997; Greger & Landberg, 1997; Monether, 1998

[3] Tanaka & Ichikuni, 1982; Alban, 1985; Trlica et al., 1985; Wolterbeek et al., 1996; Böhm et al., 1998

[4] Legrand & Asta, 1993; Huhn et al., 1995; Lippo et al., 1995; Poikolainen, 1997; Schulz et al., 1999

[5] Marion et al., 1968; Leaf, 1970; McColl, 1980; Ferm & Markkola, 1985

[6] Eltrop, 1991; Raitio, 1993; Trüby, 1995

[7] Faucherre & Dutot, 1976; Trüby, 1995; Greger & Landberg, 1997; Nabais et al., 1999; Watmough, 1999

[8] Suzuki, 1975; Kardell & Larsson, 1978; Alban, 1985; Stewart et al., 1991; Hagemeyer et al., 1992; Eklund, 1995; Hagemeyer & Schäfer, 1995; Trüby, 1995; Latimer et al., 1996; Watmough & Hutchinson,1996; Jonsson et al., 1997; Nabais et al., 1999; Watmough, 1999

[9] van Hook et al., 1977

[10] Duquesnay et al., 2000

[11] Hoyle, 1965; Leroy, 1968; Everard, 1973; Le Tacon & Totain, 1973; Auchmoody & Hammack, 1975; Bowersox & Ward, 1977; Insley et al., 1981(a, b); Heilman, 1985; Erdmann et al., 1988; McLennan, 1990; Rytter & Ericsson, 1993; Chen et al., 1998

[12] Morrison, 1985

[13] Grigal et al, 1976

[14] Bache et al., 1992

[15] Little & Martin, 1972; Buchauer, 1973; Gutenmann et al., 1992; Keller et al., 1994; Kim & Fergusson, 1994; Koricheva & Haukioja, 1995; Kozlov et al., 1995; Alfani et al., 1996a, b; Santamaria & Martin, 1998; Alfani et al., 2000; Duquesnay et al., 2000; Monaci et al., 2000

[16] Tamm, 1951; Mader & Thompson, 1969; Everard, 1973; Woodwell, 1974; Langille & Maclean, 1976; Bowersox & Ward, 1977; Lea et al., 1979a, b; McColl, 1980; Insley et al., 1981b; Ricklefs & Matthew, 1982; Prégent & Camiré, 1985; Robert et al., 1996; Chen et al., 1998

[17] Ponder et al., 1979

[18] McVickar, 1949; Ovington & Madgwick, 1958; Guha & Mitchell, 1965; Chapman in Ellis, 1966; Verry & Timmons, 1976; Santa Regina et al., 1997; Leroy, 1968; Le Tacon & Totain, 1973; Insley et al., 1981a; Erdmann et al., 1988; McLennan, 1990; Morrison, 1985

[19] Ovington & Madgwick, 1958; Ovington & Madgwick, 1959b; Ellis, 1975; Heilman, 1985

[20] Eltrop 1991; Duncan et al., 1995; Dinelli & Lombini, 1996; Greger & Landberg, 1997

[21] Riddell-Black ,1994; Punshon, 1996; Punshon & Dickinson, 1997; Monether, 1998

[22] Hutchinson & Whitby, 1977; Heinrichs & Mayer, 1980; Hogan & Wotton, 1984; Alban, 1985; Løbersli & Steinnes, 1988; Granier & Chevreuil, 1992; Klumpp et al., 1994

[23] Parker et al., 1978; Kim & Fergusson, 1994; Raitio, 1995

[24] Koricheva & Haukioja, 1995; Kozlov et al., 1995

[25] Hoyle, 1965; Ellis, 1975; Bowersox & Ward, 1977

[26] Woodwell, 1974; Chen et al., 1998; Ponder et al., 1979; Morrison, 1985; Erdmann et al., 1988; McLennan, 1990; Robert et al., 1996

[27] Guha & Mitchell, 1965; Leroy, 1968; Nilsson & Ericsson, 1986

[28] Keller et al., 1994; Alfani et al., 1996b ; Alfani et al., 2000; Duquesnay et al., 2000

[29] Memon et al., 1980; Rytter & Ericsson, 1993

[30] Duquesnay et al., 2000

[31] Wallihan, 1944; Ellis, 1975; Heilman, 1985; Morrison, 1985; Erdmann et al., 1988; McLennan, 1990

