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THE USE OF ENERGY CROPS ON METAL CONTAMINATED SANDY SOILS

Thesis submitted in fulfillment of the requirements for the degree of Doctor
(PhD) in Applied Biological Sciences

Dutch translation of the title:
Het gebruik van energiegewassen op metaalverontreinigde zandige gronden

Cover illustrations:
Harvest of energy maize, taken at the experimental site
Back illustrations:
Regrowth of willows under short rotation coppice on the experimental site

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Woord Vooraf

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Stijn Van Slycken

18 oktober 2011

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List of Abbreviations

α	Power of the statistic test
BCF	Bioconcentration factor
CEC	Cation Exchange Capacity
DM	Dry Matter
DW	Dry Weight
FM	Fresh Matter
FW	Fresh Weigth
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
n	number of repititions
OM	Organic Matter
OWS	Organic Waste Systems (Ghent, Belgium)
SRC	Short Rotation Coppice
TF	Translocation Factor
VLAREBO	Flemish regulation for soil remedediation (*Vlaams regalement betreffende de bodemsanering)*
VLAREA	Flemish regulation for prevention and management of waste) (translated from Dutch: *Vlaams reglement voor Afvalvoorkoming en –beheer*

Summary

In the 19th and 20th century (up to 1970s), historic atmospheric deposition of trace metals from metal refinery activities has resulted in elevated concentrations in agricultural soils in the Campine region. As a consequence, a surface area of at least 700 km^2 is now contaminated with several toxic metals, including cadmium (Cd), zinc (Zn) and lead (Pb). Previous studies also reported health problems in the vicinity of the smelters with increased risks for renal dysfunction, osteoporosis, lung cancer as well as other health-related issues due to a higher exposure of Cd. Furthermore, the soils in the region are characterised by a sandy texture and relatively low pH, which entails an enhanced risk for uptake of these metals in crops or leaching to the groundwater.

Conventional soil remediation approaches tend to be overly expensive considering the moderate levels of pollution and extended areas that need a treatment. Phytoremediation involves the use of plants and their associated microorganisms for the stabilisation, degradation and/or removal of pollutants from the environment. Phytoextraction in particular aims at accumulating elements into the harvestable plant parts so that the contamination can gradually be removed through harvest. The purpose of this research is to assess the feasibility of non-food crops not only as a phytoremediation option, but also forming a risk based management approach for metal contaminated agricultural soil.

In this study three non-food crops were evaluated: rapeseed (*Brassica napus* L.) for plant oil production (biodiesel), willow (*Salix*) under short rotation coppice and energy maize (*Zea mays* L.) for biogas production by anaerobic digestion. Rapeseed reached a total shoot biomass of 5.8±2.0 ton DM ha^{-1} which is in compliance with expected values. However, the seed yield was very low (0.5±0.3 ton DM ha^{-1}). Other reports, with experiments performed in the same year and region but sown on non-contaminated soils and under optimal fertilisation, showed a seed production of 3 ton DM ha^{-1}. The low biomass productivity was attributed to suboptimal fertilisation mainly of nitrogen and sulphur. Observations from the field experiment suggested that 27±14 g Cd ha^{-1} and 2.0±1.0 kg Zn ha^{-1} could be removed from the soil by harvesting rapeseed. This means that more than 250 growing cycles of rapeseed would be needed to reduce total Cd concentrations in the top soil layer from 5.0 to 2.9 mg Cd kg^{-1} (resp. the current concentration in the field plot and the site-specific threshold value of Cd for remediation of the field plot). During subsequent experiments, the growth of rapeseed failed completely. The fact that rapeseed needs to be cultivated in a rotation scheme in order to avoid possible crop failures, also contributes to lower suitability of this crop as an appropriate alternative cropping system for metal contaminated agricultural soils in the Campine region.

Secondly, the cultivation of short rotation coppice (SRC) was investigated. Short rotation coppice consists of densely planted, high-yielding varieties of either willow or poplar (*Populus*), which are harvested every two to five years. After each harvest, new shoots spontaneously re-sprout. From the biomass productivity during the first four years of growth, it could be concluded that a rotation period of four years was needed to reach maximum annual growth potential of all clones. There were strong differences observed in biomass production between clones. After four years, Zwarte Driebast had the highest biomass (~12 ton DM ha^{-1} year^{-1}), followed by Loden. In contrast, Inger achieved only a biomass production of 1.5 ton DM ha^{-1} year^{-1}. The metal extraction potential of willow was much higher than that found for rapeseed. The woody biomass itself was in general 72 g Cd and 2.0 kg Zn ha^{-1} year^{-1}. This means that in order to reduce the concentration of Cd from 6.5 mg kg^{-1} (current concentration in the field plot) to 3.0±0.3 mg Cd kg^{-1} (site-specific threshold value of Cd for remediation of the field plot), more than 170 years will be needed. Under normal conditions SRC is harvested in winter, but as the leaves contain high amount of metals, leaf harvest could increase the extraction potential by 40%. Previous reports showed that SRC from contaminated soils can be treated safely, yet it was not further assessed in the current study. Currently, the price of the wood does not guarantee a sufficient income for farmers, compaired to their current farmer income, to make it a suitable alternative cultivation. Neverthelles when the use of wood as energy source becomes more pronounced, wood prices will very likely also stimulate the adoption of SRC in the region.

A final investigated option was the use of energy maize. Under optimal fertilisation, energy maize showed an average yield of 53±10 ton FM or 20±3 ton DM ha^{-1} year^{-1}. The observed productivity levels were similar to those of the same cultivars grown elsewhere in Flanders on non-contaminated sites. As maize is a metal excluder, it contains low concentrations of metals in the different plant compartments. Due to the low concentration of metals in the plant, the extraction potential of energy maize was the lowest of the investigated crops (19±6 g Cd and 4.3±0.9 kg Zn ha^{-1} year^{-1}), implying that for remediation of the experimental site (from 5.0 to 2.5 mg Cd kg^{-1}) more than 500 years would be needed under optimistic conditions.

Lab scale batch results of 14 days, performed at OWS (Organic Waste Systems, Ghent), revealed a biogas production potential of 215±23 Nm^3 ton^{-1} FM which was similar to that of energy maize grown on a non-contaminated soil (194±4 Nm^3 ton^{-1} FM) tested under the same conditions. For obtaining optimal biogas yields, harvest must take place when the plant reaches optimal dry matter content. Although the harvest period optimises the digestibility of the biomass, it does not affect the metal extraction rates significantly. When a semi-continuous digester is fed over a period of 435 days with maize from a contaminated site, similar biogas production levels were achieved. During this semi-continuous test, concentrations of elements in the digestate were on average 3-4 times higher than those in the input material. This is similar to concentration factors observed using energy maize from a non-contaminated site. The quality and characteristics of the digestate can be estimated and evaluated in the context of legal threshold values and limitations for usage. Economic studies indicated that the average annual income of the farmers can be maintained when they convert land use from fodder maize to energy maize.

None of the tested energy crops would allow an effective short term (*e.g.* 20 - 30 years) removal of metals from the soils. However, the presence of contamination is not expected to introduce technical constraints in converting biomass with elevated concentrations into energy. The fate of the metals during subsequent processing of the biomass can be controlled, and eventual constraints in subsequent use and treatment of the secondary products can be managed. If economic viability of energy crop production can be ascertained, it can allow for a long term safe alternative use of the contaminated agricultural land. For energy maize as a cropping alternative, this was found to be the case. Growing energy crops aims at risk-reduction, and generates an alternative income for farmers, yet in the long run also generating a gradual reduction of the pollution levels. In this way, remediation is reduced to a secondary objective with sustainable risk-based land use as primary objective. This context has been adopted as ‘phytoattenuation’, setting it apart from ‘conventional’ phytoextraction.

Samenvatting

Tijdens de 19^e eeuw en 20^e eeuw (tot de jaren 70) werden de bodems in de Kempen atmosferisch aangerijkt door sporenelementen uit de metaalraffinaderijen. Dit zorgde ervoor dat ongeveer 700 km^2 langs Belgische en Nederlandse zijde aangerijkt is met metalen waaronder cadmium (Cd), zink (Zn) en lood (Pb). Diverse studies toonden aan dat in de buurt van deze raffinaderijen een veelheid aan medische problemen zoals nier disfunctie, osteoporose, kanker, enz., werden vastgesteld, door de verhoogde aanwezigheid van Cd. Doordat de Kempische bodems gekenmerkt worden door een zandige textuur met lage pH (zure bodems), is er tevens een verhoogde beschikbaarheid van metalen voor plantenopname en uitloging.

Conventionele bodemsaneringstechnieken zijn echter duur wanneer rekening wordt gehouden met de uitgestrektheid van de verontreiniging. Fytoremediatie wordt gedefinieerd als het gebruik van planten en hun geassocieerde bacteriën om een verontreiniging te stabiliseren, degraderen of te verwijderen uit een omgeving. Wanneer er gebruik gemaakt wordt van planten die metalen opnemen en verplaatsen naar bovengrondse plantendelen die verwijderd worden, ontstaat er een graduele vermindering van de verontreiniging uit het systeem. Deze saneringstechniek wordt fytoextractie genoemd. Het doel van dit onderzoek is het evalueren van de mogelijkheden om energiegewassen te gebruiken voor fytoremediatie van metaalverontreinigde landbouwgronden.

Dit onderzoek spitste zicht toe op drie energiegewassen zijnde koolzaad (*Brassica napus* L.) voor bio-olie, wilg (*Salix*) onder korteomloophout en energiemaïs (*Zea mays* L.) voor biogasproductie door middel van anaerobe vergisting. Koolzaad vertoonde een totale bovengrondse biomassa van 5.8±2.0 ton droge stof (DS) ha^{-1} opgemeten. De zaadopbrengst (0.5±0.3 ton DS ha^{-1}) was echter laag. Een gelijkaardig onderzoek in dezelfde regio maar op een niet-verontreinigde grond en onder optimale bemesting, vertoonde een zaadopbrengst van 3 ton DS ha^{-1}, waardoor besloten kon worden dat de lage opbrengst voornamelijk te wijten was aan een suboptimale bemesting van zowel stikstof als zwavel. Deze bevindingen konden echter ten velde niet bevestigd worden aangezien er tijdens de opeenvolgende jaren de groei van koolzaad telkens mislukte. Koolzaad vertoonde een extractierendement van 27±14 g Cd ha^{-1} jaar^{-1} en 2.0±1.0 kg Zn ha^{-1} jaar^{-1}. Dit betekent dat voor de sanering van een bodem met een concentratie van 5.0 mg Cd kg^{-1} naar 2.9 mg Cd kg^{-1} (respectievelijk de concentraties in het veld en de saneringsnorm van het veld) er meer dan 250 groeicycli nodig zijn. Hierbij moet bovendien opgemerkt worden dat koolzaad steeds onder een rotatie moet staan, wat bijdraagt aan de ongeschiktheid van dit gewas als alternatief landgebruik in de Kempen.

Een tweede mogelijkheid, is het gebruik van korteomloophout. Korteomloophout (KOH) wordt gedefinieerd als de teelt van snelgroeiende houtachtige gewassen waarbij de bovengrondse biomassa periodiek tot maximaal acht jaar na aanplanting of na de vorige oogst, in zijn geheel wordt geoogst. Na iedere oogst is er vanuit de afgesneden scheuten een spontane hergroei. Na het bestuderen van de groei kon besloten worden dat er voor de Kempische bodems tijdens de eerste groeicyclus pas na vier jaar groei geoogst zou moeten worden. Op die manier kan verzekerd worden dat iedere kloon zijn maximale jaarlijkse groeicapaciteit bereikt heeft. Na vier jaar groei vertoonde wilg een sterk uiteenlopende productie. Zwarte Driebast vertoonde een opbrengst van ongeveer 12 ton DS ha^{-1} jaar^{-1}, en staat in sterk contrast met dat van Inger met een opbrengst van slechts 1.5 ton DS ha^{-1} jaar^{-1}. Naast de opbrengst was ook de jaarlijkse extractie van Cd en Zn hoger dan deze van koolzaad. Wanneer enkel de houtige biomassa geoogst werd, vertoonde KOH met wilg een extractierendement van 72 g Cd en 2.0 kg Zn ha^{-1} jaar^{-1}. Hierbij vertoonden Zwarte Driebast het hoogste rendement (203±46 g Cd ha^{-1} jaar^{-1} en 5.1±1.5 kg Zn ha^{-1} jaar^{-1}), gevolgd door Loden (118±57 g Cd and 2.9±0.7 kg Zn ha^{-1} jaar^{-1}). Toch betekent dit dat de sanering van een bodem met een concentratie van 6.5 mg kg^{-1} naar 3.0 mg kg^{-1} (wat de saneringsnorm is voor het onderzochte proefveld), dit meer dan 170 jaar in beslag kan nemen. Wanneer bladeren worden meegeoogst kan het extractiepotentieel met 40% toenemen. Toch blijkt hier het grootste probleem naar haalbaarheid, de huidige prijs van het hout, die landbouwers afschrikt om KOH aan te planten. Desalniettemin zal, wanneer het gebruik van KOH als grondstof voor de energiewinnig toeneemt, de prijs van het hout stijgen alsook het gebruik van KOH in de regio gestimuleerd worden.

Een laatste energiegewas dat in het veld onderzocht werd, was energiemaïs. Onder optimale bemesting vertoonde maïs een gemiddelde opbrengst van 53±10 ton verse stof (VS) of 20±3 ton DS ha^{-1} jaar^{-1}. Deze opbrengst is gelijkaardig aan maïs geteeld elders in de Kempen en in Vlaanderen, maar op niet-verontreinigde gronden. Maïs wordt aanzien als een ‘excluder van metalen’ aangezien de concentratie aan elementen in de verschillende plantenonderdelen laag was. Daardoor vertoont energiemaïs dan ook de laagste extractierendementen van alle onderzochte energieteelten (19±6 g Cd en 4.3±0.9 kg Zn ha^{-1} jaar^{-1}). Dit betekent dat, onder optimale omstandigheden, voor de sanering van een bodem met concentratie van 5 mg Cd kg naar 2.5 mg Cd kg^{-1} (specifieke saneringsnorm voor het proefveld) meer dan 500 jaar nodig zal zijn.

Kort lopende proeven omtrent de vergistbaarheid werden uitgevoerd in samenwerking met OWS (*Organic Waste Systems*) en toonden aan dat maïs afkomstig van een metaalverontreinigde grond een vergistingspotentieel heeft van 215±23 Nm^3 ton^{-1} VS wat vergelijkbaar is met deze van maïs op een niet-verontreinigde grond (194±4 Nm^3 ton^{-1} VS). Om optimale biogasopbrengsten te verkrijgen moet de oogst plaatsvinden wanneer de plant zijn optimale droge stof gehalte bereikt heeft. Ondaks het feit dat de oogstperiode de vergistbaarheid kan optimaliseren, heeft dit geen invloed op het metaalextractiepotentieel. Bij een semi-continue proef van 435 dagen werden gelijkaardige biogasopbrengsten bekomen als deze van de korte termijnproeven. De concentratie aan metalen en nutriënten bleek 3-4 keer hoger te zijn dan deze van het gebruikte inputmateriaal. Deze op-concentratiefactor was gelijkaardig als deze uit niet-verontreinigde maïs. Deze factor laat toe om afhankelijk van de concentratie van het inputmateriaal en het type vergisting, een inschatting te maken van de samenstelling en de kwaliteit van het digestaat, waardoor deze ook gekoppeld kan worden aan de bestaande wetgeving en mogelijke toepassingen. Economische studies toonden aan dat de overschakeling van conventionele maïs naar energiemaïs in de Kempen geen nadelige invloed heeft op het inkomen van de landbouwers.

Uit deze studie blijkt dat geen enkel onderzocht gewas voor een korte termijn sanering (20-30 jaar) van metalen uit de bodem kan zorgen. Toch wordt verwacht dat de aanwezigheid van verhoogde concentraties de technische haalbaarheid voor een conversie naar energie niet beïnvloedt. Gedurende deze conversie kan het gedrag van metalen in kaart gebracht worden, waardoor de samenstelling en de kwaliteit van de bijproducten bepaald en geëvalueerd kunnen worden. Wanneer de omschakeling naar het gebruik van energiewassen economisch haalbaar is, kan een lange termijn alternatief voor de metaalverontreinigde gronden aangeboden worden. Dit werd dan ook in dit onderzoek voor energiemaïs bevestigd. Wanneer gekozen wordt voor energiegewassen wordt het veilig gebruik van de bodem en het behoud van het inkomen voor de landbouwers als primordiaal belang vooropgesteld, met op termijn een graduele vermindering van de verontreiniging. Om een duidelijk onderscheid te maken met ‘klassieke’ fytoextractie, wordt deze benadering ‘fytoattenuatie’ genoemd.

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CHAPTER 1:

INTRODUCTION

1.1. General

The contamination and subsequent degradation of European soils by trace elements and organic xenobiotics is an important and ongoing process that negatively impacts environmental quality (Mench *et al.,* 2010). Soil contamination can be a result from mining and smelting activities of metalliferous ore, application of fertilisers, sewage sludge or other biowastes containing metals, release of automobile exhausts, a pile-up of municipal wastes, *etc*. (Alloway, 1990; Jarausch-Wehrheim *et al.,* 1999; Kurz *et al.,* 1999; do Nascimento and Xing, 2006; Wei *et al.,* 2008). Even in the surrounding areas with industrial activity, increases in metal content in the soil are found (Loska *et al.,* 2004). Contamination with metals in the environment has accelerated dramatically since the beginning of the industrial revolution (Nriagu, 1979, cited from Fusco *et al*., 2005). This was also the case for the Campine region.

1.2. Campine region

From the mid-19th century to the mid-seventies, zinc (Zn) and lead (Pb) were being refined in the Campine region (northeastern region of Flanders) using a pyro-metallurgical process. Volatile metals that were not captured inside the condenser are captured on dust particles and subsequently expelled through the smoke stacks. Furthermore, discharge of waste water from this industrial operation into the surface water is reported. As a result, at least 700 km^2 in the Belgian and Dutch Campine region is now contaminated with several metals and metalloids, including Zn, Pb, Cadmium (Cd) and Arsenic (As) through atmospheric deposition. Most of the contamination is found in the upper layer of the soil (upto 30-40 cm) (Vangronsveld *et al.,* 1995a; Sonke *et al.,* 2002; Hogervorst *et al.,* 2007; OVAM, 2008).

In the Belgian Campine and in the vicinity of Zn-smelters in general, it was shown that increased exposure to Cd was leading to increased risks for renal dysfunction, osteoporosis, lung cancer, and other health-related problems (Staessen *et al.,* 1999; Kos *et al*., 2003; Nawrot *et al*., 2008). Also natural ecosystems near the smelters suffer from the toxic effects of elevated non-ferrous metal (Zn, Cd, Pb, ...) concentrations (Vangronsveld *et al*., 1995a,b, 1996; Nachtegaal *et al*., 2005).

Furthermore in the region trace elements exhibit a relatively high mobility and availability for plant uptake because the soils in that region are acidic (pH 4-6) and sandy (De Temmerman *et al*., 2003; Ruttens *et al*., 2011). As a large portion of this area is currently in agricultural use, several local vegetable harvests (*e.g*. carrot, scorzenera) cultivated for food industry have already been taken out of the market because Cd concentrations in the crops were exceeding legal threshold values for human consumption (Ruttens *et al*., 2011) (Figure 1.1).

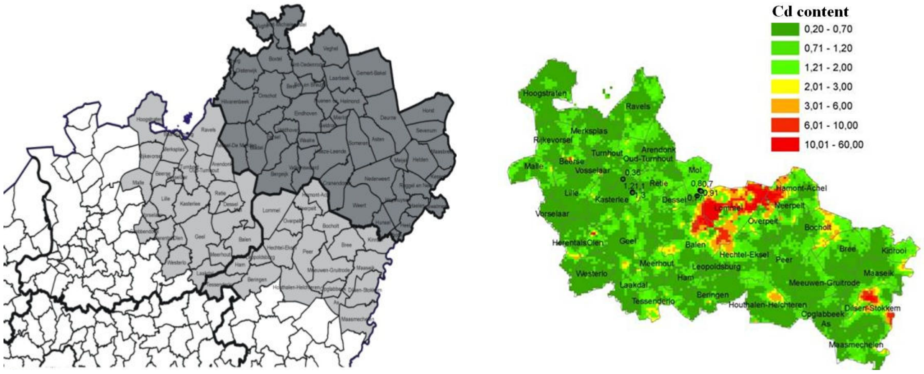

Figure 1.1. Localisation of the Campine region, with an overview of the Cd concentration (mg kg^{-1}) measured in the soil in the region (OVAM, 2008)

Therefore, remediation of these areas is needed. The use of conventional soil remediation techniques for moderate, diffusely polluted areas, like the Campine region, would be unrealistic because they are very expensive and moreover destroy the soil structure. Regional policy prescribes that these soils should be remediated, while at the same time it is desirable to keep the income of the farmers constant. Both goals can be achieved using phytoremediation in combination with the growth of energy crops (Alkorta *et al.*, 2004; Meers *et al*., 2005a,b; 2007d; Berndes *et al*., 2008). Phytoremediation is a technology that involves the use of plants and their associated microorganisms for the stabilisation, degradation and/or removal of pollutants from the environment (Garbisu and Alkorta, 2001).

1.3. Experimental site

1.3.1. Localisation and history

In order to investigate the possibilities of phytoremediation in the Campine region a contaminated site in Lommel (51°12'41" N; 5°14'32" E), was selected. This site has been diffusely contaminated with metals (including Cd and Zn) by historic industrial activities of metal smelters. The experimental site is situated 500 m NE of one of the smelters, and is property of Nyrstar Belgium. The site was used for maize cultivation until 2001 (Vangronsveld *et al*., 1995a; Hogervorst *et al*., 2007) and is part of a larger research area (~ 20 ha) used by Ghent University, Hasselt University and the Research Institute for Nature and Forest (INBO) for phytoremediation studies. The planting (Figure 1.2) and management of this site was part of a demonstration project in the context of the INTERREG-project BENEKEMPEN. This research study was funded by the Institute for the promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen, Grant IWT/CLO/50702) and Environmental and Energy Innovation Platform (MIP-Flanders (Belgium), project Phytofuel).

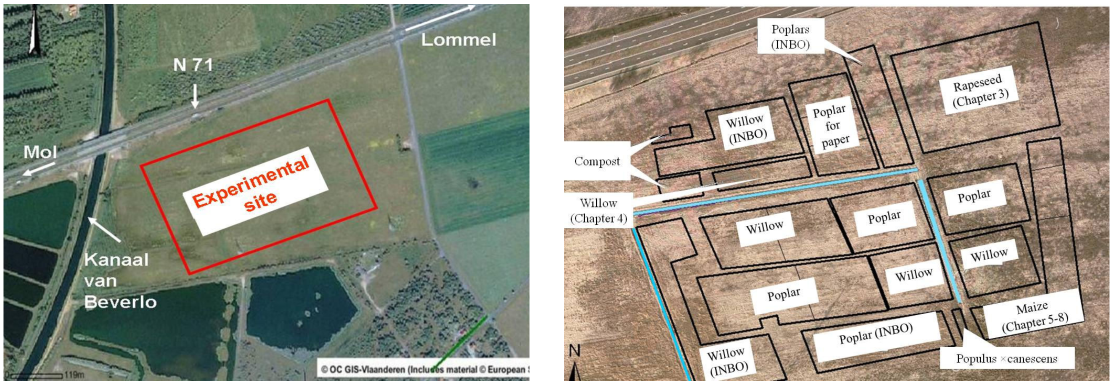

Figure 1.2. Situation and plantation scheme of the experimental site, with addition of where samples were taken for each chapter

1.3.2. Soil characterisation

Because of observed soil heterogeneity in previous experimental setups (Ruttens *et al.*, 2008, 2011), a thorough soil characterisation was performed prior to the plantation. Hereby not only the horizontal distribution of Cd, Zn and pH-H_2O in the site but also the vertical distribution of Cd and Zn was mapped.

Material and methods

For mapping the contamination levels in the field, 47 points, spread over a grid with 50 m between each sampling point, were sampled with an auger (auger, ø 3 cm, 20 cm, Eijekkamp, The Netherlands) and localised with GPS-measurements. In order to conduct a detailed information of the distributions of the Cd and Zn concentration in function of the soil depth, an auger with a cylindrical PVC tube (ø 20 cm, 30 cm height) was hammered in the ground and dug up at three different soil depths (0-30 cm, 30-60 cm, 60-90 cm). This was repeated three times at three locations where no plantation was performed. Each sample was sliced into different sections (0-5, 19-20, 20-30, 30-40, 50-60, 70-80 and 80-90 cm). All soil samples were dried at 50 °C in an oven (EU 170, Jouan s.a., Saint Herblain, France) and sieved to pass a 1 mm sieve for chemical analysis. Also soil samples were taken by the Belgian Soil Service for liming advice.

To determine the pH-H_2O, 10 g of air-dried soil was allowed to equilibrate for 24 h in 50 mL of deionised water, after stirring regularly in the beginning with a glass rod. The pH of the supernatant was measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA) (Van Ranst *et al.*, 1999). Pseudo-total soil content of Cd and Zn was determined after an *aqua regia* digestion (Van Ranst *et al.*, 1999). Certified reference sediment CRM 277 was also analysed in triplicate using the same method. Results varied from 93 to 102% of the certified *aqua regia* extractable values.

Analysis of Cd and Zn were performed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). For quality control, standards were always re-analyzed at the end of each batch of analyses. The analysis results were only retained when the measured standard concentrations were within 95 to 105% of the nominal value.

Data for mapping the contamination level were geostatistically analysed with an image processing program called IDRISI (IDRISI 32, version 132.2) to make an estimation of the variability of the field. Working with this digital image processor continuous image can be created starting from discrete points. For the presented maps, a linear interpolation is used. The use of kriging techniques is another possibility, but then more sampling points were needed. this technique allows irregularities and heterogeneities to be taken more into account in the image processing technique (Ruttens *et al*., 2008, Van Meirvenne, M., pers. communication 2008).

Pseudo-total concentration of Cd and Zn and pH throughout the field

The pseudo-total Cd and Zn concentration throughout the field is very heterogeneous, with a minimum value of 1.9 mg Cd and 136 mg Zn kg^{-1} dry soil and a maximum of 12 mg Cd and 654 mg Zn kg^{-1} dry soil. All samples exceeding their corresponding site specific remediation criteria of Flanders for Cd and some of them also those of Zn. These criteria have been adapted in 2007 to include soil pH because of its impact on metal mobility and associated environmental risks and can calculated using Equation. 1.1 and 1.2. (VLAREBO, 2009):

$$\mathrm{Cd}\ (\mathrm{mg\,kg^{-1}}) = 1.2 \cdot 10^{-0.17(5-\mathrm{z})} \qquad \text{(Equation 1.1.)}$$

$$\mathrm{Zn}\ (\mathrm{mg\,kg^{-1}}) = 0.098924 \cdot [(38.8 + 3.5 \cdot \mathrm{z}) \cdot \mathrm{x} + (22.1 + 23.5 \cdot \mathrm{z}) \cdot \mathrm{y}]^{1.13} \qquad \text{(Equation 1.2.)}$$

With: (i) x the clay content in the soil (%), if x < 2%, then a value of 2% is adopted, if x > 50%, a value of 50% is adopted; (ii) y the organic matter content in the soil (%), if y < 1%, then a value of 1% is adopted, if y > 10%, a value of 10% is adopted; and (iii) z the pH-KCl, if z < 4, then a value of 4 is adopted, if z > 7, a value of 7 is adopted.

Although great differences in the Cd and Zn concentration throughout the field were found, a certain contamination gradient could be distinguished, which corresponds to the main wind direction from the source of the pollution (Figure 1.3.) and confirms the historical background of the pollution.

The pH-H_2O in the field varied from 4.8 to 6.6 (Figure 1.4). Because of this low pH, the whole field was limed one month before the realisation of the plantation with lime (powder; zbw 53) at a dose of 6 000 kg ha^{-1} (as suggested by the Belgian Soil Service) and incorporated in the upper 25 cm soil layer using a rotary tiller. Two months after the liming activities soil sampling was repeated for analysis of pH-H_2O. Results revealed that the pH had shifted to a range from 5.6 to 6.7 (Figure 1.5).

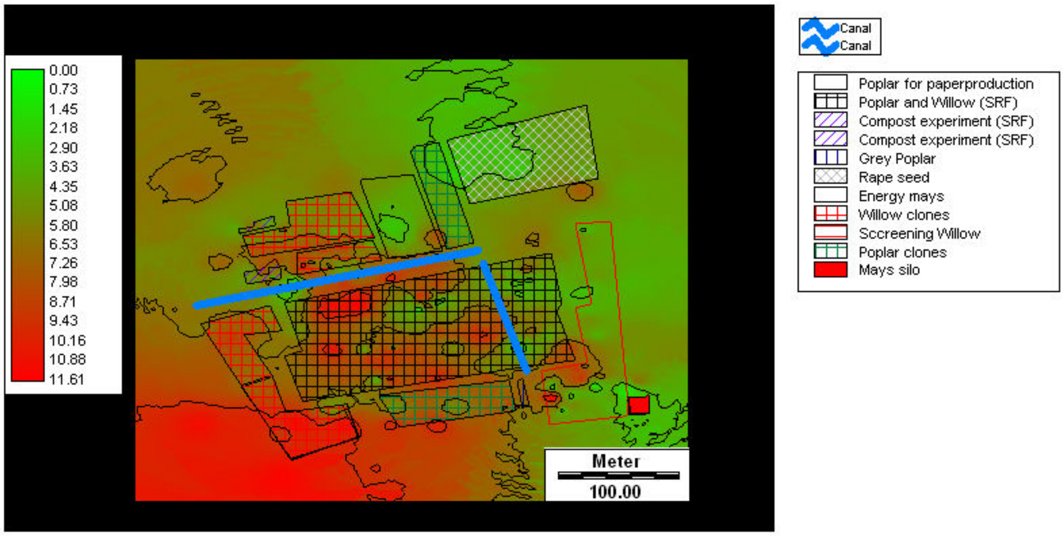

Figure 1.3. Distribution of the Cd concentration (mg kg^{-1}) in soil layer 0-25 cm, measured after an *aqua regia* destruction ties (mapping performed with IDRISI 32, version 132.2, n=47)

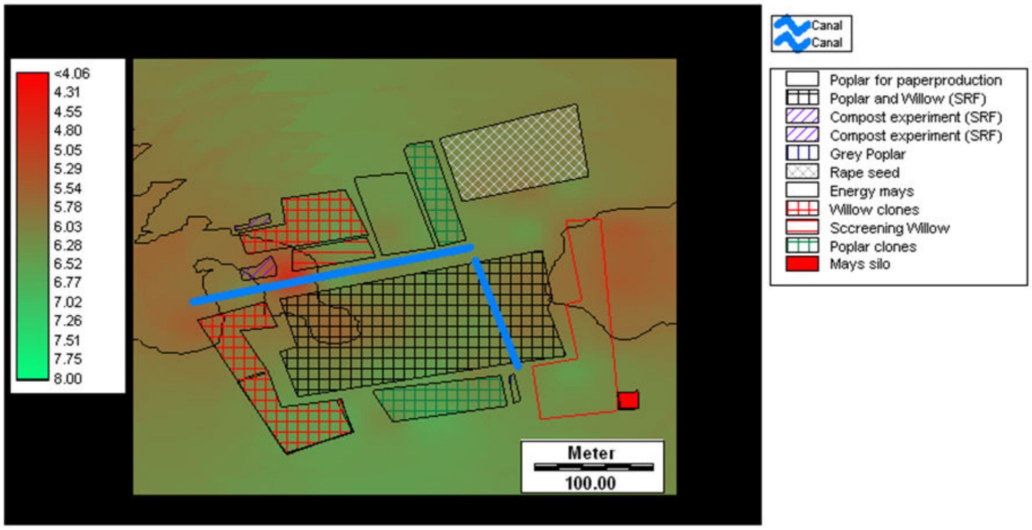

Figure 1.4. Distribution of pH-H_2O before liming (February 2006) activities taken in soil layer 0-25 cm (mapping performed with IDRISI 32, version 132.2, n=47)

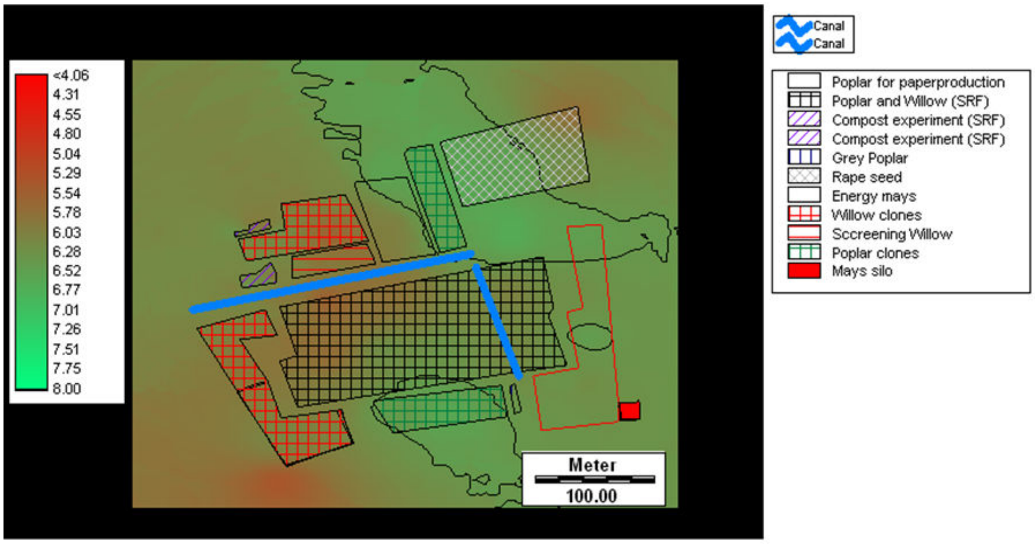

Figure 1.5. Distribution of pH-H_2O after liming (July 2006) activities taken in soil layer 0-25 cm (mapping performed with IDRISI 32, version 132.2, n=47)

Distribution of Cd in the soil

Atlhough Cd and Zn concentrations in the soil layer 0-5 cm where different, concentrations decreased with increasing soil depth. This corresponds with the fact that the contamination is a result of atmospheric deposition of metals and metalloids from the historical industrial activities of the smelting industry. As the experimental field was a former maize field, each year the field was ploughed and metals stayed therefore in the ploughlayer. This was also confirmed in other studies performed in the Campine region (Vangronsveld *et al.,* 1995a; Sonke *et al.,* 2002; Hogervorst *et al.,* 2007; OVAM, 2008) (Fig. 1.6).

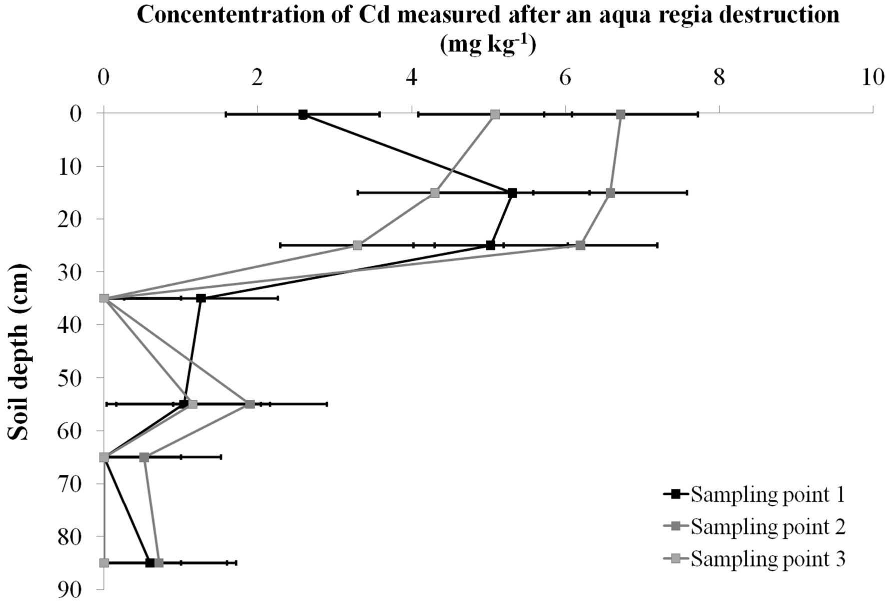

Figure 1.6. Concentration of Cd (mg kg^{-1}) measured in different soil depths at three different locations in the field (mean values, flags denote standard deviation, n=3)

1.4. Objectives and general outline

The main objective of the present work was to assess the feasibility of non-food crops not only as a phytoremediation option, but also as an alternative land use for metal contaminated agricultural soils in the Campine region. The thesis starts with a general overview (**Chapter 2**) of remediation options based on conventional sanitation, different phytoremediation strategies and physico-chemical stabilisation.

Chapter 3 reports the results of the use of rapeseed (*Brassica napus* L.) on metal contaminated soils. During this study, the growth and metal extraction potentials of rapeseed were observed under greenhouse and field conditions. Based on the field data, treatment possibilities and constraints for energy production were suggested. During the research period, the growth and metal uptake of willows (*Salix*) under short rotation coppice were observed. These results are reported and discussed in **Chapter 4**. In this chapter, also aspects as rotation period, impact of leaf fall and treatment possibilities are addressed.

In **Chapter 5**, the growth and metal uptake of different energy maize (*Zea mays* L.) varieties are discussed. Treatment option of maize is not handled in the literature. Although the experiments were performed under suboptimal conditions, feasibility parameters where positive and therefore energy maize was further investigated in **Chapter 6**. Under optimal field conditions, growth and metal uptake was re-investigated and compared with other field trials using the same maize varieties in the Campine region and in Flanders on non-contaminated sites. The biogas production potential of energy maize grown on a metal contaminated soil was investigated using biogas batch reactor tests and was compared to the biogas production potential of maize from a non-contaminated site. Based on the field data an economic feasibility study was performed for a ‘model’ farmer that wants to convert its land use from conventional maize cultivation into a phytoremediation cropping system using maize for anaerobic digestion purposes.

The possibilities for anaerobic digestion of energy maize, from contaminated agricultural sites were investigated in further detail in **Chapter 7,** along with term semi-continuous test of 435 days. This experiment also aims to investigate the fate of the metals during the digestion process, and discusses the quality of the produced digestate. Optimisation of using energy maize as a phytoremediation crop was further elaborated in **Chapter 8**. In this chapter the impact of fertilisation and harvest time on growth, metal uptake and extraction and biogas production potential are evaluated.

CHAPTER 2:

RISK BASED MANAGEMENT OF METAL CONTAMINATED SOIL

2.1. Soil remediation techniques

2.1.1. Conventional remediation

In response to a growing need to address environmental contamination, many remediation technologies have been developed to treat contaminated soil, mainly mechanically or physico-chemically based (Riser-Roberts, 1998, cited from Marques *et al*., 2009). The most commonly used techniques are listed in Table 2.1.

Table 2.1. Technologies for remediation of heavy metal contaminated soil (table based on Marques *et al.*, 2009, using Hamby, 1996; Khan *et al.*, 2004; Mulligan *et al.*, 2001; Ottosen and Jensen, 2005)

Remediation strategy	How it works
Soil washing	During this process coarse soil (sand and gravel) are separated from fine soil (silt and clay), where contaminants tend to bind and sorbs. This last soil fraction must be further treated with other technologies.
Soil vapor extraction	During this remediation process wells are installed in the area of contamination. Afterwards vacuum is applied through the wells in order to evaporate the volatile constituents of the contaminated mass, which are subsequently withdrawn through an extraction well. Subsequently, the extracted vapours are adequately treated.
Soil flushing	Soil flushing is accomplished by passing an extraction fluid through soils using an injection or infiltration process. Recovered fluids with the absorbed contaminants may need further treatment.
Solidification	Encapsulates the waste materials in a monolithic solid of high structural integrity.
Vitrification	Uses a powerful source of energy to “melt” soil at extremely high temperatures (1600–2000 °C). By this most inorganics are immobilised into a chemically inert, stable glass product and organic pollutants are destroyed.
Electrokinetics	Removes contaminants from soil by application of an electric field.
Thermal desorption	During thermal desorption the contaminated soil is excavated, screened, and heated to such temperatures that the boiling point of the contaminants is reached, and they are released from the soil. The vaporised contaminants are often collected and treated by other means.
Encapsulation	Physical isolation and containment of the contaminated material. The impacted soils are isolated by low permeability caps or walls to limit the infiltration of precipitation.
Stabilisation/ immobilisation	A stabilisation reduces the risk posed by a waste by converting the contaminant into a less soluble, immobile, and toxic form.

Conventional technologies involve the removal of metals from polluted soils by excavation, followed by treatment, and subsequently replacing the soil at its original location or disposing of it as hazardous waste (Francis *et al.*, 1999). This decontamination strategy is an *ex situ* approach which can be very expensive and can damage the soil structure and ecology (Salt *et al.*, 1995). Also *in situ* remediation techniques (*e.g.* solidification and encapsulation) are available. Although various remediation techniques are available for contaminated soils, relatively few are applicable to soils contaminated with trace elements (Vangronsveld *et al.*, 2009). Furthermore, conventional remediation techniques require high energy input and expensive machinery. In addition, they tend to destroy soil structure (Schnoor, 1997, cited from Jadia and Fulekar, 2009; Marques *et al.*, 2009).

2.1.2. Phytoremediation

Phytoremediation involves a variety of techniques and strategies that lead to contaminant degradation, removal (through accumulation or dissipation), transfer or immobilisation (Mench *et al.,* 2010). It makes use of the naturally occurring processes by which plants and their microbial rhizosphere organisms sequester, degrade or immobilise pollutants for cleaning soils or water bodies contaminated with metals or organic pollutants (Pilon-Smits, 2005). The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source. It takes advantage of the fact that a living plant can be considered as a solar-driven pump, which can extract and concentrate particular elements from the environment (Raskin *et al.*, 1997). As for conventional treatments, phytoremediation also has advantages and constraints that are a direct result of the biological aspect of this type of treatment system (Singh *et al.*, 2003).

The main advantage of phytoremediation is that it is on average a ten-fold cheaper than engineering-based remediation methods, such as soil excavation, soil washing or burning, or pump-and-treat systems (Glass, 1999, cited from Marques *et al.*, 2009). This type of soil remediation takes place at much lower cost than the conventional methods (Kumar *et al.*, 1995; Rulkens *et al.*, 1998; Weyens *et al.*, 2009a,b). Furthermore it requires minimal site disturbance and maintenance, reduces exposure of the polluted substrate to humans, wildlife and environment, resulting in a low cost and a high public acceptance. Phytoremediation is a technique that is best suited for the remediation of slightly to moderately polluted areas. It turns out to be a valuable alternative, especially for the treatment of large contaminated areas with diffuse pollution (Pilon- Smits, 2005; Mench *et al.*, 2009; Weyens *et al.*, 2009a,b). Phytoremediation overcomes shortcomings of physical and chemical remediation methods such as the destruction of soil structure, secondary pollution, and high costs (Mench *et al.*, 2009).

However, the use of phytoremediation has also some disadvantages. Phytoremediation is limited by the climatic, environmental conditions of the site to be cleaned, such as temperature, altitude, soil type, and the accessibility for agricultural equipment (Schmoger *et al.*, 2000). Large-scale applications of phytoremediation still faces a number of obstacles, including the levels of contaminants (being toxic for the organisms involved in remediation), the bioavailable fraction of the contaminants (being too low), depth of the remediation (limited to the depth of the rooting system) and, in some cases, evapotranspiration of volatile organic pollutants from soil or groundwater to the atmosphere (Pilon-Smits, 2005; Weyens *et al.*, 2009a,b). Another disadvantage is that (as any biological approach) this technology is not capable for full decontamination because it is limited to the plant available fraction of the trace elements (Vangronsveld *et al.*, 2009).

It must also be noticed that high metal concentration in the standing crop may pose a risk for herbivores (Vandecasteele *et al.*, 2002, Van Nevel *et al.*, 2007). Therfore studies determining the risk for toxicfaction for various mammal and bird species must be made. Hereby also secondary poisoning through the plant-herbivoreepredator pathways should be taken into account and are critical for a correct assessment of the risks posed by the standing crops with elevated concentration of metals (Van Nevel *et al.*, 2007).

2.2. Phytoremediation strategies

Various phytoremediation strategies are possible for the remediation of metal contaminated soils, using different plant properties (Salt *et al.*, 1998; Pilon-Smits, 2005) (Figure 2.1). In the following sections, the various strategies are discussed in further detail.

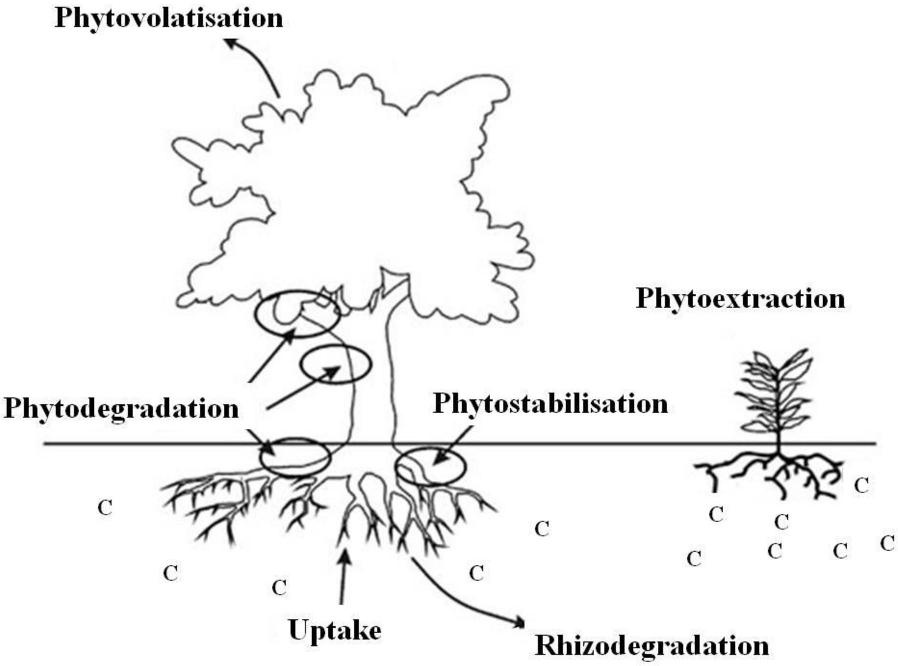

Figure 2.1. Main types of phytoremediation, together with an indication of where the technique take place, C representing a contaminant compound (adapted from Pilon-Smits, 2005)

2.2.1. Rhizo- and phytodegradation

Degradation refers to the breakdown of the contaminant into smaller constituents, or its transformation to a metabolite. This degradation can happen in the rhizosphere by microbial organisms, as well as within the plant itself. Phytodegradation occurs when a plant has taken up the contaminant into its tissues, and enzymes within the plant transform the compound, often into molecules that can be more readily broken down or released in root exudates. If degradation or transformation occurs in the rhizosphere by microbial organisms, the technique is referred to as rhizodegradation or phytostimulation (Arthur *et al.*, 2005).