---

[32] Tamm, 1951; Guha & Mitchell, 1965; Hoyle, 1965; Leroy, 1968; Le Tacon & Totain, 1973; Woodwell, 1974; Grigal et al, 1976; Verry & Timmons, 1976; Bowersox & Ward, 1977; Hutchinson & Whitby, 1977; Lea et al., 1979a, b; Ponder et al., 1979; McColl, 1980; Insley et al., 1981b; Ricklefs & Matthew, 1982; Alban, 1985; Ferm & Markkola, 1985; Trlica et al., 1985; Nilsson & Ericsson, 1986; Capelli et al., 1989; Rytter & Ericsson, 1993; Kim & Fergusson, 1994; Riddell-Black, 1994; Duncan et al., 1995; Alfani et al., 1996a; Dinelli & Lombini, 1996; Robert et al., 1996; Santa Regina et al., 1997

[33] Jordan, 1975; Punshon, 1996; Punshon & Dickinson, 1997; Monether, 1998

[34] Riddell-Black, 1994; Duncan et al., 1995; Dinelli & Lombini, 1996

[35] Buchauer, 1973; Parker et al., 1978

[36] Hogan & Wotton, 1984; Kozlov et al., 1995; Santamaria & Martin, 1998; Duquesnay et al., 2000; Monaci et al., 2000

[37] Little & Martin, 1972; Løbersli & Steinnes, 1988; Granier & Chevreuil, 1992; Gutenmann et al., 1992; Keller et al., 1994; Alfani et al., 1996a; Djingova et al., 1996

[38] Hutchinson & Whitby, 1977; Alban, 1985; Trlica et al.,1985; Capelli et al., 1989; Kim & Fergusson, 1994; Alfani et al., 1996a

[39] Wallihan, 1944; McVickar, 1949; Mader & Thompson, 1969; Ellis, 1975; Langille & Maclean, 1976; Memon et al., 1980; Insley et al., 1981a; Morrison, 1985; Prégent & Camiré, 1985; Bernier & Brazeau, 1988; Erdmann et al., 1988; McLennan, 1990; Chen et al., 1998

[40] Tamm, 1951; Guha & Mitchell, 1965; Hoyle, 1965; Leroy, 1968; Le Tacon & Totain, 1973; Woodwell, 1974; Grigal et al, 1976; Verry & Timmons, 1976; Bowersox & Ward, 1977; Lea et al., 1979a, b; Ponder et al., 1979; McColl,1980; Insley et al., 1981b; Ricklefs & Matthew, 1982; Ferm & Markkola, 1985; Nilsson & Ericsson,1986; Rytter & Ericsson, 1993; Robert et al., 1996; Santa Regina et al., 1997

[41] Alban, 1985; Trlica et al., 1985; Keller et al., 1994; Kozlov et al., 1995; Alfani et al., 1996a; Djingova et al., 1996; Alfani et al., 2000; Duquesnay et al., 2000

[42] Leroy, 1968; Everard, 1973; Ellis, 1975; Ponder et al., 1979; Santa Regina et al., 1997

[43] Little & Martin, 1972; Capelli et al., 1989; Koricheva & Haukioja, 1995; Alfani et al., 1996a; Robert et al., 1996

[44] Little & Martin, 1972; Buchauer, 1973; Jordan, 1975; Hogan & Wotton, 1984; Løbersli & Steinnes, 1988; Bache et al., 1992; Keller et al., 1994; Kim & Fergusson, 1994; Klumpp et al., 1994; Koricheva & Haukioja, 1995; Kozlov et al., 1995; Stjernquist et al., 1998

[45] Jordan, 1975; Eltrop, 1991; Dinelli & Lombini, 1996

[46] Little & Martin, 1972; Buchauer, 1973; Hutchinson & Whitby, 1977; Capelli et al., 1989; Granier & Chevreuil, 1992; Koricheva & Haukioja, 1995; Alfani et al., 1996a, b; Djingova et al., 1996

[47] Little & Martin, 1972; Capelli et al., 1989; Keller et al., 1994; Kim & Fergusson, 1994; Koricheva & Haukioja, 1995; Alfani et al., 1996a; Santamaria & Martin, 1998; Alfani et al., 2000; Monaci et al., 2000

[48] Guha & Mitchell, 1965; Robert et al., 1996

[49] Robert et al., 1996