2.2.2. Phytostabilisation

Phytostabilisation is defined as a technique where plants are used to reduce the mobility and bioavailability of environmental pollutants. It uses plants and associated microbes for long-term containment of contaminants in solid matrices through mechanical and (bio)chemical stabilisation (Figure 2.2). The development of a well-developed vegetation cover (i) strongly reduces horizontal and vertical spreading of the contaminants, (ii) reduces risks of percolation to groundwater and/or (iii) prevents or minimises the incorporation of the contaminant into the food chain by protecting us against direct inhalation or ingestion of contaminated particulates (Vangronsveld and Cunningham, 1998; Vangronsveld *et al.*, 1995a; Mench *et al.*, 2007, cited from Mench *et al.*, 2010; Mendez and Maier 2008; Vangronsveld *et al.*, 2009).

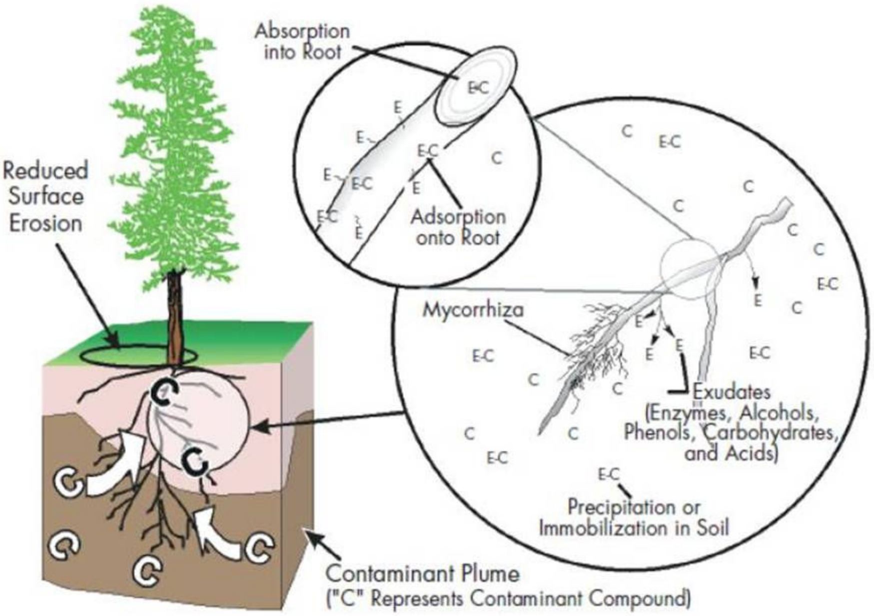

Figure 2.2. Phytostabilisation of contaminated soil, C representing a contaminant compound and E exudates (enzymes, alcohols, carbohydrates, acids) (adapted from Yaapar *et al.*, 2008*)*

Establishing of a vegetative cover may be relevant when topsoil decontamination by (phyto)extraction is not possible due to constraints of phytotoxicity, timescale, when plant, element uptake may provide an exposure risk to livestock (Conesa *et al.*, 2007) and when no alternative treatment is available (Mench *et al.,* 2010).

Phytostabilisation can be adapted to a variety of sites and situations, with different conditions (*e.g.* soil pH, salinity, soil texture, metal levels, and contaminant types) through careful selection not only of the appropriate plant species but also of the applied amendments (Berti and Cunningham, 2000, cited from Marques *et al.*, 2009). It is particularly suited for sites where the land value is small compared to the cost of soil excavation and landfilling (Robinson and Anderson, 2007, cited from Robinson *et al.*, 2009; Marques *et al.*, 2009). It is not a technology for designated clean-up of contaminated soils, but a management strategy for stabilising (inactivating) contaminants that are potentially toxic (Vangronsveld and Cunningham 1998; Vangronsveld *et al.*, 1995a; Mench *et al.*, 2007, cited from Mench *et al.*, 2010; Mendez and Maier, 2008). Monitoring of the contaminants must be part of any successful management scheme when phytostabilisation is used as a remediation tool or as a temporary measurement awaiting a more definitive remediation (Glick, 2003; Renella *et al.* 2008; Mench *et al.,* 2010; Vangronsveld *et al.*, 2009).

Phytostabilisation requires that the site be permanently vegetated, thus limiting future land use options. Nevertheless, periodical harvest of the biomass can result in the production of sources for bio energy purposes. The timing and nature of the harvest is critical, since it will necessarily decrease evapotranspiration from the site and as such potentially increase contaminant leaching. If the contaminant is translocated into the above ground biomass and this biomass is removed, also gradual reduction of the contaminant in the soil is achieved (Robinson *et al.*, 2007). However, when the emphasis lies on effective metal removal through harvest, the technique is referred to as phytoextraction.

2.2.3. Phytovolatilisation

If the contaminant is taken up by the roots, translocated to the leaves and volatised through stomata, where gas exchange occurs, the phytoremediation technique is called phytovolatilisation (Vroblesky *et al*., 1999). The chemical conversion of toxic elements into less toxic and volatile compounds is a possible strategy for detoxification of metal ion contaminants, resulting in the removal of specific harmful volatile elements (*e.g.* mercury (Hg) and selenium (Se)) from soil and plant foliage to the atmosphere (Raskin *et al*., 1997; Zayed *et al*., 2000).

Phytovolatilisation has the advantage that the vegetation does not require regular harvesting. The crop can be left on-site until the soil's element concentrations comply with environmental regulations (Robinson *et al*., 2006). Nevertheless, this technique can only be adapted to elements which can be transformed into a volatile component. Also it must be taken into account that, if a toxic volatile compound is emitted by plants, the fate of the gas in the atmosphere should be determined as a part of the risk assessment (Pilon-Smits, 2005). As in our study Cd and Zn are investigated, phytovolatilisation is not possible and will not be further discussed.

2.2.4. Phytoextraction

Phytoextraction is based on the use of plants that accumulate trace elements and organics into the harvestable parts (Kumar *et al*., 1995; Salt *et al*., 1998). When the produced plant biomass is removed from the environment a gradual remediation of the soil is achieved (Figure 2.3). It is suggested that plants used for phytoextraction should have following characteristics: (i) Plants should have a profuse root system; (ii) exhibit a rapid growth rate; (iii) have the potential to produce a high biomass in the field; (iv) accumulate high levels of the metal in the harvestable parts; and (v) be easily harvestable (Vangronsveld *et al*., 2009).

2.3. Phytoextraction of metal contaminated soils

During the history of phytoremediation different approaches have been conceptualised: (i) the use of natural hyperaccumulator plants with exceptional metal-accumulating capacities; (ii) induced or chemically assisted phytoextraction. This latter is achieved by introducing soil amendments (*e.g.* chelators or acidifying amendments) in order to increase trace element mobility in the soil. (iii) A final approach is the utilization of fast growing plants by which the high biomass can compensate the low concentration in the plant parts and therefore create similar extraction rates as those of hyperaccumulating plants (Meers *et al*., 2005a; Salt *et al*., 1998; Vangronsveld *et al*., 2009).

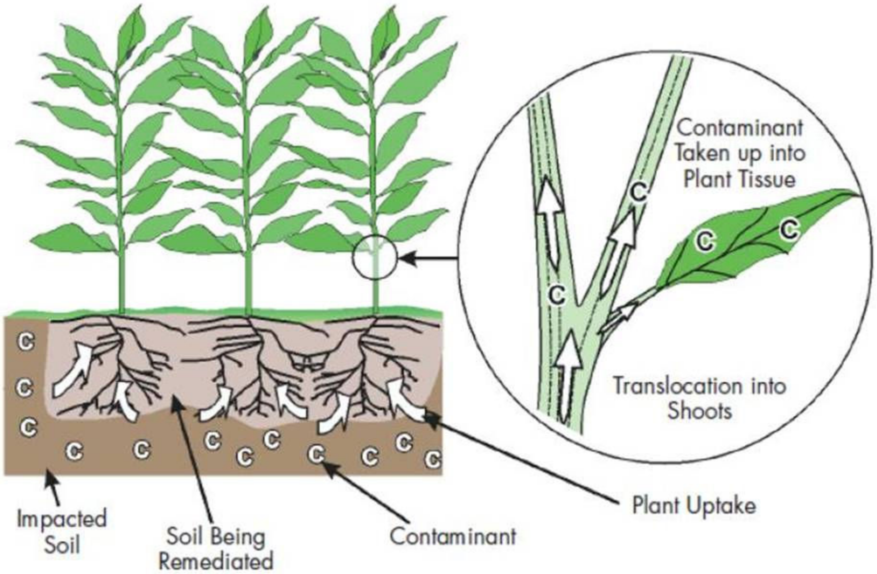

Figure 2.3. Phytoextraction of contaminated soil, C representing a contaminant compound (adapted from Yaapar *et al*., 2008)

2.3.1. Hyperaccumulators

Hyperaccumulating plants

Initially, only plants with natural metal accumulating characteristics and hyperaccumulating plants were explored (Chaney, 1983, cited from Marques *et al*., 2009; Garbisu and Alkorta, 2001; Vassilev *et al*., 2002). Hyperaccumulator plants are plants that accumulate contaminants to concentrations 10 to 100 times those found in ‘normal’ plants (Brooks *et al*., 1977, cited from Marques *et al*., 2009). According to Reeves and Baker (2000), the term *hyperaccumulator*, describing a plant with a highly abnormal level of metal accumulation, appears to have been first applied by Jaffré *et al*. (1976), who reported high Ni concentrations in *Sebertia acuminate* (Marques *et al*., 2009). An attempt to give greater precision to the definition of hyperaccumulation was made by Reeves (1992) for Ni: A hyperaccumulator of Ni is a plant in which a Ni concentration of at least 1 000 mg kg^{-1} (or pro mille) has been recorded in the dry matter of any aboveground tissue in at least one specimen growing in its natural habitat (Marques *et al*., 2009). This definition was then used for defining hyperaccumulation of other elements than Ni, including 10 000 mg kg^{-1} dry weight of shoots for Zn and Mn, 1 000 mg kg^{-1} for Co, Cu, Ni, As and Se, and 100 mg kg^{-1} for Cd (McGrath and Zhao, 2003). The current definition of a hyperaccumulator is more precise: the concentration of the metal in the shoot must be higher than: 1.0% for Zn and Mn, 0.1% for Al, As, Se, Ni, Co, Cr, Cu, and Pb, and 0.01% for Cd. In addition, the shoot to root concentrations ratio must be invariably higher than 1 (Marques *et al*., 2009).

The use of hyperaccumulating plants was mostly studied with *Thlaspi caerulescens* (McGrath *et al.* 1993, cited from Vangronsveld *et al*., 2009; Garbisu and Alkorta, 2001; Vassilev *et al*., 2002). The major mechanism in tolerant plant species appears to be the compartmentalisation of metal ions (*i.e.* sequestration in the vacuolar compartment or cell walls), which excludes them from cellular sites where processes such as cell division and respiration occur, thus providing an effective protective mechanism (Chaney *et al*., 1997). The use of hyperaccumulators has some major drawbacks: they are mostly specialised in the accumulation of only one specific element, while many polluted soils contain more than one contaminant. Also, most hyperaccumulator species have a limited ecological range and are therefore not easy to grow on other soils. In addition, there is a lack of technology for their large-scale cultivation and in understanding biological and environmental factors involved in metal hyperaccumulation. A further disadvantage is the generally low biomass production.

Ebbs *et al.* (1997) concluded that, although *T. caerulescens* showed ten times higher Cd concentrations, its corresponding biomass production was also ten times less as compared to other crops used in that study.

Phytomining and biofortification

The low biomass production can become less important when the elements that are extracted are very valuable. In such cases, the phytoextraction technique is referred to as phytomining. Phytomining of Ni was proven to be economically feasible in the USA (Chaney *et al.*, 2007). Also in Europe (Albania), successful field experiments using a Ni hyperaccumulator *(Alyssum murale*) have been reported (Bani *et al.*, 2007). However, for elements such as Zn, phytomining is not economically feasible as the Zn price at the world market is actually too low to make "zinc-recycling" cost-effective (Robinson *et al.*, 2009). Biofortification on the other hand aims at increasing the concentration of essential elements (*e.g.* Ca, Cu, Fe, Mg, I, Se, Zn) in crops in order to improve human health and agricultural productivity. The most commonly deficient elements in the diet of humans are Fe and Zn (Branca and Ferrari, 2002; White and Broadley, 2005).

2.3.2. The use of biomass crops for phytoextraction

General

Besides the use of hyperaccumulating plants, research has also been reporting the use of fast-growing crops such as maize (*Zea mays* L.), ryegrass (*Lolium* spp.) and triticale (×*Triticale* spp.*)* for phytoextraction. Although plant concentrations are much lower than those of hyperaccumulators, total metal removal could be equal than those of hyperaccumulating plants because of their high biomass production (Vassilev *et al.* 2002; Meers *et al.*, 2005b; Hernández-Allica *et al.*, 2008).

Research on other potential phytoremediation crops, started by evaluating plant species within the *Brassicaceae* family (Vamerelli *et al.*, 2010). The use of *Brassica* was not only tested under greenhouse condition, but also under field conditions. Rapeseed (*Brassica napus* L.) cultivated in the mid-North of Europe proved to be moderately resistant when on a multi-metal contaminated soil. It produces higher biomasses compared to metal (hyper)accumulators from the same family and good accumulation levels in shoots. Although data showed a degree of variability in the plant genotype with respect to metal uptake potential, rapeseed is seen as an appropriate candidate for phytoextraction (Grispen *et al*., 2006; Van Ginniken *et al*., 2007; Hernández-Allica *et al*., 2008).

Herbaceous or woody biomass species may be promising in view of their ability to produce high biomass which can compensate for the low concentrations of contaminants in their tissues (Vamerelli *et al*., 2010). When the contamination that needs to be remediated is deeper than 20–30 cm, the choice of *Salix* or *Populus* will have the advantage (Laureysenss *et al*., 2003, 2004a,b, 2005a,b; Marques *et al*., 2009; Vangronsveld *et al*., 2009). *Salix* species are not hyperaccumulators, but it was shown that among different clones there are high accumulators of Cd and Zn together with a great tolerance to Cd, Zn, and Cu (*e.g.* Landberg and Greger, 1994, 1996; Vervaeke *et al*., 2003; Meers *et al*., 2005). The potentials of willows on different types on abandoned farmland (Ledin, 1996); brownfields (Dickinson and Pulford, 2005); land with application of sewage sludge or wood ash (Dimitriou and Arronson, 2005); dredged sediments (Vervaeke *et al*., 2003;Vandecasteele *et al*., 2005; Meers *et al*., 2005), landfills (Ettala, 1988; Nixon *et al*., 2001), soils contaminated with radionuclides (Vandenhove *et al*., 2001) but also on marginally contaminated arable soils (Klang-Westin and Eriksson 2003; Dickinson and Pulford, 2005; Meers *et al*., 2007d; Ruttens *et al*., 2011).

Maize has also been tested for their potential for phytoextraction of Cd and other metals such as Ni, Cu, Pb and Zn (Meers *et al*., 2007d; Wu *et al*., 2007; Murakami and Ae, 2009). As maize is characterized as a metal excluder, it is often studied in association with the use of chelators, application of sulphur, mycorrhizae, bacteria and co-planting with hyperaccumulators (Vamerelli *et al*., 2010), which will be addressed later (2.3.3).

Besides these crops also the use of sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum* L.), cereals, grasses, hemp (*Cannabis sativa* L.), flax (*Linium usitatissimum* L.), showed promising results (Angelova *et al*., 2004; Meers *et al*., 2005b; Nehnevajova *et al*., 2007; Vangronsveld *et al*., 2009; Vamerelli *et al*., 2010).

Determining the extraction potential

The potential of a certain plant species for metal extraction is often examined using the bioconcentration (BCF) and translocation factors (TF). The bioconcentration factor is defined as the metal concentration ratio in the plant to that in the soil, while the translocation factor is defined as the metal concentration ratio of shoot to root. Plants with a BCF factor higher than 1 are considered as accumulators, while metal excluder species have a BCF lower than 1 (Baker 1981, cited from Zhao *et al*., 2003; McGrath and Zhao, 2003; Zhao *et al*., 2003). On metal contaminated agricultural sandy soil rapeseed shows a BCF factor varying between 0.6–1.5 for Cd and 0.87–3.1 for Zn. willows on the other hand showed bioconcentration factors of 4.8 for twigs (Grispen *et al*., 2006; Ruttens *et al*., 2011). Maize on the other hand is clearly an excluder as it has a BCF of 0.6 (Meers *et al*., 2010).

Nevertheless, these parameters are only reflecting plant metal uptake and translocation to the aerial plant parts and therefore it is better to discuss the total amount of metals that is being removed from the soil in relation to the total amount of metals present in the soil (Mertens *et al*., 2005, 2006), together with the time needed for remediation (Van Nevel *et al*., 2007). The time needed for phytoextraction can be estimated using the following formula (Equation 2.1) (Robinson *et al*., 2006):

$$t = \frac{M_i(x)_{max} - M_f}{P_M \cdot B_M} \qquad \text{(Eq. 2.1.)}$$

With (i) t the time in years; (ii) x the spatial position (latitude, longitude) ; (iii) $M_i(x)_{max}$ the maximum initial metal burden (mg ha^{-1}) in the affected area; (iv) M_f the target metal burden (mg ha^{-1}); (v) P_M the average metal concentration in the plant (mg kg^{-1} DM); and (vi) B_M the biomass production of the plant (kg^{-1} DM ha^{-1} $year^{-1}$). The concentration (P_M) is a function of the available metal concentration in the soil. However, this formula is an estimate. When metals are removed from the soil by successive cropping, the plant available metal concentration decreases. Although in some situations, the change may be proximately linear, in most cases it is logarithmical (Robinson *et al*., 1999; Van Nevel *et al*., 2007). Also the plant biomass itself will change over time. As metal concentration in the soil decreases, response of the plant will change by reduced metal stress. Besides metals, nutrients will be removed from the soil and if no fertilisation, pest control or crop rotation is applied, productivity levels may decrease. Furthermore, this calculation is based on a single metal contamination, while in practice multi-contaminated soils will be subjected for phytoextraction (Van Nevel *et al*., 2007; Robinson *et al*., 2009). Nevertheless, this calculation can be used as an estimate for comparing predicted remediation times between different crops.

Treatment of harvested biomass

When evaluating the potential of a crop for phytoextraction, the treatment possibilities/constraints of the harvested biomass needs to be evaluated. Hereby, energy crops are showing great potential (Schwitzguébel *et al*., 2002; Gosh and Singh, 2005; Schröder *et al*., 2008; Mench *et al.,* 2010). When phytoextraction is combined with proper treatment of the biomass, the whole process can even be turned into a profit-making operation (Robinson *et al*., 2009), the decontamination time becomes less important (Van Nevel *et al*., 2007; Robinson *et al*., 2009; Meers *et al*., 2010). As for decontamination time, studying the treatment of the harvested biomass after the phytoextraction process has also been a cause of concern (Ernst, 2005). Such metal-rich and potentially hazardous biomass requires appropriate management, storage or disposal so that it does not pose an environmental risk (Ghosh and Singh, 2005). The crops can be used in different ways, each having advantages and disadvantages (Sas-Nowosielska *et al*., 2008). The possible treatment options include dumping, composting, compaction, incineration, gasification, pyrolysis, direct disposal, liquid extraction, fibre, package and paper, livestock bedding, wood chips for soil amendment, oil production, thermal-oxidation (solvolysis) and production of syn-gas for green chemistry, and synthetic biofuel and bioethanol (Kumar *et al*., 1995; Bridgewater *et al*., 1999; Vangronsveld *et al*., 2009; Mench *et al.,* 2010).

Economic feasibility

The replacement of a traditional crop into a phytoremediation crop is only viable if it is a financially attractive long term option with no side effects (Thewys, 2008). This economic feasibility is important to upscale the result from lab-scale into a long term strategy of managing metal contaminated soils (Vassilev *et al*., 2004). These economic studies can be done by making a cost-benefit analysis, but also by incorporating the alternatives, and environmental benefits (*e.g.* Witters *et al*., 2009; Thewys *et al*., 2010a,b).

2.3.3. Assisted phytoextraction

The extraction potential can be enhanced by changing the availability for plant uptake. This can be achieved by means of adding chemicals. To understand how plant uptake can enhanced, the uptake under normal conditions will be discussed.

Plant uptake

Plants extract and accumulate elements from the soil solution. The uptake of elements takes place at the first few millimetres of the root tips. Two different uptake routes have been reported: (i) passive uptake, where ions are moved through the porous cellwalls. This uptake is driven by the electric potential gradient across the plasma membrane or through ion channels; (ii) a second pathway consists of an active process by which ions move through the symplast. This transport is substrate-specific and energy dependent and is driven by membrane proteins with transport functions (Williams *et al*., 2000; Blaylock and Huang, 2000, cited from Vangronsveld *et al*., 2009; Pilon-smits, 2005).

Metals are present in the soil under various form/species: (i) free metal ions; (ii) soluble metal compounds in the soil solution; (iii) exchangeable ions sorbed onto inorganic solid phase surfaces; (iv) precipitated as insoluble inorganic metal compounds (carbonates, phosphates, etc.); (v) metals complexed by soluble or insoluble organic material; and (vi) metals bound in silicate minerals (Dube *et al*., 2001). Metals that are considered as available for plant uptake are those that exist as soluble components in the soil solution or are easily desorbed or solubilised by root exudates or other components of the soil solution. Often this portion only represents a small part of the total metal content of the soil (Blaylock and Huang, 2000, cited from Vangronsveld *et al*., 2009).

The presence of plant available metals depends on various soil physico-chemical factors, such as the degree of complexation with soluble ligands, environmental conditions, redox conditions, the type and density of the charge on soil colloids, the reactive surface area, cation exchange capacity, biological activity and the soil pH (Harter, 1983, cited from Marques *et al*., 1999; Norwell, 1984, cited from Marques *et al*., 2009; Taiz and Zaiger, 2002).

Besides soil factors, the plant itself can enhance the availability of elements in the soil. Plants can release metal chelators in the rhizosphere. The complexed metal form is then transported into the plant through a transport protein specific for that metal. Plants can also extrude H^+ via ATPases, which replace cations at soil CEC (cation exchange capacity)-sites, making metal cations more bioavailable. Also, by altering the pH an elecotrochemical gradient is formed, which facilitates this transport. The availability can also be enhanced by bacterial chelators such as siderophores, organic acids, and phenolics, which can release metal cations from soil particles and make them more available for plant uptake (Von Wiren *et al*., 1996; Taiz and Zeiger, 2002).

Chemically assisted phytoextraction

Besides the production of chelators produced and released by the plant material, one can also increase the availability by adding chemical agents to the soil and is referred to as 'chelant-assisted' or 'chelant-induced' phytoextraction (Nowack *et al*., 2006; Meers *et al*., 2008). These chelants form complexes with free ions in the soil, therefore increasing their solubility and stimulating the plant uptake. This technique is not only used for various metals such as Cd, Zn and Ni, but also for pollutants which are not easily available, such as Pb (Blaylock *et al*., 1997; Saifullah *et al*., 2009; Vangronsveld *et al*., 2009). Enhanced uptake can be achieved by adding both inorganic and organic agents to the soil, although the latter appears to be more effective in increasing the solubility of metals (Schmidt, 2003; Quartacci *et al*., 2005, 2006; Meers *et al*., 2008; Saifullah *et al*., 2009; Tack and Meers, 2010; Vamerelli *et al*., 2010).

Although many reports clearly show the applicability of chelators under lab-scale conditions, the technique has many disadvantages. A major drawback of this technique is the fact that as the solubility increases, also risk of leaching increases. Furthermore, great attention must be paid to the application time. Several publications claim that plants must have reached their full size before adding mobilising agents. In addition, studies reported that the plant metal uptake and/or biomass production may decrease after chelator additions to the soil and thus reduce phytoextraction performance. Phytotoxicity symptoms have been reported when mobilising agents are applied (Meers *et al*., 2008; Neugschwandtner *et al*., 2008; Marques *et al*., 2009; Mench *et al.,* 2010).

Among various synthetic chelators, ethylenediamine tetraacetate (EDTA) has been tested most intensively. It was shown that it not only improves the translocation from the soil to the roots but even facilitates the transport to the shoot (Blaylock *et al*., 1997; Lombi *et al*., 2001; Meers *et al*., 2008). However, EDTA is poorly biodegradable (Nörtemann, 1999) and therefore increases potential risks for leaching (Wenzel *et al*., 2003).

An alternative to EDTA, is the ethylenediamine disuccinate (EDDS) (Meers *et al*. 2004). For some metals it shows a solubilisation effect greater than that of EDTA at equimolar concentrations and pH. In contrast to EDTA, no toxic effects of EDDS on soil biota were found and EDDS is readily biodegradable in the soil (Vandevivere *et al*., 2001; Wenzel *et al*., 2003; Tandy *et al*., 2004). Besides EDTA and EDDS, other substances that have also been reported in literature, including Nitrilotriacetic acid (NTA), diethylene triamine pentaacetic acid, nitriloacetate, … (Wenger *et al*., 2003; Quartacci *et al*., 2005; Meers *et al*., 2008; Tack and Meers, 2010).

Also inorganics can be used for enhancing the plant available fraction. It has been shown that the addition of chloride selectively enhances the uptake of Cd and Pb but not of Cu (Lee and Chon, 2001, cited from Meers *et al*., 2008; Meers *et al*., 2003). By applying elemental sulphur or physiologically acid fertilisers, such as ammonium sulphate, a slight acidification of the soil is induced which could increase the availability for plant uptake (Kayser *et al*., 2000; Puschenreiter *et al*., 2001). The feasibility of all the different types of chelating agents can be found in Meers *et al.* (2008) and Tack and Meers (2010) for further reading.

Microbial assisted phytoextraction

Besides chemical chelators, the efficiency of phytoextraction can be improved by manipulation of the plant associated community. In this approach the associated bacteria of a plant are isolated, modified to exhibit desired characteristics and re-inoculated in the soil (Lebeau *et al*., 2008; Weyens *et al*., 2009a; Vangronsveld *et al*., 2009).

The modification exists of equipping the bacteria with (i) a metabolic pathway for the synthesis of natural chelators in order to improve metal availability for plant uptake and translocation or (ii) with a metal sequestration system to reduce phytotoxicity (Lodewyckx *et al*., 2001; Valls and de Lorenzo, 2002) (Figure 2.4).

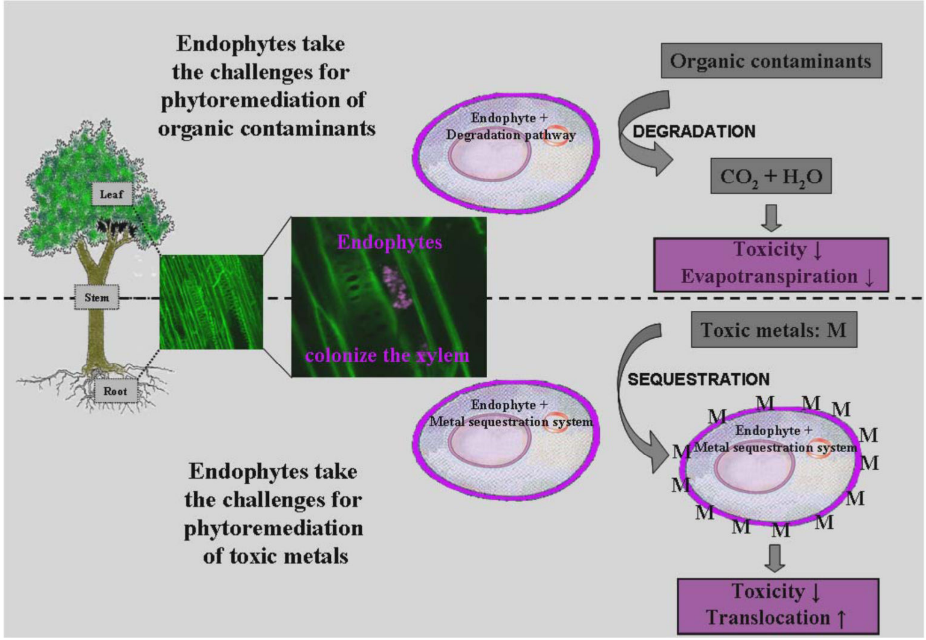

Figure 2.4. Different strategies of endophytes for improving phytoremediation of organic contaminants and toxic metals, M represents toxic metals (Weyens *et al*., 2009a)

Microbially assisted phytoextraction can be achieved by the use of endophytic bacteria, which are defined as bacteria colonising the internal tissues of plants without causing symptoms of infection or negative effects on their host (Schulz and Boyle, 2006, cited from Weyens *et al*., 2009b). In comparison with rhizosphere and phyllosphere bacteria, endophytic bacteria are likely to interact more closely with their host. In these very close plant-endophyte interactions, plants provide nutrients and residency for bacteria, which in exchange can directly or indirectly improve plant growth and health (Mastretta *et al*., 2009).

Direct plant growth promoting mechanisms may involve production of plant growth regulators. An indirect benefit can be achieved by bacteria preventing growth or activity of plant pathogens (Weyens *et al.*, 2009a,b). Plant growth-promoting bacteria stimulate root formation by plants and the formation of siderophores. Bacteria mainly facilitate the growth of plants in the presence of high levels of metals (Lebeau *et al*., 2008; Weyens *et al*. 2009b; Glick, 2010). Also, bacterial support of root growth-promoting ectomycorrhizal fungi, and more specific, arbuscular mycorrhizal fungi, have been shown to enhance plant tolerance to biotic and abiotic stresses, including the presence of high levels of heavy metals (Leyval *et al*., 2002, cited from Marques *et al*., 2009; Vangronsveld *et al*., 2009; Zimmer *et al*., 2009).

The use of non-genetically modified breeding track (such as mutagenesis) can also be used for enhancing the extraction potential. This can be an alternative to the use of genetically modified organisms (GMO), which is currently proclaiming itself as an enhancement phytoextraction strategy (Vangronsveld *et al*., 2009; Glick, 2010; Mench *et al.,* 2010). This usage remains however controversial: (i) currently, there is no convincing GMO for such a purpose, since several genes are required to significantly improve plant performance (rather than a single gene expression); (ii) the ecotoxicological/ecological consequences of using GMO's are far from being satisfactory understood; and (iii) the answer of using GMO depends on public perception and acceptance. More detailed information about the achievements and drawbacks in GMOs in view of trace element phytoextraction is provided by Krämer and Chardonnens (2001), and Mejare and Bülow (2001) (Vangronsveld *et al*., 2009; Glick, 2010; Mench *et al.,* 2010).

2.4. Stabilisation of metals using amendments

Another risk based soil remediation strategy, which is not phytoremediation related, is the application of single or combined amendments. Adding these amendments in the soil, reduces the availability of the pollutants for plant uptake and thus limits eventual toxicity to plants, allowing revegetation of contaminated sites (Vangronsveld *et al.*, 1995a,b, 1996). By incorporating these amendments into the soil, the labile contaminant pool and phytotoxicity is decreased (Mench *et al.*, 2006). Ideally, soil amendments should be easy and safe to handle and apply, non-toxic to the plants, easy to produce, and inexpensive. Soil amendments that have little to no economic value are preferred to more expensive materials (Berti and Cunningham, 2000, cited from Marques *et al.*, 2009; Marques *et al.*, 2007).

Both successes and failures have been reported on the use of stabilising soil amendments. A thorough evaluation of the overall effect of ameliorants and the developing ecosystem and the sustainability (durability) of trace element immobilisation in contaminated soils is crucial for the acceptance of inactivation/stabilisation strategies (Vangronsveld *et al.*, 2009). Hereby, a comprehensive study of biological, physico-chemical evaluation methods, eco-toxicological methods on short and long term should be applied.

Many types or amendments have shown their capacity to reduce the metal availability in soils. Various studies (*e.g.* Clemente *et al.*, 2006; Madejón *et al.*, 2006; Marques *et al.*, 2007) have shown that the soil application of organic matter amendments, such as manure or compost, to metal contaminated soil led to a significant reduction in the amount of Zn leached through the soil. In combination with plants, the reduction in metal percolation increased up to 80%. In fact, organic matter amendments are amongst the most promising additives, especially due to their low commercial cost and consequently added value of their application for soil remediation purposes. Their application provides organic matter which improves soil physical properties, water infiltration, and water-holding capacity. They also contain essential nutrients for plant growth (Marques *et al.*, 2009). Also other amendments such as lime, phosphate, cyclonic ashes, beringite, steel shots, red mud (also known as bauxite residue) and clays have their potential as amendment that influence plant available metals (Chen *et al.*, 2000; Gebelen *et al.*, 2003; Adriano *et al.*, 2004; Gray *et al.*, 2006; Madejón *et al.*, 2006; Ruttens *et al.*, 2006, 2010). In particular, liming has been considered as an important management tool in reducing the toxicity of metals in soils (Gray *et al.*, 2006; Madejón *et al.*, 2006).

CHAPTER 3:

FEASIBILITY OF GROWING RAPESEED (*BRASSICA NAPUS* L.) ON METAL CONTAMINATED AGRICULTURAL SOILS

Redrafted from Van Slycken S., Croes S., Guisson R., Witters N., Meers E., Adriaensen K., Ruttens A., Vangronsveld J., De Jonghe W., Thewys T. and Tack F.M.G. (2011d). Feasibility of growing *Brassica napus* L. on metal contaminated agricultural soils. International Journal for Phytoremediation. Submitted for publication.

Abstract

Phytoextraction is a technology that exploits the natural properties of plants and their associated microorganisms to remove toxic trace elements from contaminated soils. Plants with a high biomass production and high element accumulation capabilities are most suited. In this study, rapeseed (*Brassica napus* L.) was investigated as a potential plant to remove cadmium (Cd) and zinc (Zn) from contaminated soils in the Campine region (Belgium). Plant growth evaluated in a pot experiment under different contamination levels revealed no significant differences between treatments and with a non-contaminated site. In a subsequent field experiment with pseudo-total concentration of 5.0±1.2 mg Cd and 261±56 mg Zn kg^{-1} dry soil, total shoot biomass reached 5.8±2.0 ton ha^{-1}. Seed yield was low (0.5±0.3 ton DM ha^{-1}), mainly due to suboptimal fertilisation of nitrogen and sulphur. Extraction rates (27±14 g Cd ha^{-1} and 2.0±1.0 kg Zn ha^{-1}) were higher than those of plant species with a metal excluder strategy. Nevertheless, calculated remediation periods are long and field trials were not always successful.

3.1. Introduction

The largest challenge in phytoremediation is finding the right plant for a specific type of contamination (Herrero *et al.*, 2003). It is suggested that, besides accumulating good amounts of metals into their above ground biomass, plants should tolerate relatively high levels of metals in the soil, while maintaining rapid growth rates and reaching a high biomass in the field (Alkorta *et al.*, 2004; Sappin-Didier *et al.*, 2005; Hernández-Allica *et al.*, 2008).

Taking these criteria into account, rapeseed (*Brassica napus* L.) was suggested as a suitable candidate for phytoextraction (Grispen *et al.*, 2006; Ben-Ghnaya *et al.*, 2007). Also in the context of energy crops, rapeseed is very promising. Rapeseed is worldwide used as an oil producing crop which can be adopted easily in crop rotation farming systems. In Europe it has been cultivated since the 14th century. Today, rapeseed oil is mainly used in food applications, but to a growing extent also in biofuel production. Phytoextraction using *B. napus* might have the potential to become a profitable activity when combined with commercialisation of the produced oil, especially when considering the increasing oil prices during the recent years. Belonging to the family of the *Brassicaceae*, rapeseed was reported to be capable of accumulating relatively elevated metal concentrations in its aerial plant parts (Ebbs *et al.*, 1997). Also on multi-contaminated soil, rapeseed is moderately tolerant (Marchiol *et al.*, 2004; Angelova *et al.*, 2008; Purakayastha *et al.*, 2008). Because of this tolerance, rapeseed is often used in research studies on enhancing metal uptake by using chelates (*e.g.* Wenzel *et al.*, 2003; Cui *et al.*, 2004; Zaier *et al.*, 2010) or bacteria (*e.g.* Farwell *et al.*, 2007; Dell' Amico *et al.*, 2008; Sheng *et al.*, 2008). Although Meers *et al.* (2005b) concluded that the total extraction potential of rapeseed was low, Grispen *et al.* (2006) found that phytoextraction using rapeseed could become a profitable practice and consequently a sustainable alternative non-food crop for farmers dealing with metal contaminated agricultural soils.

The main objective of this chapter is to evaluate the growth and phytoextraction potential of rapeseed. Plant growth and Cd and Zn uptake by rapeseed were evaluated in a pot experiment with different contamination levels of Cd and Zn. Subsequently, biomass production, metal uptake and extraction potential were evaluated during a field experiment.

3.2. Material and Methods

3.2.1. Experimental set-up

Pot experiment

A first research on the site specific phytoremediation potential of rapeseed, was performed during a pot experiment with two different contamination levels (Table 3.1). In the experimental site of this research (Cfr. 1.3) a gradient in soil contamination was found (Ruttens *et al*., 2008). Based on these data, two locations with a different contaminated level (referred to as soil A and soil B) were selected. At each location, 10 kg of soil was collected from the top soil layer (0–25 cm), similar to samplings performed by Meers *et al.* (2006, 2007b). Subsequently, the samples were air-dried and sieved through through a 1 mm sieve. In order to study the impact of metal contamination on growth, a non-contaminated soil (referred to as soil R) was also used in the experimental setup. This soil is a sandy loam soil and is classified as arable farming land (Meers *et al*., 2006).

Pots with a diameter of 14 cm and a total volume of 1.5 L were used. To avoid soil loss, a root canvas and filter paper (white ribbon, MN 640 m, Macherey-Nagel, Düren, Germany) were placed at the bottom of each pot. Eighteen pots were filled with 1 kg of soil (six pots for each soil). To optimise the nutritional state, pots were fertilised a few days before the experiment started, with 200 mL of a modified 100% Hoagland-solution, containing 0.0018 mg L^{-1} $MnCl_2.4H_2O$, 0.0029 mg L^{-1} H_3BO_3, 0.230 mg L^{-1} $NH_4H_2PO_4$, 0.490 mg L^{-1} $MgSO_4.7H_2O$, 0.0498 mg L^{-1} $FeSO_4.7H_2O$, 0.708 mg L^{-1} $Ca(NO_3)_2.4H_2O$ and 1.02 mg L^{-1} KNO_3 (Hewitt, 1966). Plants were grown in a greenhouse under a lightning with a constant tubular lightning (OSRAM, L58W/640) between 6 am and 6pm (Figure 3.1).

Figure 3.1. Overview of the greenhouse experiment using rapeseeds (photo's: Van Slycken, S.)

In each pot, 20 seeds of one cultivar, rap1 of the field experiment, were randomly sown and after five days, thinned to 15 plants per pot. In order to maintain a moisture content of 20% (which corresponds with 60% of the field capacity), plants were watered every two days with distilled water. At the end of the experiment (7 weeks), shoots were collected, fresh biomass was determined, washed with distilled water and oven-dried at 50 °C (Dieser Memmert-Schrank, Germany). After oven drying, the different plant parts were weighted again. The shoot biomass was pulverised using a Culatti DCFH 48 grinder for analysing metal concentrations.

Field experiment

To evaluate the phytoremediation potential of rapeseed under field conditions, a field of 2 200 m^2 was subdivided into eight subplots of 45 m by 6 m. The initial soil pH-H_2O varied widely within the test site and was rather low (4.8–6.6). Before sowing, the field was limed and ploughed for homogenization (final soil pH varied between 6.1 and 8.1) and fertilised with approximately 50 kg nitrogen ha^{-1} using liquid pig manure. Pesticide and herbicide control were also in accordance to common agricultural practices. Four commercially available *Brassica napus* varieties (Remy, PR45D01, Robust, PR46W31)given use by KWS and Pioneer and reffered to as rap1-rap4, were sown on September 10, 2006 with a density of 60 seeds m^{-2}, making sure that each cultivar was sown on two randomly chosen subplots. The cultivars were selected for their capacity to adapt to the climatic conditions in Flanders. All cultivars were listed as a 00 variety (CCAPS, 2009).

In June 2007, before harvest, five plant and soil samples were taken in each plot. Each sample consisted of all the plant material in a sampled surface area of 1 m^2. In each plant sampling area, six soil samples were taken with an auger (auger for arable land, ø 13 mm, operation length 25 cm, Eijkelkamp, Giesbeek, the Netherlands) and mixed for obtaining a bulked sample. Soil samples from both experiments were dried at 50 °C in an oven (EU 170, Jouan s.a., Saint Herblain, France) and sieved to pass a 1 mm sieve.

3.2.2. Soil Analysis

For determination of the soil pH-H_2O, 10 g of air-dried soil was allowed to equilibrate for 24 h in 50 mL of deionised water, after it was stirred regularly in the beginning with a glass rod. To determine pH-KCl, 50 mL of 1 M KCl was added to 10 g of air-dried soil and allowed to equilibrate for 10 min (Van Ranst *et al.*, 1999). The pH of the supernatant was measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA), calibrated with pH 4.0 and 7.0 standards. The Cation Exchange Capacity (CEC) of the sediment was determined by first saturating the soil matrix with NH4$^+$, then desorbing the NH4$^+$ by K$^+$ and measuring the quantity of the NH4$^+$ in the leachate (Van Ranst *et al.*, 1999). Organic matter was determined using the method described by Walkley and Black (Allison, 1965).

Pseudo-total soil contents of Cd and Zn were determined after *aqua regia* digestion (Van Ranst *et al.*, 1999). Certified reference sediments BCR141R and BCR142R were also analyzed in triplicate using the same method. Results varied from 93 to 102% of the certified *aqua regia* extractable values. Meers *et al.* (2007b) observed a good correlation between shoot accumulation of Cd and Zn and their measured concentration in a soil $CaCl_2$ extraction. $CaCl_2$ extractions were performed by allowing 10 g air-dry soil to equilibrate in 50 mL 0.01 M $CaCl_2$ for 2 h, filtering it over a white ribbon filter (MN 640 m, Macherey-Nagerl, Düren, Germany). For quality control, standards were re-analyzed at the end of each batch of analyses. Results were only accepted when the measured standard concentrations were within 95 to 105% of the certified value (*i.e.* taking into account possible artifacts due to the dilution of certified stock solutions). Analysis of Cd and Zn in both extractions were performed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). For each soil sample the corresponding legal threshold limits (mg kg^{-1}) for remediation of agricultural soils were calculated according to the Flemish regulations (VLAREBO, 2009, as explained in 1.3.2).

Pseudo-total Na, K, Ca and Mg content were measured on the *aqua regia* digested samples The nutritive status of P, Na, K, Ca en Mg was estimated using a NH_4-OAc-EDTA extraction. Extractions were carried out by allowing 20 g air-dry soil to equilibrate in 100 ml 1 M NH_4-OAc-EDTA for 30 min and filtering it over a white ribbon filter (white ribbon, MN 640 m, Macherey-Nagel, Düren, Germany). Na, K, Ca and Mg concentration in both extraction were measured using ICP-OES, while P was determined using the method of Scheel (Van Ranst *et al.*, 1999).

3.2.3. Plant analysis

All fresh shoots (shoot$_{total}$) were firstly weighted, divided into shoot$_{bottom}$ (shoot part until 25 cm height), shoot$_{rest}$ (rest of the shoot) and seeds, weighted, washed with distilled water, and oven-dried at 50 °C (Dieser Memmert-Schrank, Germany). After oven drying, the different plant parts were weighted again, grounded using a Culatti DCFH 48 grinder and sieved through a 1 mm sieve for metal analysis.

Ground plant samples were ashed at 450 °C. The ash was dissolved in nitric acid before element analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA) and inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, PerkinElmer, Waltham, MA, USA). Certified reference material of rye grass (BCR281) and spinach (SRM Spinach 1570a) was analyzed in triplicate using the same method. Results varied from 93 to 102% of the certified values.

For each cultivar the bioconcentration factor (BCF) was calculated. This factor is defined as the metal concentration ratio in the plant to that in the soil, while the translocation factor is defined as the metal concentration ratio of shoot to root. These two factors reflect plant metal uptake, and metal translocation to the aerial plant parts under the specific soil conditions. Plants with a BCF higher than 1 are considered as accumulators, while metal excluder species have a BCF lower than 1 (Baker 1981, cited from Zhao *et al*., 2003; McGrath and Zhao 2003; Zhao *et al*., 2003).

3.2.4. Statistical analysis

Statistic analyses were performed using SPSS 11.0 (SPSS Inc.) and Excel (Miscrosoft Inc.) software packages. For statistical analysis normality was checked using a Kolmogorov-Smirnov test. Significance of observed differences between the results of the soil analysis was assessed by using an ANOVA (significance level α=0.05) combined with a Tukey HSD test (when homoscedasticity was found) or with a Dunnet C test (when no homoscedasticity was found). As the Cd and Zn concentration in the *aqua regia*-destruction were different between the plots of the different cultivars, significance of differences between growth, metal concentration and metal uptake were investigated using ANCOVA.

3.3. Results

3.3.1. General soil properties

According to the USDA triangle, soils were characterised as ‘Sand’, whereas the reference soil of the pot experiment is classified as ‘sandy loam’ (Table 3.1). From the NH_4OAc-EDTA (Table 3.2), and the CEC (Table 3.1 and 3.2), a high nutritive status can be attributed to these soils (Van Ranst *et al.*, 1999). Nutritive status of the reference soil (soil R) is very similar to that of the contaminated soils from the pot and field experiment.

Table 3.1. General soil properties of the non-contaminated and contaminated soils of the pot experiment and the general soil properties of the experimental field, OM = organic matter, CEC = cation exchange capacity (mean ± standard deviation, n=6)

	pH-H_2O	**pH-KCl**	**OM (%)**	**CEC mol(+) kg^{-1}**	**Clay/Silt/Sand (%)**
Soil R	7.0±0.1	5.6±0.1	3.8±0.1*	7.4	9/24/67*
Soil A	6.2±0.1	5.1±0.1	3.4±0.2	5.7	4/8/77**
Soil B	7.1±0.1	6.4±0.1	5.0±0.1	7.6	4/8/77**
Field	6.5±0.3	5.9±0.3	5.0±0.1	7.6	4/8/77**

* = Parameters described by Meers *et al.*, 2006
** = Parameters described by Meers *et al.*, 2010

Table 3.2. Total concentration and nutritive state of Ca, Mg, Na and K (mg kg^{-1}) using a NH_4OAc-EDTA extraction of soils used in the pot experiment and those measured in the experimental field (mean ± standard deviation, different letters represent different significance level at α=0.05 of the different plots, n=5 for the pot experiment and n=10 for the field experiment)

	Ca_{tot}	**Ca_{nutr}**	**Mg_{tot}**	**Mg_{nutr}**	**Na_{tot}**	**Na_{nutr}**	**K_{tot}**	**K_{nutr}**
Soil R	1435±23a	872±25a	511±6a	84±3a	87±1a	83±1a	511±2a	502±4a
Soil A	949±40b	774±11b	225±8b	26±1b	81±4a	11±1b	354±19b	62±1b
Soil B	2288±45c	1941±36c	268±39	45±1c	188±171ab	19±1b	346±1b	63±1b
Field	1716±31d	1223±259	186±25	31±3b	30±8b	11±5b	291±49b	55±13b

Table 3.3. Cd and Zn concentrations, measured by *aqua regia* destruction and $CaCl_2$-extraction (mg kg^{-1}) of the soils used in the pot. 'Thresholds' are legal threshold limits (mg kg^{-1}) for remediation of agricultural soils according to the Flemish regulations (VLAREBO, 2009) (mean ± standard deviation, different letters represent different significance level at α=0.05 of the different plots, n=5)

		Soil R	Soil A	Soil B	Field
Cd	***aqua regia***	0.60±0.60a	3.5±0.1b	7.2±0.1c	5.0±1.2d
	$CaCl_2$	<DL	0.42±0.02a	0.23±0.01b	0.28±0.09b
	Threshold	2.5	2.1±0.1a	3.5±0.1b	2.9±0.4a
Zn	***aqua regia***	31±2a	244±5b	417±32c	261±56b
	$CaCl_2$	0.56	29±1a	8.1±0.2b	17±7b
	Threshold	271	226±11a	397±6b	299±34c

Table 3.4. Cd and Zn concentrations, measured by *aqua regia* destruction and $CaCl_2$-extraction (mg kg^{-1}) of the soils from the field experiment. 'Thresholds' are legal threshold limits (mg kg^{-1}) for remediation of agricultural soils according to the Flemish regulations (VLAREBO, 2009) (mean ± standard deviation, different letters represent different significance level at α=0.05 of the different plots, n=10)

		Field experiment			
		rap1	**rap2**	**rap3**	**rap4**
Cd	***aqua regia***	6.2±1.6a	4.9±1.0b	4.6±0.7b	4.4±0.7b
	$CaCl_2$	0.34±0.05a	0.24±0.04b	0.31±0.12b	0.23±0.07b
	Threshold	2.8±0.3a	3.2±0.2a	2.7±0.4a	3.0±0.4a
Zn	***aqua regia***	318±64a	252±45b	246±43b	237±36b
	$CaCl_2$	16±4a	9.9±3.0b	16±10b	9.6±3.9b
	Threshold	326±41a	286±26a	285±25a	302±33a

The pseudo-total Cd and Zn concentration was higher in soil B compared to soil A (Table 3.3.). The metal concentrations collected from the field plot (Table 3.4.) were higher than those measured in soil A and lower than those measured in soil. The total and available Cd and Zn concentrations in the soil on which rap1 were sown were significantly higher in comparison to the concentrations measured for the other plots. Total Cd and Zn concentrations in soil A and total Cd and Zn concentrations in soil B were above the legal threshold values for soil remediation with an agricultural land use (VLAREBO, 2009). All soil samples used during the field experiment contained Cd concentrations higher than the legal threshold value for Cd and was set at 5.0 (Table 3.3).

3.3.2. Pot experiment

The fresh biomasses of the shoots differed significantly between all contamination levels (Table 3.5). Plants grown in soil A had significantly lower root fresh weights in comparison to plants grown in soils B and R. When the shoot and root dry weights were considered, no significant differences were found between the three different soils.

Table 3.5. Fresh (FW) and dry weight (DW) of the shoots of 15 plants grown for 51 days on a non-contaminated soil (Soil R) and two metal contaminated soils (Soil A and B) and concentration of Cd and Zn in the shoots (mean ± standard deviation, different letters represent different significance level at α=0.05, n=5)

	Productivity (g pot^{-1})		**Concentration (mg kg^{-1} DW)**	
	FW	**DW**	**Cd**	**Zn**
Soil R	2.1±0.1a	0.17±0.0.3a	0.35±0.02a	69±7a
Soil A	1.2±0.1b	0.14±0.02a	6.0±0.4b	781±42b
Soil B	1.5±0.1c	0.16±0.02a	8.1±1.4c	430±35c

Total Cd and Zn shoot concentration differed significantly between the three different soils (Table 3.5). Total Cd concentrations in the shoots correlated with the total concentrations in the soil (Pearson correlation coefficient 0.73, significant at the 0.05 level (2-tailed)). Plants grown in the reference soil showed the lowest Cd, while plants grown in soil B accumulated the highest amounts of Cd. The highest Zn concentration was observed in plants grown on soil A. The shoot metal concentrations differed significantly between soils A and B.

Plants grown in soil A had a BCF for Cd and Zn of respectively 1.7±0.1 and 3.2±0.1, plants grown in soil B respectively 1.1±0.2 and 1.0±0.1. Although the total Cd and Zn concentrations were higher in soil B, the bioconcentration factors of plants grown in soil A were higher, due to the higher metal availability in soil A.

3.3.3. Field experiment

The total aboveground dry biomass varied between 4.7±1.8 (rap3) and 7.0±2.5 (rap4) ton DM ha^{-1} and did not differ significantly between cultivars (Figure 3.2). The average dry biomass productivity in the field augmented to 5.8±2.0 ton DM ha^{-1}. Similar to the total aboveground biomass, rap1 showed the highest seed productivity (0.61±0.44 ton DM ha^{-1}), and rap2 the lowest (0.48±0.25 ton DM ha^{-1}), although it was grown on a plot with higher Cd and Zn concentrations (Table 3.4). Nevertheless no significant differences could be found, and was on average 0.53±0.21 ton DM ha^{-1}. For the purpose of obtaining maximal metal extraction potential, the entire shoots were harvested, while in practice plants are cut about 20 cm above ground level. If only the shoot dry biomass above 25 cm is taken into account (shoot$_{rest}$), a dry biomass productivity of 3.7±1.7 ton DM ha^{-1} was obtained.

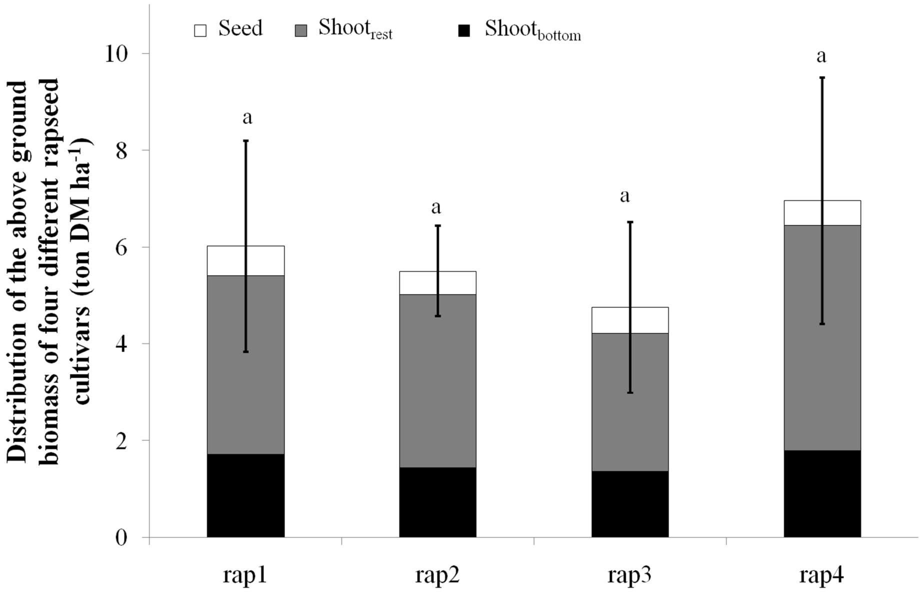

Figure 3.2. Dry weights (ton DM ha^{-1}) of the different aerial plant parts of the four investigated rapeseed varieties (rap1-rap4) on the field (mean values, flags denote the standard deviation of the total dry weight, n=10)

The concentrations of Cd and Zn (mg kg^{-1} DM) in the different plant parts are presented in Table 3.6. Metal concentrations were highest in the lowest part of the shoots (shoot$_{bottom}$) and lowest in the seeds. Cultivar rap1 accumulated significantly more Cd and Zn in the different plant parts in comparison to the other cultivars. As metal concentrations in the lower shoot parts were higher than those of the upper shoot parts, the bioconcentration factors were also higher for the lowest shoot parts (Table 3.7). However, the bioconcentration factors of the whole shoots were not significantly different between cultivars. No significant differences were found between the bioconcentration factors calculated from the data of the field and the pot experiment.

Table 3.6. Concentration of Cd and Zn (mg kg^{-1}dry matter) in the above ground plant parts (shoot$_{bottom}$, shoot$_{rest}$, shoot$_{total}$, seed) of the four rapeseed cultivars in the field experiment (mean ± standard deviation, different letters represent different significance level at α=0.05 of the different plots, n=5 for the pot experiment and n=10 for the field experiment)

		rap1	rap2	rap3	rap4
Shoot $_{bottom}$	**Cd**	8.0±1.7a	5.3±0.5b	5.4±1.7b	6.1±1.4b
	Zn	496±117a	367±74b	384±110ab	411±98ab
Shoot $_{rest}$	**Cd**	6.2±1.8a	4.4±0.7ab	3.7±1.2b	5.2±1.6ab
	Zn	436±113ab	343±53ab	292±75c	437±113b
Shoot$_{total}$	**Cd**	6.3±1.1a	4.7±0.6b	4.2±0.9b	4.8±1.3ab
	Zn	427±77a	350±57a	324±85a	379±103a
Seed	**Cd**	1.3±0.4a	0.54±0.12b	0.74±0.18b	0.65±0.21b
	Zn	97±13a	81±6c	67±5b	80±7c

Table 3.7. Bioconcentration factor of the different above ground plant parts for Cd and Zn content (shoot$_{bottom}$, shoot$_{rest}$, shoot$_{total}$, seed) of the four rapeseed cultivars in the field experiment (mean ± standard deviation, different letters represent different significance level at α=0.05 of the different plots, n=10)

		rap1	rap2	rap3	rap4
Shoot$_{bottom}$	**Cd**	1.4±0.5a	1.1±0.3a	1.2±0.2a	1.4±0.5a
	Zn	1.6±0.6a	1.5±0.5a	1.6±0.4a	1.8±0.7a
Shoot$_{rest}$	**Cd**	1.0±0.4ab	1.0±0.3ab	0.78±0.20a	1.2±0.5b
	Zn	1.4±0.5a	1.4±0.4a	1.2±0.3a	2.0±0.7a
Shoot$_{total}$	**Cd**	1.1±0.4a	1.0±0.2a	0.88±0.16a	1.2±0.4a
	Zn	1.5±0.5a	1.4±0.4a	1.3±0.3a	1.8±0.7a
Seed	**Cd**	0.22±0.08a	0.12±0.04b	0.16±0.04ab	0.15±0.07ab
	Zn	0.31±0.09ab	0.33±0.07ab	0.28±0.05a	0.35±0.09b

The extraction potential of rapeseed is crucial in order to achieve an effective phytoextraction. The Cd and Zn extraction potentials of rapeseed, based on the current field experiment, are represented in Figure 3.3 and Figure 3.4.

Significant differences were found in aboveground Cd and Zn accumulation between cultivars. Cultivar rap1 accumulated significantly more Cd in the base of the shoot (shoot$_{bottom}$) than cultivars rap2 and rap3. Cultivar rap4 accumulated significantly more Cd and Zn in the above shoot part (shoot$_{rest}$) than cultivar rap3. Also the whole shoot (shoot$_{total}$), without the seeds, of cultivar rap1 accumulated significantly more Cd than cultivar rap3. Based on the experimental setup a total removal by the above ground biomass of 27±14 g Cd ha^{-1} year^{-1} and 2.0±1.0 kg Zn ha^{-1} year^{-1} could be obtained. The extraction potential of the seeds was low, due to low bioconcentration factors and low yields. The extraction potential of the seeds represents only 1% for Cd and Zn of the total aboveground extraction potential of rapeseed. If a normal seed yield could be achieved, the extraction potential would be augmented by 5-10%. The lowest part of the shoot represented 25-35% of the total extraction potential. Therefore, it is advisable to harvest the whole plant when phytoextraction is considered.

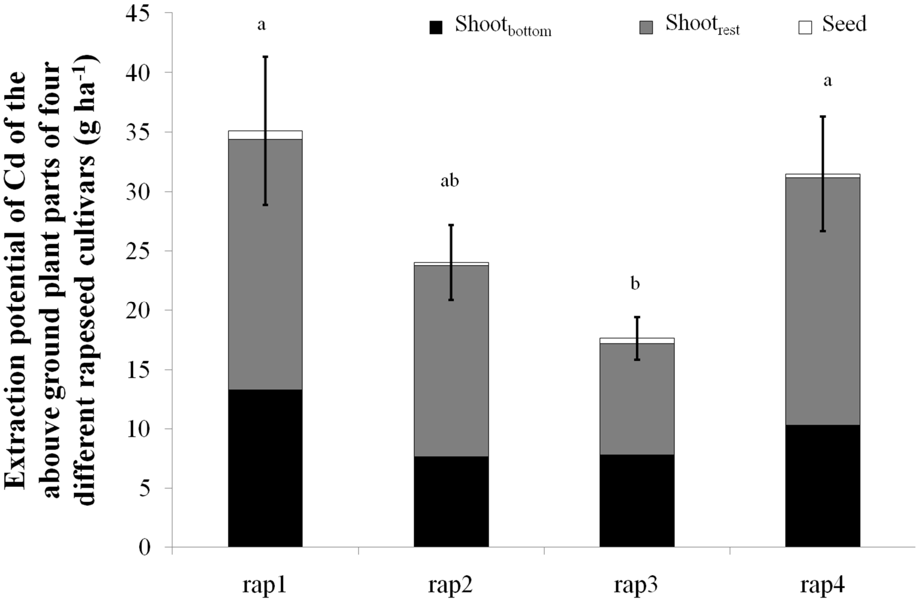

Figure 3.3. Extraction potentials of Cd (g ha^{-1} year^{-1}) of the different aerial plant parts of the four investigated rapeseed varieties (rap1-rap4) (mean values, flags denote standard deviation of the total extraction potential, different letters represent different significance level at α=0.05, n=10)

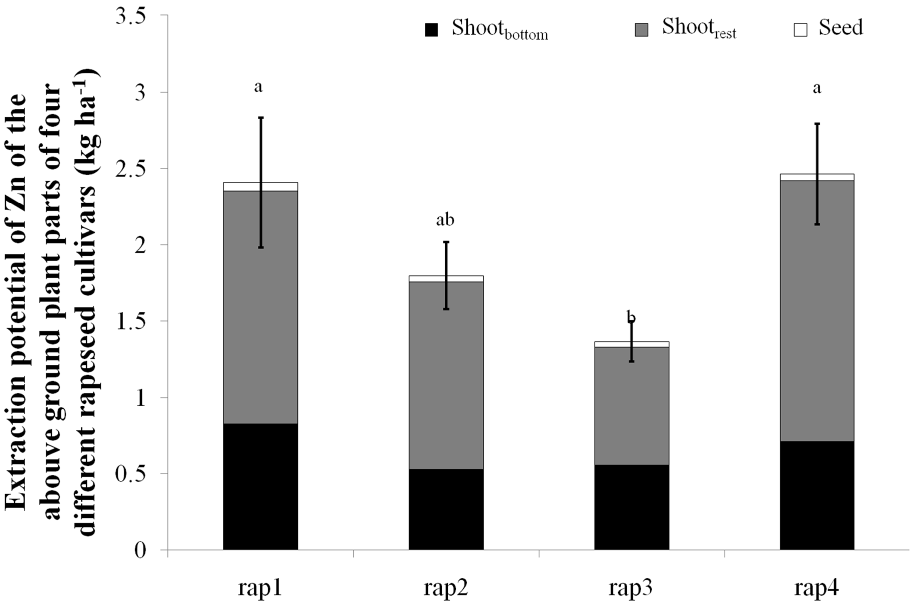

Figure 3.4. Extraction potentials of Zn (kg ha^{-1} year^{-1}) of the different aerial plant parts of the four investigated rapeseed varieties (rap1-rap4) (mean values, flags denote the standard deviation of the total extraction potential, n=10)

3.4. Discussion

A challenge for phytoextraction studies is to find the right plant for a specific contaminated site. Although similar extraction potentials can be obtained between plant species, a choice between plants with trace element hyperaccumulation or high biomass productivity must be made, taking the tolerance of the plants against the pollutants into account. Furthermore, it is essential to test this under the specific soil conditions (Herrero *et al.*, 2003; Hernández-Allica *et al.*, 2008). Pot experiments can be adopted to estimate the plant phytoextraction potential on a specific soil, because metal concentrations in seedlings can be used to estimate metal concentrations in mature plants (Lehn and Bopp, 1987). By incorporating different contamination levels and a non-contaminated soil, conclusions can be made concerning plant tolerance to the contamination level. Shoots, harvested from the pot experiment, showed a bioconcentration factor higher than 1, indicating that rapeseed has the capability to translocate and accumulate metal concentrations in its aerial plant parts. Nevertheless, a field experiment delivers more realistic information about biomass productivity and metal accumulation potential in the plants.

In the subsequent field experiment, shoot biomass production reached the expected values (Figure 3.2), while seed production was rather low in comparison to normal seed yields in Flanders (4-5 ton ha^{-1}, when a moisture content of 9% is considered) (García Cidad *et al.*, 2004, Lamont *et al.*, 2005). The used fertilisation regime probably contributed to the low biomass productivity. This is confirmed when the results are compared with data from a field experiment in a non-contaminated area, also in the Campine region (Geel) with varieties rap2 and rap4 where yields of 3 Mg seeds ha^{-1} where reported (Hooibeekhoeve, 2006).

Fertiliser application should be adapted to sowing date, soil type, application time (Diepenbrock, 2000; Barłóg and Grzebisz, 2004; Sharifi *et al.*, 2009), but also to climate conditions, mineral nitrogen concentrations, the organic sources of mineralized nitrogen (Ozer, 2003), *etc*. This nitrogen application was furthermore proposed, because rapeseed was sown on sandy soils in September. After winter a supplementary fertilisation is advised in order to fully stimulate the growth and flowering, inclusive seed production (van der Mheen, 2003). Therefore, it can be concluded that the fertilisation was efficient enough for plant growth during the first month after sowing. After the winter period, plants needed a second dose of N for further growth and maturation of the plant. Due to this, the seed yield could be doubled (Sharifi *et al.*, 2009). Furthermore van der Mheen (2003) and Lamont *et al.* (2005) recommend an additional 40 to 60 kg S ha^{-1} (by fertilisation with a sulphate containing nitrogen fertiliser). For rapeseed and for *Brassica* sp. in general, S improves the synthesis of proteins, oil content and glusinolates. The impact of S on seed quality is more significant than the impact of the load of N as it has been reported that insufficient doses of S inhibits the seed production (Fismes *et al.*, 2000; Malhi *et al.* 2007; Čeh *et al.* 2008; Sharifi *et al.*, 2009). The importance of fertilisation for rapeseed production in the Campine region was proven by great differences in rapeseed productivity with and without fertilisation. Rapeseed sown on fertilised non-contaminated soils had a seed production of 3 Mg ha^{-1}, whereas almost no seed yield was found in plots without fertilisation (Hooibeekhoeve, 2006). These findings could not be confirmed during our field experiment in 2007 and 2008, as growth of rapeseed failed.

When growing agricultural crops in polluted regions, the distribution of toxic metals over the different plant parts is a crucial issue (Angelova *et al.*, 2005). Results of our field experiment indicate that metal concentrations were highest in the lowest part of the shoot and lowest in the seeds (Table 3.6) and are similar to other studies of rapeseed in the Campine region (Grispen *et al.*, 2006). From a legal perspective, all total Cd concentration in soil samples were above the legal threshold values for soil remediation (VLAREBO, 2009) (Table 3.4).

Assuming a specific soil density of 1 400 g dm^{-3}, a plough layer depth of 25 cm (in which the contamination is quite homogeneously situated) and a linear extraction potential, more than 250 growing cycles of rapeseed would be needed to reduce total Cd concentrations in the top soil layer from 5.0 to 2.9 mg Cd kg^{-1} (which is the site-specific threshold value of Cd for remediation in the current experiment).

Moreover, one has to take into consideration that rapeseed must preferably be cultivated under a rotation scheme of three years with wheat/barley or even under a scheme of four years in combination with other crops. Studies showed that the yield of wheat can be 10% higher when it is sown after winter rapeseed, but also the yield of rapeseed itself increased using a rotation system. Sowing rapeseed after rapeseed can result in a high risk of crop failure, due to species specific infection diseases (van der Mheen, 2003; Lamont *et al.*, 2005). Therefore, remediation periods can be even longer. Estimations of this remediation period could not be made as no data on metal uptake and yield of those rotation crops under the specific conditions in the contaminated Campine region were available.

In the phytoremediation context it is necessary to foresee an appropriate treatment of the biomass, preventing any risks to the environment which could turn phytoremediation into a profit making operation (Sas-Nowosieleska *et al.*, 2004; Chaney *et al.*, 1997; Ghosh and Singh, 2005). Hereby the seed can be processed into bio-oil, in which also a press cake is obtained. This cake or meal is mostly used as fodder (Lamont *et al.*, 2005; Van Ginneken *et al.*; 2007). Therefore metal concentrations should be in accordance with the European standards for animal food (maximum 1 mg kg^{-1} fresh material for Cd for general feed stuffs, standard is represented when a moisture content of 12% is considered). Treatment of the cake in the non-food eliminates possible re-entrance of the metals in the food chain. There appears to be marginal effect of metals in the biomass, neither on the economic viability, nor on the net energy production over a whole life cycle (Witters *et al.*, 2011a,b). Besides the processing of the seed, the shoot material also should be utilized in a safe way. As concentrations in this material are exceeding the previously mentioned threshold values for food or fodder, alternative conversion techniques, such as (co)-digestion or combustion are preferable.

3.5. Conclusion

The explorative studies of rapeseed showed no significant differences in dry weight between different contaminated soils and a non-contaminated soil. The accumulation of metals into the seedlings revealed promising aspects towards the potentials cultivating rapeseed on metal contaminated agricultural soils. During the subsequent field experiment rapeseed reached a total shoot biomass of 5.8±2.0 ton DM ha^{-1}. However, the seed yield was low (0.5±0.3 ton DM ha^{-1}), mainly due to suboptimal fertilisation of nitrogen and sulphur. Extraction rates (27±14 g Cd ha^{-1} and 2.0±1.0 kg Zn ha^{-1}) were higher than those of plant species with a metal excluder strategy. Nevertheless, calculated remediation periods are long and field trials were not always successful.

CHAPTER 4:

FIELD EVALUATION OF WILLOWS (*SALIX*) UNDER SHORT ROTATION COPPICE FOR PHYTOREMEDIATION

Redrafted after Van Slycken, S., Meiresonne, L., Witters, N., Meers, E., Van Peteghem, P., Thewys, T., Vangronsveld, J. and Tack, F.M.G. (2011a). Field evaluation of willows (*Salix*) under short rotation coppice for phytomanagement of metal polluted agricultural soils. International Journal of Phytoremediation. Accepted for publication.

Abstract

Short rotation coppice (SRC) might be a promising phytoremediation crop since it uses fast growing, high biomass producing tree species with often a sufficient metal uptake. This study evaluates growth, metal uptake and extraction potentials of eight willow clones (Belders, Belgisch Rood, Christina, Inger, Jorr, Loden, Tora and Zwarte Driebast) on a metal contaminated agricultural soil, with a total Cd and Zn concentration of 6.5±0.8 and 377±69 mg kg^{-1} soil, respectively. Although, during the first cycle, on average generally low productivity levels (3.7 ton DM ha^{-1} year^{-1}) were obtained in this soil, certain clones exhibited more acceptable productivity levels (*e.g.* Zwarte Driebast 12.5 ton DM ha^{-1} year^{-1}). Even at low biomass productivity levels, SRC showed promising extraction potentials of 72 g Cd and 2.0 kg Zn ha^{-1} year^{-1}, which is much higher than for example energy maize or rapeseed. Cd and Zn removal can be increased by 40% if also leaves are harvested. However, at the moment, the price of wood remains the most critical factor in order to implement SRC as an acceptable, economically feasible alternative crop on metal contaminated agricultural soils.

4.1. Introduction

Over the past years cultivation of energy crops has gained increasing interest because of the environmental benefits they offer in terms of reduction of greenhouse gases as a carbon dioxide neutral source of energy. In addition, they form a potential source of income and employment to rural communities (Bell *et al.*, 2006). If biomass has to play a significant role in the world's energy future, the use of dedicated energy crops is essential. Short rotation coppice (SRC) is gaining popularity in Europe (Evans *et al.*, 2010). It consists of densely planted, high-yielding varieties of either willow (*Salix*) or poplar (*Populus*), harvested in 2–5 year cycles. After the harvest, new shoots spontaneously resprout in the following spring. The total production period can extend to 30 years, depending on the sprouting of the stools (Defra, 2004). The wood is used mainly as a biomass fuel, not only for industrial energy power plants, but also for heating application of homes or farms (Licht and Isebrandt, 2005). However, due to the increasing importance of energy crops, a competition of land use between biomass production for food, material and energy is emerging (Gold and Seuring, 2011). This conflict could be avoided by growing energy crops on marginal, poor, degraded or polluted soils (Weyens *et al.*, 2009b; Ruttens *et al.,* 2011).

Some willow species have the capacity to accumulate significant amounts of Cd in aboveground biomass compartments (Rosselli *et al*., 2003; Jenssen *et al*., 2009). In recent years, research has focused on planting willow on abandoned farmland (Ledin, 1996); brownfields (Dickinson and Pulford, 2005); land with application of sewage sludge or wood ash (Dimitriou and Arronson, 2005); dredged sediments (Vervaeke *et al*., 2003; Meers *et al*., 2005); landfills (Ettala, 1988; Nixon *et al*., 2001); soils contaminated with radionucleides (Vandenhove *et al*., 2001); and also marginally contaminated arable soils (Klang-Westin and Eriksson 2003; Dickinson and Pulford, 2005; Meers *et al.*, 2006).

The objectives of this chapter are the evaluation of (i) the biomass productivity at field scale on sandy, metal contaminated soils in the Campine region; (ii) metal extraction potentials; (iii) the optimal rotation cycle in this region; (iv) the various subsequent biomass usage possibilities and (v) the economic feasibility for farmers when they convert their metal contamined (*i.c*. Cd and Zn) land use to this phytoremediation system with short rotation coppice.

4.2. Material and Methods

4.2.1. Experimental setup

For the purpose of the current screening experiment, 2.100 m^2 was reserved and eight commercially available willow clones were selected. A selection of willow clones was made based on Swedish research and on the experience of other Belgian and Dutch breeding experiments and programs (Ruttens *et al*., 2008). The final selection consisted of 'Belders' (*S. alba* L. var. *alba*), 'Belgisch Rood' (*S.* x *rubens* var. *basfordiana*) (Zwaenepoel *et al.*, 2005), 'Christina' (*S. viminalis*), 'Inger' (*S. triandra x S. viminalis*), 'Jorr' (*S. viminalis*), 'Loden' (*S. dasyclados*), 'Tora' (*S. schwerinii* x *S. viminalis)* and 'Zwarte Driebast' (*S. triandra*). Belders, Belgisch Rood and Zwarte Driebast are representing three willow cultivars coming from the Netherlands (Zwaenepoel *et al.*, 2005), whereas Christina, Inger, Jorr, Loden and Tora are five commercial Swedish clones.

The plot was divided into 32 subplots of 28 x 23 m. Each clone was planted in quadruplicate and distributed over the 32 plots randomly. The design consisted of a double row design with alternating inter-row distances of 0.75 and 1.5 m, and a spacing of 0.6 m between cuttings within the rows ('Swedish design'), accommodating an overall planting density of 15 000 cuttings per hectare (Figure 4.1).

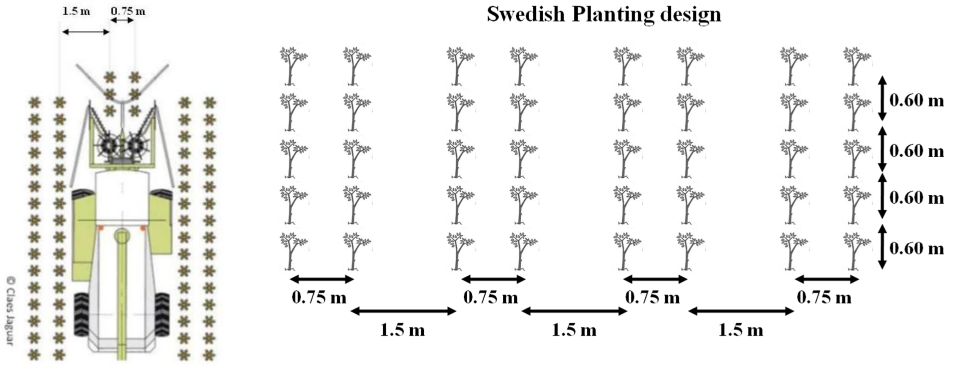

Figure 4.1. Plantation scheme of short rotation coppice using the Swedish design (adapted from Defra, 2004; De Somviele *et al.*, 2009)

For planting, 25 cm long dormant unrooted and untreated hardwood cuttings were chosen (Figure 4.2). In order to promote optimal growth, mechanical weeding was carried out in the inter-row in the first year of growth. On a yearly base, plant and soil samples were taken at the end of September in each repetition plot. In the first year six plants were harvested in the middle plantation row of each plot in order to have sufficient plant material for analysis. The middle row was chosen in order to avoid border effects. During the following years four plants were harvested similarly. Sampling was performed on places where no effect was observed of previous sampling. Spread over the plant sampling area, six soil samples were taken with an auger (auger for arable land, ø 13 mm, operation length 25 cm, Eijkelkamp, Giesbeek, the Netherlands) and mixed in order to obtain a bulked sample.

Figure 4.2. Planting of short rotation coppice on the experimental field (photo's: Ruttens, A., Meiresonne, L. and Van Slycken, S.)

4.2.2. Soil analysis

Soil samples were dried at 50 °C in an oven and sieved to pass a 1 mm sieve. To determine the pH-H_2O, 10 g of air-dried soil was allowed to equilibrate for 24 h in 50 mL of deionized water, after stirring regularly in the beginning with a glass rod. The pH of the supernatant was measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA) (Van Ranst *et al.*, 1999).

Pseudo-total soil content of Cd, Pb and Zn was determined after *aqua regia* digestion (Van Ranst *et al.*, 1999). Certified reference sediment CRM 277 was also analysed in triplicate using the same method. Results varied from 93 to 102% of the certified *aqua regia* extractable values.

$CaCl_2$ was used as an estimator of the most mobile/phytoavailable fraction of the metals present in the soil. Extraction was performed by allowing 10 g air-dry soil to equilibrate in 50 mL 0.01 M $CaCl_2$ for 2 h and filtered over a white ribbon filter (MN 640 m, Macherey-Nagerl, Düren, Germany).

Analysis of Cd and Zn were performed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Results varied from 93 to 102% of the certified *aqua regia* extractable values. For quality control, standards were always re-analyzed at the end of each batch of analyses. The analysis results were only retained when the measured standard concentrations were within 95 to 105% of the nominal value.

For each soil sample the corresponding legal threshold limits (mg kg^{-1}) for remediation of agricultural soils were calculated according to the Flemish regulations (VLAREBO, 2009, as explained in 1.3.2) usnign the measured pH and an organic matter content as determined in Chapter 3..

4.2.3. Plant analysis

The total aerial plant was divided into wood and leaf fraction and weighed for determination of total fresh biomass production. It was subsequently washed with distilled water, and oven-dried at 50 °C (Dieser Memmert-Schrank, Germany), after which dry biomass was determined. Each plant fraction was pulverzised using a Culatti DCFH 48 (Culatti AG, Zürich, Switzerland) grinder and sieved over a 1 mm sieve. Grounded plant samples were ashed at 450 °C. The ash was dissolved in nitric acid before element analysis (Cd and Zn) using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA).

4.2.4. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. For statistical analysis, normality was checked using a Kolmogorov-Smirnov test. Significance of observed differences was assessed by using an ANOVA combined with a Tukey HSD test (when homoscedasticity was found) or with a Dunnet C test (when no homoscedasticity was found).

4.3. Results

4.3.1. Soil properties

The soil has a 'sand' texture according to the USDA triangle (Meers *et al*., 2010). Due to the liming, the overall pH-H_2O (6.6±0.2) became suited for growing short rotation forestry (Ledin, 1996). No significant differences were found between the plots, suggesting homogenous soil conditions throughout the test field. Pseudo total concentration of Cd and Zn in the test field were 6.5±0.8 mg Cd and 377±69 mg Zn kg^{-1} soil. In Flanders, remediation criteria for metals are site specific as they are a function of clay, organic matter content and pH (VLAREBO, 2009 and explained in Meers *et al*. (2010)). For the specific site these were set at 3.0±0.3 mg Cd and 378±14 mg Cd kg^{-1}.

4.3.2. Biomass production of shoots and leaves

During the first year no significant differences in growth were observed (Table 4.1). Only Belders and Belgisch Rood showed a low production level. The low production level of Belgisch Rood was due to damage by rabbits in the field. During the second year the growth of Belders and Belgisch Rood improved and was more in line with the other clones. Although no significant differences could be measured between the clones, Zwarte Driebast showed the highest biomass production level (Table 4.1).

After three years of growth, the willows exhibited an average aboveground biomass production of 8.3 ton DM ha^{-1}, with significant differences between the investigated willow clones (Table 4.1). Zwarte Driebast achieved significantly higher biomass production in comparison to all other clones. The lowest biomass production was found for Inger. After four years the maximum biomass productivity achieved was about 14 ton DM ha^{-1}, with Zwarte Driebast still showing the highest biomass, followed by Loden, Tora and Jorr (Table 4.1). Based on the samples of year 3 and 4 no significant differences were found between the total biomass of Belders and Jorr. Those of Zwarte Driebast and Loden on the other hand showed a notable increase in growth during the last year. If the total biomass is divided by the years of growth, it becomes clear that most clones still remained at similar yearly biomass productivity levels.

Table 4.1. Biomass of shoots and leaves (ton DM ha^{-1}) after the first four years of growth (mean ± standard deviation, different letters within one column represent differences significant at the level of α=0.05, n=4)

	Clone	Year 1	Year 2	Year 3	Year 4
Stem	**Belders**	0.075±0.004a	1.9±0.1a	9.9±3.3a	7.1±4.6a
	Belgisch Rood	0.082±0.041a	0.98±0.37a	4.1±2.9a	/
	Christina	0.22±0.07a	0.85±0.55a	4.4±1.2a	6.7±6.0abcd
	Inger	0.45±0.16a	1.6±0.6a	3.8±0.8a	5.6±3.5abc
	Jorr	0.64±0.16a	1.3±0.2a	9.0±1.5a	7.8±4.8abc
	Loden	0.60±0.28a	1.7±0.5a	8.5±1.6a	17±9d
	Tora	0.39±0.11a	1.2±0.2a	7.1±0.7a	10±8ab
	Zwarte Driebast	0.72±0.34a	2.6±0.7a	21±5b	49±28d
Leaves	**Belders**	0.029±0.006a	0.43±0.18a	1.9±0.4a	3.9±2.6a
	Belgisch Rood	0.029±0.011a	0.19±0.05a	1.3±0.4a	/
	Christina	0.068±0.012a	0.25±0.12a	2.2±0.4a	0.8±0.5a
	Inger	0.11±0.02a	0.27±0.06a	2.2±0.5a	0.4±0.3a
	Jorr	0.14±0.03a	0.27±0.06a	4.5±1.5a	3.4±2.2a
	Loden	0.16±a0.06	0.32±0.07a	2.7±1.0a	3±2a
	Tora	0.10±0.02a	0.25±0.07a	1.9±1.4a	3±2a
	Zwarte Driebast	0.18±0.07a	0.27±0.08a	3.1±0.6a	5±6a

When examining the annual biomass of the leaves (Table 4.1), no significant differences were found between the biomass production of the leaves of the different investigated clones. It should be noted that these measurements were characterized by high standard deviations. In general the biomass production of the leaves increased with time, where the leaf productivities of year 3 and 4 (on average 2.7 ton DM ha^{-1}) were similar, and significantly different from the first 2 years. Measurements indicated that during the sampling period the leaf biomass decreased for certain clones. This was due to the fact that the leaf biomass was defined as the total leaf biomass that was still on the sampling tree at period of harvest. Fallen leaves were not taken into account.

4.3.3. Metal concentration and extraction potential of the plant compartments

During the growth period of four years, Cd shoot concentration of Inger and Jorr were significantly higher in year four than in year two (Table 4.2). For the other clones the concentrations did not significantly change during the growing period. The concentration of Zn measured in the shoots in year two and year four differed significantly for all the clones with exception of clone Loden. Concentrations were always lowest in Belders, followed by Zwarte Driebast and Jorr. The highest concentrations in the stem were always found in Loden, followed by Tora, Belgisch Rood, Christina and Inger. Concerning the foliar concentration of Cd and Zn, no significant differences were found over the different years. General conclusions are similar to those for the concentrations in the stem: concentrations were highest for Loden and Tora, followed by Christina and Belgisch Rood. The lowest concentrations were found for Belders, Zwarte Driebast and Jorr.

Based on the biomass production levels and the concentration of the shoots, our experiments showed an annual extraction of 72 g Cd and 2.0 kg Zn ha^{-1} year^{-1}. The highest extraction potentials were measured for Zwarte Driebast (203±46 g Cd and 5.1±1.5 kg Zn ha^{-1} year^{-1}), followed by Loden (118±57 g Cd and 2.9±0.7 kg Zn ha^{-1} year^{-1}) (Figure 4.3 and 4.4). During the harvest also leaf material was included. Leaf harvest represented a Cd and Zn extraction of 78±67 g Cd and 6,4 kg Zn ha^{-1}. The foliar extraction potential of Cd was highest for Jorr (198±44 g Cd ha^{-1}), followed by Jorr (90±26 g Cd ha^{-1}), Loden (90±57 g Cd ha^{-1}) and Zwarte Driebast (80±72 g Cd ha^{-1}). For Zn the highest extraction potential was found for Zwarte Driebast (13±10 kg Zn ha^{-1}), followed by Tora (9;7±1.0 kg Zn ha^{-1}), Jorr (8.1±2.2 kg Zn ha^{-1}) and Loden (6.7±4.2 kg Zn ha^{-1}). The high standard deviation of Zwarte Driebast was due to the high variation in biomass measurements.

Table 4.2. Cd and Zn concentrations (mg kg^{-1} DM) in shoot and leaves after 1, 2, 3 and 4 years of growth in the different plant compartments (mean ± standard deviation, different letters within one column represent differences significant at the level of α=0.05, n=4). During year four no measurements of Belgisch Rood could be performed

		Stem				Leaves			
		Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 1**	**Year 2**	**Year 3**	**Year 4**
Cd	**Belders**	12±12a	4.3±1.1a	6±1.6a	8.3±3.4a	12±2a	14±2abc	13±3a	10±3a
	Belgisch Rood	17±11a	15±1b	17±1ab	/	68±11b	42±5abcd	45±4ab	/
	Christina	11±3a	15±1b	17±4ab	21±6ab	26±12ab	45±8acd	56±10ab	48±4c
	Inger	10±1a	13±4acab	10±1ab	22±2ab	27±5ab	33±10abcd	31±8ab	38±3c
	Jorr	10±4a	10±1ab	12±1a	18±4ab	18±4ab	15±2ab	13±1a	27±1b
	Loden	19±10a	30±6c	28±6b	28±13b	51±20b	62±3cd	57±13b	60±24bcd
	Tora	16±2a	18±3b	21±2ab	21±1ab	56±20b	70±25abcd	66±11ab	76±7d
	Zwarte Driebast	12±2a	12±2ab	13±5a	17±3ab	25±6ab	26±6abcd	22±8a	11±1a
Zn	**Belders**	191±30a	148±22a	179±24a	291±128a	1643±178a	1845±82ab	2447±619a	1413±577ab
	Belgisch Rood	333±120a	275±28abc	273±21ab	/	4449±1638a	2764±450ab	2954±377a	/
	Christina	324±90a	452±75bc	508±73ab	640±60ab	2618±1350a	3499±617b	5061±645a	3092±176acde
	Inger	298±49a	333±105abc	276±66ab	844±65b	3221±856a	2974±313b	2368±100a	2956±197acde
	Jorr	223±21a	277±75ab	323±14ab	642±153ab	2169±306a	1439±124a	3974±148a	2430±137abcd
	Loden	323±170a	548±152bc	579±73b	682±160ab	3033±1360a	3849±638ab	4427±1078a	4057±996ade
	Tora	415±91a	435±114abc	486±40ab	631±72ab	4305±1598a	3979±838ab	4193±241a	3756±326ade
	Zwarte Driebast	281±85a	222±28ab	329±35ab	414±59a	2758±1026a	2545±127b	2813±741a	1867±290b

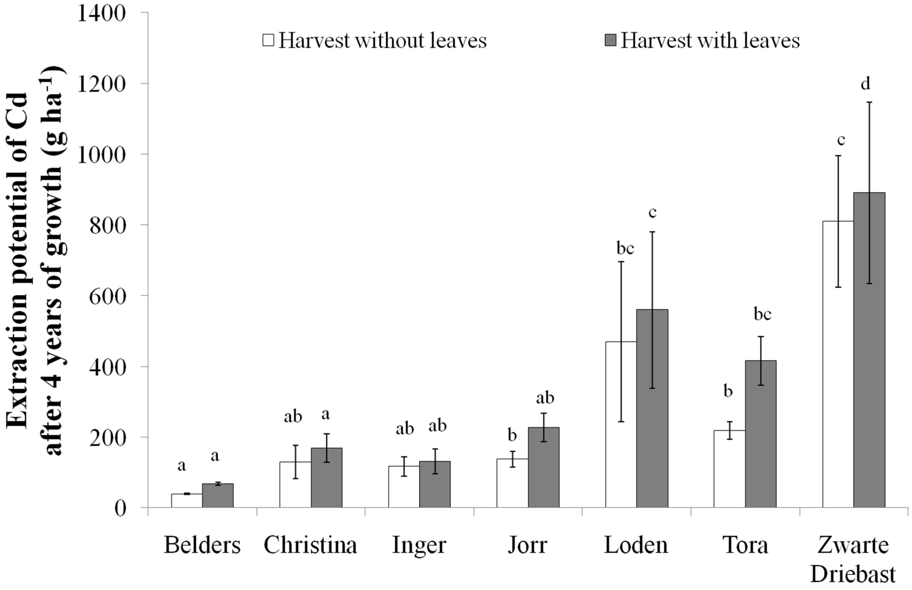

Figure 4.3. Cd (g ha^{-1} year^{-1}) removal by the shoots with and without the leaves of the eight observed willow clones at the field. As no measurements of Belgisch Rood could be made, the extraction potential was not calculated. (Mean values, flags denote the standard deviation, letters represent different significance level of concentration per clone at α=0.05, n=4)

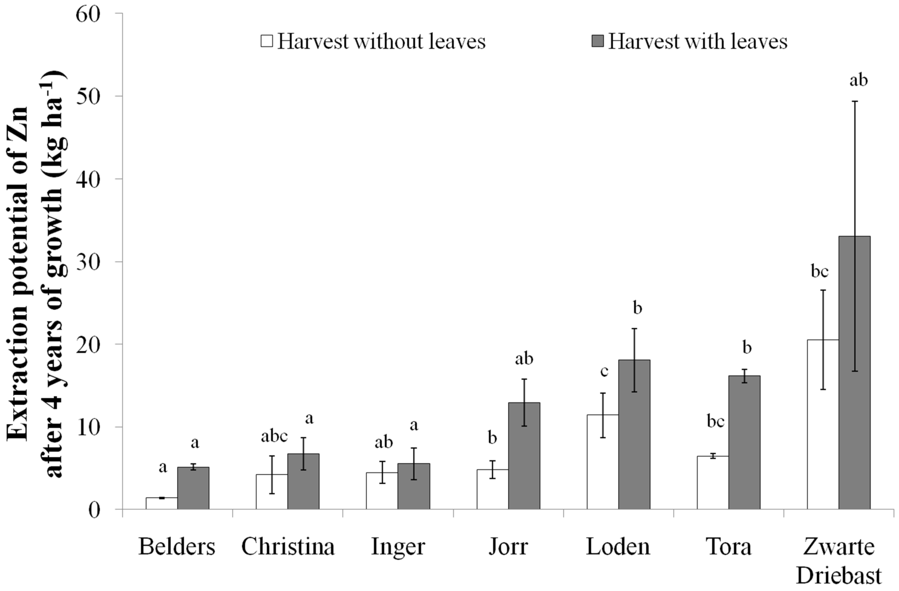

Figure 4.4. Zn (kg ha^{-1} year^{-1}) removal by the shoots with and without the leaves of the eight observed willow clones at the field. As no measurements of Belgisch Rood could be made, the extraction potential was not calculated. (Mean values, flags denote the standard deviation, letters represent different significance level of concentration per clone at α=0.05, n=4)

4.4. Discussion

In general, a trace element phytoextraction protocol consists of the following elements: (a) cultivation of the appropriate plant/crop species on the contaminated site; (b) removal of harvestable trace element-enriched biomass from the site and (c) post harvest treatments to reduce volume and/or weight of biomass for disposal as a hazardous waste, or for its recycling to recover the trace elements that may have an economic value (Ruttens *et al*., 2011). If a proper treatment can be achieved, then the remediation process can provide a complete feasible alternative in comparison to the conventional remediation techniques (Chaney *et al.*, 1997; Ghosh and Singh, 2005; Vangronsveld *et al*., 2009).

Biomass productivity levels of SRC depend on the specific site conditions, clonal selection, climatic conditions, plant spacing and management (Adegbidi *et al*. 2001; Dimitriou and Arronson, 2005; di Nasso *et al*., 2010). After four years of growth, the willows showed on average a biomass production of 14 ton DM ha^{-1} or 3.7 ton DM ha^{-1} year^{-1} (Table 4.1). The observed mean biomass productivity levels (3.7 ton DM ha^{-1} year^{-1}) after four years were low, but in comparison to general expected productivity levels of willows under short rotation coppice (6 to 12 ton DM ha^{-1} year^{-1}). Other studies with short rotation coppice on non-contaminated soil condition in Flanders and in Western Europe also reported similar lower productivity (Meers *et al*., 2007; Vande Walle, 2007; Ruttens *et al*., 2011).

Great differences were found between the observed clones, with Zwarte Driebast showing the highest productivity level (~12 ton DM ha^{-1} year^{-1}), followed by Loden (~4.3 ton DM ha^{-1} year^{-1}). The productivity of Zwarte Driebast is highly in contrast to that of the clone with the lowest productivity level, namely Inger with an average yearly biomass production of only 1.5 ton DM ha^{-1} year^{-1}. Although Zwarte Driebast is showing the highest productivity, it showed high variability between the plots. In one of the sampling plots a biomass production level as high as 18 ton DM ha^{-1} year^{-1} was found. No satisfactory explanation for this high variability in growth could be made, nor in contamination level nor in nutrient conditions. Loden also showed a good biomass productivity with lower variability, which confirms results of earlier studies of Loden on a metal contaminated site (Meers *et al*., 2005). When applying SRC, mono-clonal application is not recommended. Multi-clonal plantations are creating a buffer against diseases or other unforeseen failures of a clone and are therefore consistently showing higher productivity levels than good yielding clones grown as monoclonal stands (Dawnson and McCracken, 1995; Willebrand *et al*., 1993). Therefore, a mixture of the best-performing clones is advisable instead of a mono-culture consisting solely of the single best performing clone.

Productivity levels can also be optimised by using the proper rotation length. Some willow clones are already reaching constant productivity levels after two years, while other clones are reaching their maximum after several years of slow growth (Kuipers, 2003). Moreover, when the rotation periods are longer, higher stem diameters will be found, which can have repercussions on the harvestability of the stems (Kuipers, 2003). The rotation period must be adjusted to the site specific conditions. In the current experiment most of the clones are having their highest increase in productivity at year three (Table 4.1), except Belders and Jorr. As mentioned, based on the samples, no significant differences were found between the total biomass after three and four years and therefore a decrease in productivity is observed. Loden and Zwarte Driebast were, in contrast to the other clones, still showing increased productivity levels after the third year of growth. The suggested rotation period of 3-4 years (Bullard *et al.*, 2002, Weih, 2004, Dimitriou *et al.*, 2006) is therefore in accordance with our observations. Nevertheless, a longer rotation period of four years is advisable for ensuring the culmination point of growth of all clones.

The rate of metal transfer to the aboveground biomass is also important when evaluating phytoextraction performances. Considering the current experimental site, where a Cd of 6.5±0.8 mg kg^{-1} was measured in the soil and that needs to be reduced to 3.0 mg Cd kg^{-1} (which is the site specific threshold value for remediation), and assuming (i) a specific soil density of 1 400 g dm^{-3}, (ii) a plough layer depth of 25 cm and (iii) a linear extraction potential, more than 170 years would be needed. This period can be lowered using the appropriate set of clones (Klang-Westin *et al.*, 2002). When extraction potential would be achieved solely by clones with the highest extraction potential, such as Loden and Zwarte Driebast, the remediation period could drop under 100 years. In comparison to other reported energy crops like energy maize (Meers *et al.*, 2010) and rapeseed (Chapter 3) for which more than 300 years are needed for soil remediation, willows showed to be the best performing plant species.

As leaves contain 3 to 5 times higher concentrations of metals than the stem, a harvest including leaves could increase the effectiveness of the phytoextraction process. Therefore it was chosen to harvest in fall, in contrary to other studies where harvest is performed in winter (Dimitriou and Arronson, 2005, Maxted *et al.*, 2007) (Figure 4.5). Indeed, when the metal extraction by the leaves is included, the total extraction potential after four years is augmented by about 40%. This impact of leaf harvest on extraction potential is clone specific. The highest impact of leaves was found for Tora, Belders and Jorr. The impact of the leaves on extraction effectiveness for Loden and Zwarte Driebast is lower. If there would be an annual removal of the leaves, the extraction potential could even be doubled. The extraction periods are similar to those reported in other studies using willows on contaminated soils in Flanders (Meers *et al*., 2007; Ruttens *et al*., 2011) and elsewhere (Hammer *et al*., 2003).

Figure 4.5. Willows after four years of growth followed by the harvest (photo's: Van Slycken, S.)

Leaf harvest has an additional effect. Through decomposition of fallen leaves, metals return to the soil. This was investigated for different tree species on a sandy acidic contaminated soil on a neighboring site (Van Nevel *et al*., 2011). They reported higher Cd and Zn concentration in the topsoil layer under aspen (*Populus tremuloides* Michx) plantation, which have a similar accumulation potential as willow (Ruttens *et al.*, 2011). In order to achieve an increase of metals in the top soil layer, the metal accumulation through the decomposition must be higher than the uptake of metals. The total amounts of Cd and Zn per ha entering the soil through leaf fall are much lower than the amounts of Cd and Zn that are extracted by harvest, meaning that at the moment no recontamination was observed. Nevertheless, this should be further evaluated during regrowth observation of SRC on metal contaminated soils.

With an energy output of about 19 times higher than its input, willow wood is mainly used as a biomass fuel (Ledin, 1996; Ingemarsson *et al.*, 1999). Different treatments, including thermal, chemical and biological methods can be applied. In this study, combustion, gasification and pyrolysis will be addressed. In case of combustion the biomass is used to generate electricity in existing steam-turbine power plants. This technique is the oldest, simplest but most inefficient technology (Smart *et al*. 2005; Evans *et al*., 2010). Research on the fate of metals during the combustion describes that during this process metals will end up in the different ash-fractions (bottom ash, cyclone fly ash, filtered fly ash and flue dust). Contrary to macro-nutrients, which will be mostly concentrated in the bottom ashes, elements like Cd and Zn will accumulate in the fly ashes. The metal balance is not only depending on the separation technique, but also on the wood/bark proportion of the biomass (Narodoslawsky and Odenberg, 1996; Odenberg *et al*., 1997).

A second usage strategy is gasification, where a gas and a residual fraction are formed. Similar to combustion, metals will be distributed over the different ash fractions and Cd and Zn will mainly accumulate in the filter fly ash fraction. New techniques, specification of the biomass and adaptation of the gasification plant can influence the pathway of metals (Vervaeke *et al*., 2006). A third option is when biomass is heated in total absence of oxygen. This process is called pyrolysis. A distinction is made between fast and slow pyrolysis. In the current study, only fast pyrolysis is discussed. In this process, the biomass is rapidly heated at moderate temperatures (723 K) with a vapor residence time of only a few seconds, very often a maximum of 3 seconds. The hot gases then need to be cooled quickly. During this process, besides a heat and ash fraction, also a bio-oil fraction is produced. Since Cd becomes volatile at 823 °C, the Cd present in the biomass does not enter the bio-oil. Also using a hot-gas filter will prevent the occurrence of Cd in the bio-oil. The efficiency of the process, together with the quality of the oil originating from willows grown on a contaminated site are similar to those of a non-contaminated site. Even when the process is performed with a mixture of wood and leaves, only minor differences are found in productivity and quality of the products (Lievens *et al*., 2009; Stals *et al*., 2010a,b).

A final and crucial argument to consider SRC on metal contaminated agricultural soils is the economic feasibility. Willows are very attractive for (co)-combustion because they have a low ash content, low nitrogen content, and are free of iron, sand or stone particles. The price of the wood, however, depends on the legal status of the wood (Vanaken, N., pers. communication, February 2009). The classification of woody biomass is important for the determination of the appropriate emission directive when (co-)combusted. In Flanders, legislation on emissions is determined in VLAREM I and II. VLAREM I defines the different types of installations and the obligations that follow out of that, while VLAREM II handles the actual emission regulation of each type. We only found maximum concentrations relevant to our case study for Pb (30 mg kg^{-1}). As Pb concentrations measured in the willow samples from the experimental field are situated below these standards, the biomass produced on the metal contaminated land can be used without any problem. Thus, the price paid for this wood will be equal to that of uncontaminated wood, fluctuating in a very volatile market around € 50-100 ton^{-1} DM. This price does not guarantee a sufficient income for farmers comparable to their current income. When other conversion techniques are considered, the main conclusion remains the same. The most pragmatic question is not the conversion feasibility but the price of the woody biomass at the market (Thewys and Kuppens, 2008; Witters *et al.*, 2009). Disposal costs of ashes from all conversion techniques are rather low since the total amount of ashes is low. This should lead to the stimulation of SRC as an alternative crop on contaminated land. However, farmer's acceptance cannot be neglected and will be determined by the ability of local, regional and federal policy makers to inform farmers in an intensive, exact and complete way to reduce obstacles (Witters *et al.*, 2009).

4.5. Conclusion

On the sandy soils in the Campine region, only a few commercially available willow clones achieved an acceptable biomass production of nearly 12 ton DM ha^{-1} year^{-1}. In combination with their ability to accumulate metals in the stem and leaves, SRC exhibits higher metal extraction potential (72 g Cd and 2.0 kg Zn ha^{-1} year^{-1}) than other energy crops such as energy maize or rapeseed. The removal of the leaves could even augment the extraction potential by 40%. Nevertheless, remediation periods still remain too long in order to be of practical use for effective soil cleanup. Economic and environmental benefits can be in favour of SRC as a safe alternative use of these contaminated soils that are not anymore suited for safe food production. A gradual decrease of metals in the top layer over a longer term effectuated by metal extraction can be considered as a secondary beneficial side effect. Combustion, gasification, and pyrolysis are options to convert the produced biomass into energy. When the use of wood as energy source becomes more pronounced, wood prices will very likely also stimulate the adoption of short rotation coppice for energy production in the region.

CHAPTER 5:

GROWTH AND METAL UPTAKE OF ENERGY MAIZE (*ZEA MAYS* L.) ON METAL CONTAMINATED SOILS

Redrafted from Meers, E., Van Slycken, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., Thewys, T. and Tack, F.M.G. (2010). The use of bio-energy crops (*Zea mays* L.) for 'phytoattenuation' of heavy metals on moderately contaminated soils: a field experiment. Chemosphere, 78, 35-41.

Abstract

Worldwide there are numerous regions where conventional agriculture is affected by the presence of elevated amounts of plant-available trace elements, causing economic losses and food and feed quality and safety. The Belgian and Dutch Campine regions are a first-class example, with approximately 700 km^2 diffusely contaminated by historic atmospheric deposition of Cd, Zn and Pb. In this region, the primary land use is agriculture, which is frequently confronted with crops exceeding the European standards for heavy metal contents in food and feed stuffs. Phytoremediation as a soil remediation technology only appears feasible if the produced biomass might be valorised in some manner. In the current case, we propose the use of energy maize (*Zea mays* L.) aiming at risk-reduction and generation of an alternative income for agriculture, yet in the long run also a gradual reduction of the pollution levels. Since the remediation aspect is demoted to a secondary objective with sustainable risk-based land use as first objective, we introduce the term ‘phytoattenuation’: this is in analogy with ‘natural attenuation’ of organic pollutants in soils where also no direct intended remediation measures but a risk-based management approach is implemented. In the current field experiment, cultivation of energy maize could result in 33 000-46 000 kWh of renewable energy (electrical and thermal) per hectare per year which by substitution of fossil energy would imply a reduction of up to 21 ton ha^{-1} $year^{-1}$ CO_2 if used to substitute a coal fed power plant. Metal removal is very low for Cd and Pb but more significant for Zn with an annual reduction of 0.4-0.7 mg kg^{-1} in the top soil layer.

5.1. Introduction

As already mentioned in Chapter 3, the largest challenge in phytoremediation is sometimes finding the right plant for a specific type of contamination (Herrero *et al.*, 2003). In the context of achieving phytoextraction, mostly two trends were observed: (i) the use of hyperaccumulating plants; or (ii) the application of chemical agents to enhance metal accumulation in high biomass producing crops. Although extensive efforts have been spent on phytoremediation research for removal of heavy metals from the soil, no convincing field data have been presented which allow its broad practical application as a short-term alternative for conventional remediation techniques.

Application of hyperaccumulators is limited by the generally low biomass productivity of hyperaccumulating species, while chemically induced hyperaccumulation is impaired by the various environmental risks, *e.g.* metal leaching and toxic effects on microbes, involved with deliberate mobilization and increasing bioavailability of heavy metals. Comparing four biomass producing agronomic crops either treated with or without metal mobilising soil amendments, Meers *et al*. (2005b) concluded that in order for phytoextraction to evolve into a suitable technique, either the extraction efficiency requires to be further increased or the produced biomass needs to be economically valorised, *e.g.* bioenergy. The previous chapter concludes that if establishment and the valorisation of the crop could be optimal, the main drawback of phytoextraction (the long required remediation time) could become invalid. Nevertheless, for rapeseed there still were some management problems (Cfr. Chapter 3) as for short rotation coppice (Cfr. Chapter 4), the final price of the wood remained at the moment a bottleneck.

A third crop that is investigated as an alternative crop on metal contaminated agricultural soils is energy maize (*Zea mays* L.) (Meers *et al.*, 2010). These are cultivars of *Zea mays* that are used for energy production purposes, *e.g.* by producing biogas through anaerobic digestion. Such energy maize and biogas production represent a new branch of agriculture, which has been emerging at large scale over the past five to ten years. Meers *et al*. (2005b) hereby also observed that of four biomass producing crops, tested on moderately contaminated calcareous dredged sediment derived surface soils, *Z. mays* exhibited the highest biomass potential on moderately metal contaminated land, with the lowest metal accumulation in the harvestable plant parts. By using this metal excluder specie a risk reduction of metals in the produced biomass could be achieved.

Therefore the main objectives of his chapter was to (i) evaluate the biomass productivity potential of this crop under field condition (ii) to evaluate metal extraction potentials on a metal contaminated soil in the Campine region; and (iii) to screen subsequent biomass treatments possibilities.

5.2. Material and Methods

5.2.1. Experimental setup

For the purpose of this experiment 4 050 m^2 were subdivided in six plots of 50 m by 13.5 m. Six commercially available *Z. mays* cultivars (Atletico, Fransico, KWS1393, PR34B39; PR39F58, La Fortuna) provided by different breeders (KWS and Pioneer) and referred to as Z1-Z6, were sown on the plots. The cultivars were selected by the plant breeders for their capacity to adapt to the climatic conditions in Flanders. Their FAO-number (Food and Agriculture Organization of the United Nations) varied, yet was always larger than 220, which is ideal for silage maize. This FAO-number represents the maturity date of the cultivar based on a uniform, standardized method (Jugenheimer, 1958, cited from Marton *et al.*, 2003) and is therefore a measure of the greenness of the maize at a certain harvest date. The higher the number, the longer it will take a plant to achieve full ripeness. Prior to sowing, and in accordance with the other experiments, the field was limed and ploughed for homogenization to achieve a final pH that varied between 6.1 and 7.4 (Cfr. 1.3). The crops were sown in May 5, 2006 at a density of 95 000 seeds ha^{-1}, with due attention for good agronomic practices. The field was fertilised at a low nitrogen dose of approximately 50 kg ha^{-1} using liuid pig manure.

Harvest and sampling took place in October 23, 2006 (Figure 5.1). Within each cultivar block four combined plant and soil samples were taken, at locations randomly distributed over these blocks. Each individual plant sample consisted of a mixture of all plants in a sampled surface area of 1 m^2. Spread over this area also a mixed soil sample was taken (auger for arable land, ø 13 mm, operation length 25 cm, Eijkelkamp, Giesbeek, the Netherlands).

Figure 5.1. Growth, harvest and silaging of maize (photo's: Van Slycken, S.)

5.2.2. Soil analysis

Soil samples were dried at 50 °C in a soil oven (EU 170, Jouan s.a., Saint Herblain, France) and sieved to pass a 1 mm sieve. To determine the pH-H_2O, 10 g of air-dried soil was allowed to equilibrate for 24 h in 50 mL of deionized water, after stirring regularly in the beginning with a glass rod. The pH of the supernatant was measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA) (Van Ranst *et al.*, 1999). For pH-KCl, 50 mL of 1 M KCl was added to 10 g of air-dried soil and allowed to equilibrate for 10 min, after stirring regularly in the beginning with a glass rod. The pH of the supernatant was then measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA), calibrated using pH 4.0 and 7.0 standards (Van Ranst *et al.*, 1999).

Pseudo-total soil metal content was determined after *aqua regia* digestion (Van Ranst *et al.*, 1999). Cd and Zn Analysis were performed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Certified reference sediment CRM 277 was also analysed in triplicate using the same method. Results varied from 93 to 102% of the certified *aqua regia* extractable values.

An assessment of the most mobile/phytoavailable fraction of the heavy metals present in the soil, was made by $CaCl_2$ extraction. Meers *et al.* (2007b) observed a good correlation between shoot accumulation of heavy metals and $CaCl_2$ extraction. Extraction was performed by allowing 10 g air-dry soil to equilibrate in 50 mL 0.01 M $CaCl_2$ for 2 h, filtering it over a white ribbon filter (MN 640 m, Macherey-Nagel, Düren, Germany) and analysing metal (Cd, Zn) concentration in the filtrate using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). For quality control, standards were always re-analysed at the end of each batch of analyses. The analyses results were only accepted when the measured standard concentrations were within 95 to 105% of the certified value (*i.e.* taking into account possible artifacts due to the dilution of certified stock solutions).

For each soil sample the corresponding legal threshold limits (mg kg^{-1}) for remediation of agricultural soils were calculated according to the Flemish regulations (VLAREBO, 2009, as explained in 1.3.2) usnign the measured pH and an organic matter content as determined in Chapter 3..

5.2.3. Plant analysis

Plant samples collected in the field were weighed for determination of fresh weight biomass production, washed with distilled water, and oven-dried at 50 °C. Plants were then subdivided in shoot, cobs and grains. The individual plant compartments were pulverised using a Culatti DCFH 48 grinder and sieved over a 1 mm sieve. Ground plant samples were ashed at 450 °C and dissolved in nitric acid before element analysis (Cd and Zn) using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). For the determination of Cd content in the grains inductively coupled plasma mass spectrometry (ICP-MS; ELAN DRC-e, PerkinElmer, Waltham, MA, USA) was used.

5.2.4. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Miscrosoft Inc.) software packages. For statistical analysis normality was checked using a Kruskall Wallis test. Significance of observed differences was assessed by using an ANOVA combined with a Tukey HSD test.

5.3. Results

5.3.1. Soil properties

The Flemish remediation criteria for heavy metals (VLAREBO, 2009) for this experimental site are respectively 2.9±0.2 mg kg^{-1} Cd and 426±10 mg kg^{-1} Zn (Table 5.1). No significant differences were found between the total Cd and Zn concentration in the different plots, suggesting homogenous soil conditions throughout the test-field. The $CaCl_2$-extractable fraction of metals soil fraction of heavy metals were respectively 4-10% of total Cd and 4-7% of total Zn in the soil. The concentration of Cd in the $CaCl_2$-fraction for Z1 was higher than those of Z5 and Z6, and showed large variations in measurements which could influence further results.

Table 5.1. pH-H_2O, concentration of Cd and Zn measured by *aqua regia* destruction (mg kg^{-1}), $CaCl_2$extraction (mg kg^{-1}), together with the legal threshold limits (mg kg^{-1}) for remediation of agricultural soils according to the Flemish regulations (VLAREBO, 2009) for the different test plots (mean ± standard deviation, different letters represent different significance level at α=0.05, n=4)

		Cd			Zn		
	pH-H_2O	***aqua regia***	**$CaCl_2$**	**threshold**	***aqua regia***	**$CaCl_2$**	**threshold**
Z1	6.7±0.4a	5.7±2.1a	0.55±0.24a	2.6±0.4a	297±78a	21±11a	411±25a
Z2	6.8±0.2a	5.4±1.5a	0.40±0.15ab	3±0.3a	300±75a	11±3a	429±14a
Z3	7.0±0.2a	6.2±1.1a	0.29±0.03ab	2.8±0.3a	289±48a	11±4a	423±10a
Z4	6.8±0.3a	6.7±2.3a	0.39±0.11b	3±0.3a	355±85	16±5a	429±14a
Z5	6.7±0.3a	5.0±2.2a	0.31±0.09b	2.8±0.4a	266±96a	15±7a	423±22a
Z6	6.9±0.2a	8.9±2.5a	0.39±0.0.9ab	3.2±0.2a	398±73a	17±6a	442±9a

5.3.2. Biomass production

The experiment of 2006 showed that the total fresh weight biomass production ranged from 36 to 52 ton ha^{-1} for the six cultivars, with an average dry matter content of 30%. Hereby no significant differences could be found between the total dry weight of the different cultivars under study (Table 5.2).

Table 5.2. Dry weights (ton ha^{-1}) of the different aerial plant parts, of the six (Z1-Z6) investigated maize varieties (mean ± standard deviation, different letters represent different significance level at α=0.05, n=4)

	Stem	Cob	Grain	Total
Z1	4.4±0.8a	1.9±0.7a	8.1±2.0a	15±3a
Z2	3.9±0.6a	1.9±0.3a	9.8±2.5a	16±2a
Z3	4.0±1.3a	2.0±0.8a	9.4±3.4a	14±5a
Z4	4.3±1.2a	1.6±0.3a	7.7±3.4a	16±5a
Z5	3.1±0.7a	1.2±0.4a	7.1±1.9a	11±3a
Z6	2.7±0.7a	1.7±0.2a	6.5±1.1a	12±2a

The dry weight of the plant is mostly determined by the grain (up to 70%). Under normal conditions, a higher productivity in the range of 60-70 ton FW ha^{-1} would be expected for energy maize.

5.3.3. Metal concentration and extraction potential of the plant compartments

For all metals under this study, concentrations in grains were the lowest (Table 5.3). This means that, the percentage of metals accumulated in the grains is very low compared to the total mass extracted and translocated to harvestable plant parts, although the grain biomass fraction represented the majority of the produced biomass (Table 5.2).

Table 5.3. Concentration of Cd and Zn (mg kg^{-1} dry matter) of the different aerial plant parts, of the six (Z1-Z6) investigated maize varieties (mean ± standard deviation, different letters represent different significance level at α=0.05, n=4)

	Cd				Zn			
	Stem & leaves	**Cob**	**Grain**	**Total**	**Stem & leaves**	**Cob**	**Grain**	**Total**
Z1	1.8±0.3a	0.47±0.17 a	0.10±0.15a	0.66a	547±94a	220±34a	0.80±0.007a	191a
Z2	2.2±0.4a	0.65±0.19a	0.06±0.02a	0.64a	541±156a	166±61a	0.66±0.04b	151a
Z3	1.8±0.9a	0.65±0.23a	0.07±0.04a	0.68a	433±63a	102±7ac	0.65±0.08ab	143a
Z4	1.9±0.2a	0.80±0.70a	0.07±0.06a	0.62a	500±43a	83±11ac	0.78±0.06b	144a
Z5	1.8±0.4a	0.50±0.10a	0.05±0.01a	0.58a	549±131a	343±99a	0.67±0.08b	189a
Z6	2.1±0.4a	0.58±0.29a	0.06±0.03a	0.62a	468±31a	108±11a	0.81±0.10ab	124a

Multiplying the measured biomass production of shoots, cobs and grains by the observed concentrations, delivers the total metal removal capacity of the crop per hectare (Figure 5.2 for Cd and 5.3 for Zn). Cultivars Z5 and Z6 exhibited the lowest performance both from a biomass production perspective and metal concentration/accumulation perspective. Cultivars Z1-Z4 exhibited a metal extraction potential in the same range, with Z1 showing the highest Zn removal and Z2 showing the highest Cd removal capacity. Z5 and Z6 exhibited significantly lower uptake than the other four cultivars for all metals under evaluation, yet this difference is attributed more to overall biomass productivity (Figure 5.2 for Cd and 5.3 for Zn) than metal concentrations in the plants.

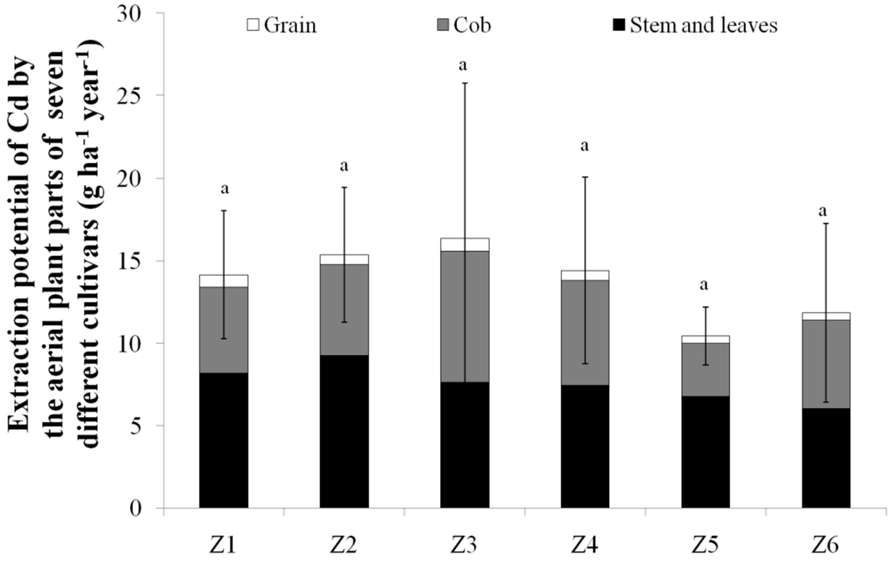

Figure 5. 2. Extraction potentials of Cd (g ha^{-1} year^{-1}) of the different aerial plant parts of the six investigated maize varieties (Z1-Z6) (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=4)

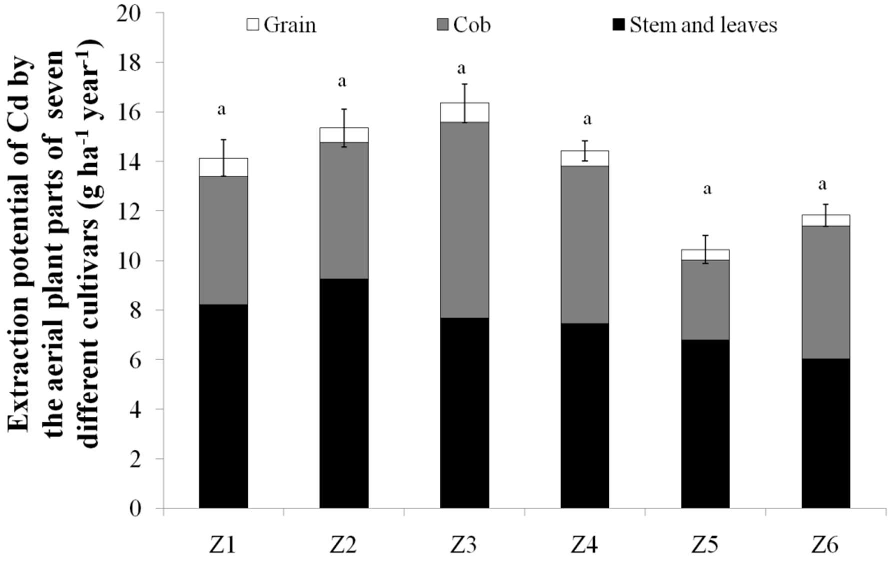

Figure 5. 3. Extraction potentials of Zn (kg ha^{-1} year^{-1}) of the different aerial plant parts of the six investigated maize varieties (Z1-Z6) (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=4)

5.4. Discussion

Due to probably sub-optimal fertilisation and the drought period, the biomass productivity of energy maize was low. The Royal Meteorological Institute of Belgium reported that in the spring of 2006 very low precipitation was measured in March and that for the summer in June and July various heat waves (periods of 5 d above 25 °C with minimum 3 d above 30 °C) were measured and catalogued as very exceptional. Also precipitation amount in June and July (resp. 25 and 48 mm while a monthly rainfall of 70 mm is expected, KMI, 2011) were exceptional and the average rainfall in the Campine region was even reported as very excpetional (KMI, 2011). Similar findings on the impact of drought in the period was also reported in other regions throughout Europe (Eumetsat, 2009), which resulted in a low agricultural output of all grains in Western Europe in the season 2006, in particular the Campine region.

Of the total amount of Cd and Zn in aerial plant parts, respectively 6.3 and 0.3% were allocated to the grains. Most metals accumulated in the stem and leaves, representing 82% of total accumulated aboveground Cd and 87% of total Zn. Similar findings were reported by Jarausch-Werheim *et al.* (1999), who investigated Zn concentration in maize grown on different fields treated with anaerobically-digested dehydrated sludge. These authors also found that Zn was primarily stored in shoot parts rather than being translocated to the grains. They stated this translocation mechanism protects the plant against toxification of its reproductive organs, yet it also implies accumulation in the shoot above safety criteria for use as fodder. Low metal accumulation in the grains can indeed be attributed to a translocation barrier that prevents accumulation of undesired trace elements in the seeds of plants, a protective mechanism designed to protect its descendants. Nevertheless, the metal concentration in the current experiment exceeded the legal thresholds for contaminants in food and feed stuffs. The European standards for animal feed are 1.14 for Cd for general feed-stuffs or 34 mg kg^{-1} for green fodder material with a moisture content of 12%. No such criteria exist for Zn, which is an essential element. Cd concentration in the shoots exceeds the maize fodder standards. Therefore, it is safer to target the harvested biomass for non-food purposes, as Flemish legal threshold values for metal concentrations in biomass aimed for bioenergy production by means of anaerobic digestion are less strict than for animal feed, at 6 mg kg^{-1} for Cd and 900 mg kg^{-1} for Zn.

During the anaerobic digestion process, biomass is converted into biogas and digestate. Under mesophilic digestion conditions, biogas productivity varies between 180-220 Nm^3 ton FW energy maize, averaging at 200 Nm^3 ton FW as a rule of thumb. Methane content of biogas originating from energy maize is about 52% (Amon *et al.*, 2004, cited from Walla and Schneeberger, 2008.; 2007; Van der Voort *et al.*, 2008; Wijnholds, 2008). With a caloric value of methane equal to 9.95 kWh per Nm^3 CH_4, each ton biomass (FW) can yield biogas with a gross energy content of approximately 1035 kWh. Finally, converting this gas into useful forms of electric and thermal energy by means of a combined heat and power engine (CHP) with 40% electric and 40% thermal efficiency would result in 414 $kWh_{electricity}$ and 414 $kWh_{thermal\ energy}$ ton^{-1} maize (FW). For the produced biomass production in our field experiment, this implies a total annual production per hectare of 15 000-21 000 $kWh_{electricity}$ in addition to 18 000-25 000 $kWh_{thermal\ energy}$ ha^{-1}. In Flanders, this equals the average annual energy consumption of 4-6 households (average consumption in Flanders 3 450 $kWh_{electricity}$ per household). In addition, substitution of fossil energy by this renewable energy implies a reduction in CO_2 emission of 15-21 ha^{-1} $year^{-1}$ if used to substitute a coal power plant or a reduction of 6.5-9.5 ton ha^{-1} $year^{-1}$ if used to substitute a natural gas power plant.

The low metal accumulation in the grains may be of particular interest when different end-uses are envisaged for the metal-rich fodder material and the metal-poor grains. Current agronomic practices for *Z. mays,* however, do not allow simultaneous harvest and isolation of grains from the shoot biomass. Either the entire shoot is harvested and shredded for fodder maize applications (animal feed), or the grains are harvested with the residual shoot biomass discarded back on land. For the moment, harvesting the entire plant and using the harvested biomass for non-food/non-feed applications, is the only practical option if a combination with phytoremediation by means of gradual attenuation is aimed at. However, currently state-of-the-art agro-technology is moving towards the development of harvesting hoppers which can harvest and separate both plant compartments, with several examples of this presently available in Germany (Meers *et al.*, 2010). In the context of phytoremediation the removal of the discarded material needs also a proper treatment.

Overall metal removal per ha varied between 6.5-10 for Cd and 1.4-2.8 kg ha^{-1} for Zn and are in the same order of magnitude as other field experiments on metal contaminated soil with a full cropping stage of maize (Kurz *et al*., 1999; Lombi *et al*., 2001). Expressing this as annual concentration reductions in the top soil layer this translates to 0.002-0.003 for Cd and 0.41-0.81 mg kg^{-1} year^{-1} for Zn, assuming a specific soil density of 1400 g dm^{-3} and a plough layer depth of 25 cm. These extraction values are far too low for allowing effective short term soil remediation.

5.5. Conclusion

Data suggest that energy maize can create a risk-based land use of metal contaminated soils. Hereby the biomass can be used for the production of renewable energy. Based on the current data the cultivation of energy maize in the region could result in the production 30000-42000 kWh$_{el+th}$ of renewable energy per hectare. When supposing the substitution of coal powered power plant, this would imply a reduction of up to 21 ton ha^{-1} year^{-1} CO_2. Due to sub-optimal fertilisation and an extreme drought period in Europe and in the Campine region, biomass productivity levels were not representative. The low accumulation of metals in the plant results in long remediation periods.

CHAPTER 6:

FEASIBILITY STUDY OF ENERGY MAIZE (*ZEA MAYS* L.) ON METAL CONTAMINATED AGRICULTURAL SOILS

Redrafted from Van Slycken S., Witters N., Meers, E., Peene, A., Michels E., Adriaensen K., Ruttens A., Vangronsveld J., Du Laing G., Thewys T. and Tack, F.M.G. (2011b). Safe use of metal contaminated agricultural land by cultivation of energy maize (*Zea mays* L.). Environmental Pollution. Accepted for publication.

Abstract

Production of food crops on metal contaminated agricultural lands in the Campine region (Belgium) can be problematic as legal threshold values for safe use of the crops can be exceeded. Conventional sanitation of vast areas is too expensive and alternatives need to be investigated. *Zea mays* on a metal contaminated soil in that region showed an average yield of 53±10 ton fresh or 20±3 ton dry biomass ha^{-1}. Cd concentrations in the whole plant (0.96±0.29 mg kg^{-1}) were just below legal thresholds for contaminants in feed stuffs (1.14 mg kg^{-1}) but were meeting the current threshold values for usage for anaerobic digestion (6 mg kg^{-1} for Cd). Biogas production potential did not differ between maize grown on contaminated (215±23 Nm^3 ton^{-1} FM) and non-contaminated (194±4 Nm^3 ton^{-1} FM) soils. Results suggested favourable perspectives, allowing farmers to generate profitable non-food crops, although effective soil cleaning would be extremely slow. Nevertheless, a valuable, sustainable alternative use can be proposed for moderately contaminated soils on which conventional agriculture is impaired.

6.1. Introduction

Agricultural soils may be contaminated by metals as a result of industrial activities, *e.g.* mining and smelting of metalliferous ores, and agricultural practices, *e.g.* application of metal-contaminated fertilisers, sewage sludge and other biosolids (Kurz *et al.*, 1999). Previous chapters, together with other studies (Vangronsveld *et al.*, 2009; Thewys *et al.*, 2010a,b) showed that biomass with elevated concentrations of metals may safely be used for renewable energy production. Although effective short term cleaning of the soil (< 20 years) is an unrealistic expectation, a safe and economic alternative is offered for use of the metal contaminated agricultural lands. Therefore, the focus of the research shifted towards creating safe use of the contaminated soils (Fässler *et al.*, 2010; Robinson *et al.*, 2010).

Within this option previous chapter indicated that the production of energy maize (*Zea mays* L.) on metal contaminated soils would fit within a risk-based land use of metal contaminated soils. As metal accumulation levels in plant material are low, the impact of the trace elements on the subsequent processing of the biomass for bioenergy production could be limited. Especially if maize is not used for food or fodder, but for anaerobic digestion. Therefore, it is suggested that if the biomass productivity of maize is acceptable, and the treatment is not technically impaired, maize still fits into the above mentioned strategy.

The objectives of this chapter are therefore to (i) evaluate growth and yield performance under optimal fertilisation conditions; (ii) establish the potential of the biomass for biogas production by means of anaerobic digestion; and (iii) assess the economic feasibility for farmers that convert their land use to a non food crop, *in casu* energy maize.

6.2. Material and Methods

6.2.1. Experimental setup

For the purpose of this experiment 1 890 m^2 were subdivided in 42 plots of 4.5 m by 10 m. Within these plots, seven commercially available *Z. mays* cultivars (Atletico, Fransisco, KWS1393, PR34B39, PR39F58, PR38H20, La Fortuna), provided by KWS and Pioneer and referred to as Z1-Z7, were sown *ad random* in repetition blocks (six plots per cultivar). Cultivars were selected by the plant breeders for their capacity to adapt to the Flemish climatic conditions. Their FAO-number varied, yet was always larger than 220, which is ideal for silage maize. This FAO-number represents the maturity date of the cultivar based on a uniform, standardized method (Jugenheimer, 1958, cited from Marton *et al*., 2003) and is therefore a measure of the greenness of the maize at a certain harvest date. The higher the number, the longer it will take a plant to achieve full ripeness (KWS, 2011).

The crops were sown on May 15, 2007 at a density of 95 000 seeds ha^{-1} and harvested in October 15, 2007, with due attention for good agronomic practices. The field was fertilised with liquid pig manure (Table 6.1), at a rate of approximately 170 kg N ha^{-1}, which is the most common fertilisation regime of maize in the area. Common agricultural practices were adopted for land tillage and pest control.

Table 6.1. Density (ρ), dry matter (DM)-content and total N, P and K content of the fertilisation used in the experiments (n=3)

ρ (kg m^{-3})	DM-content (%)	N (mg kg^{-1} DM)	P (mg kg^{-1} DM)	K (mg kg^{-1} DM)
1035	6.1±0.1	27±1	24±1	46±2

Just before harvest, a plant and soil sample was taken in each replication plot. One plot of each cultivar was sampled twice. The plant sample consisted of taken 6 neighbouring plants. The total plant was divided into its different plant organs (stem, leaves, bract, rachis, grain) for biomass determination and metal analysis. Spread over each plant sampling area, six soil samples were taken with an auger (auger for arable land, ø 13 mm, operation length 25 cm, Eijkelkamp, Giesbeek, the Netherlands) and mixed in order to obtain a bulked sample.

6.2.2. Soil Analysis

Soil samples were dried at 50 °C in an oven (EU 170, Jouan s.a., Saint Herblain, France) and sieved to pass a 1 mm sieve. Soil conductivity was measured with a WTW LF 537 electrode (Wisschenschaftlich Technischen Werkstäten, Weilheim, Germany) after equilibration for 30 min in deionized water at a 5:1 liquid:solid ratio and subsequent filtering (white ribbon, MN 640 m, Macherey-Nagel, Düren, Germany). To determine the pH-H_2O, 10 g of air-dried soil was allowed to equilibrate for 24 h in 50 mL of deionized water, after stirring regularly in the beginning with a glass rod. For determination of pH-KCl, 50 mL of 1 M KCl was added to 10 g of air-dried soil and allowed to equilibrate for 10 min (Van Ranst *et al*., 1999). The pH of the supernatant was then measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA), calibrated using pH 4.0 and 7.0 standards (Van Ranst *et al*., 1999).

Pseudo-total soil concentrations of Cd and Zn were determined after *aqua regia* digestion (Van Ranst *et al.*, 1999). Analysis was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Certified reference sediment CRM 277 was analyzed in triplicate using the same method. Results varied from 93 to 102% of the certified *aqua regia* extractable values. $CaCl_2$ extraction was used to asses the most available (phytoavailable) fraction of the metals present in the soil (Meers *et al*. 2007a). Extraction was performed by allowing 10 g air-dry soil to equilibrate in 50 mL 0.01 M $CaCl_2$ for 2 h, filtering it over a white ribbon filter (MN 640 m, Macherey-Nagerl, Düren, Germany) and analyzing metal (Cd and Zn) concentration in the filtrate using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). For quality control, standards were always re-analysed at the end of each batch of analyses. The analysis results were only retained when the measured standard concentrations were within 95 to 105% of the nominal value.

Nitrogen concentration in the soil was determined using a Kjeldahl destruction, while P was determined using the method of Scheel (Van Ranst *et al.*, 1999). Pseudo-total Na, K, Ca and Mg content were measured on the *aqua regia* digested samples using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Total P content was determined by colorimetry (Jenway 6500 spectrophotometer, Jenway, Essex, UK) using the method of Scheel on the *aqua regia* destruction (Van Ranst *et al.*, 1999). The nutrient state (P, Na, K, Ca en Mg) was estimated using a NH_4-OAc-EDTA extraction. Extractions were carried out by allowing 20 g air-dry soil to equilibrate in 100 ml 1 M NH_4OAc-EDTA for 30 min and filtering it over a white ribbon filter (white ribbon, MN 640 m, Macherey-Nagel, Düren, Germany). Elemental analysis was performed with using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Nutritive P content was determined by colorimetry (Jenway 6400 spectrophotometer, Jenway, Essex, UK) using the method of Scheel a NH_4OAc-EDTA extract (Van Ranst *et al.*, 1999).

For each soil sample the corresponding legal threshold limits (mg kg^{-1}) for remediation of agricultural soils were calculated according to the Flemish regulations (VLAREBO, 2009, as explained in 1.3.2) using the measured pH and an organic matter content as determined in Chapter 3.

6.2.3. Plant analysis

Plant samples collected in the field were subdivided in stem, leaves, bract, rachis and grain, weighed for determining fresh weight biomass production, washed with distilled water, and oven-dried at 50 °C (Dieser Memmert-Schrank, Germany). The individual plant parts were pulverised using a Culatti DCFH 48 grinder and sieved over a 1 mm sieve. Ground plant samples were ashed at 450 °C. The ash was dissolved in nitric acid (Van Ranst *et al*., 1999) before element analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA) and inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, PerkinElmer, Waltham, MA, USA). With known dry biomass and metal concentration in the different plant compartments, the average metal content of the whole plant was calculated.

6.2.4. Batch tests for determination of biogas production potential

For the purpose of this experiment, samples of the ensilaged maize from the field experiment (Chapter 4) were taken and transported to OWS (Organic Waste Systems, Ghent, Belgium) for determination of its biogas production potential. Samples were compared with the biogas production of reference maize silage (maize grown on a non-contaminated soil), which was collected by OWS. Batch tests for determination of the biogas production potential were based on ISO, 15985, ASTM D 5511-94 and DIN 38414 and consisted of adding a small amount of substrate (50-100 g fresh material) to a large amount of active inoculum (1 kg fresh material) which was stabilised prior to the start of the test. After thorough stirring 2 L reactors were filled with the mixture. It was fermented at 52 °C during 14 days (Figure 6.1).

The biogas production was measured by volumetric displacement of water. Produced volumes were converted into standard conditions of temperature and pressure (temperature=273 K, atmospheric pressure=1 013 hPa). By comparison with reference reactors with only inoculum, the net production of biogas was calculated in Nm^3 kg^{-1} of substrate. The Biogas Production Potential of the sample equals the net biogas production after digestion during 7 days, expressed in Nm^3 biogas ton^{-1} fresh sample.

Figure 6.1. Experimental setup of batch tests for determination of biogas production potential (photo's: Peene, A., OWS, Belgium)

From each silage also a subsample was oven-dried at 50 °C (Dieser Memmert-Schrank, Germany, and then pulverised using a Culatti DCFH 48 grinder and sieved over a 1 mm sieve for Cd and Zn analysis as mentioned in 6.2.3.

6.2.5. Economic feasibility study

For the economic analysis, consider farmers who are growing maize, as fodder for their cattle and pigs, with an average acreage of 40 ha, consisting of 20 ha grass and 20 ha fodder maize. These 20 ha fodder maize were replaced by energy maize. This energy maize was then considered to be sold for energy conversion purposes. non-contaminated fodder maize for cattle and pigs is bought from outside the contaminated area. As a result, agricultural yearly net income per hectare (R) will be diminished with purchase and transport costs of fodder maize and supplemented with revenues from selling energy maize (Thewys *et al*. (2010a,b)).

The change in revenue (R) from remediation activities per hectare including the compensation for transport costs is given by:

$$R = (P + D_e \cdot T) \cdot mE - (P + D_f \cdot T) \cdot mF + S \qquad \text{(Eq. 6.1.)}$$

With (i) P the price of fodder and of energy maize per ton fresh material (€ ton^{-1} FM), in the deterministic case assumed equal for both energy and fodder maize; (ii) mE and mF the fresh yield of energy and fodder maize respectively, per hectare (ton FM ha^{-1}); (iii) S the energy premium per hectare (€ 45 ha^{-1}); (iv) D_e the distance from the farm to a central digester, (v) D_f the distance of maize field from a clean region to the farm; and (vi) T the transport cost per ton per kilomter (€ ton^{-1} km^{-1}). Detailed information and calculations can be found in Thewys *et al*. (2010b).

Deterministic calculations (*i.e.* using deterministic values for each of the variables) were performed using a spreadsheet (Excel, Microsoft Inc.). To take into account uncertainty concerning the value of variables, Monte Carlo simulation was performed by means of the software Crystal Ball (Decisioneering Inc.). A run in this simulation calculates the change in net agricultural income (R) according to values randomly taken from a predefined value range of the variables. Ranges were defined as the most likely value (*i.e.* the deterministic value) ±10%. The distributions of the variables have a triangular shape as there is insufficient data to fit any other distribution but the minimum, maximum and most likely values are known or presupposed based on expert information. Performing numerous runs (in our study 20000), this technique calculates numerous income changes (R), resulting in a distribution of income changes together with the probability to obtain a positive income change (Prob(R>0)) resulting from the conversion from fodder to energy maize.

6.2.6. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. For statistical analysis, normality was checked using a Kolmogorov-Smirnov test. Significance of observed differences was assessed by using an ANOVA combined with a Tukey HSD test (when homoscedasticity was found) or with a Dunnet C test (when no homoscedasticity was found). Differences between the biogas production potential of energy maize from a contaminated and a non-contaminated soil was checked using a T-test.

6.3. Results

6.3.1. Soil properties

Total and extractable (except N) contents of main elements are presented in Table 6.2. Based on content of the main elements in the NH_4OAc-EDTA-extract and the CEC (Cfr. Chapter 3), the nutritive state of the soil was evaluated as 'high' (Van Ranst *et al.,* 1999). Site-specific remediation criteria for elements in Flanders (VLAREBO, 2009) have been adapted in 2007 en 2008 to include soil pH because of its impact on metal mobility and associated environmental risks (Meers *et al.*, 2010). The elevated metal concentrations in soils (Table 6.3) may result in elevated metal concentrations in plants and therefore present a risk of metal transfer to the food chain (Vangronsveld *et al.*, 1995a; Smeets *et al.*, 2005). The concentrations of metals in the *aqua regia* and in the $CaCl_2$-extract (Table 6.3) showed no significant differences between the sampled plots and were similar to previous experiments (Chapter 4,5).

The concentrations of Cd and Zn in the *aqua regia* extracts were 5.3±1.5, 179±36 and 289±15 mg kg^{-1} respectively. No significant differences were found between the pH of the different plots and the pH-H20 was on average 6.3±0.2 and pH-KCl was on average 5.6±0.3. The pH-H_20 was slightly lower to the previous year (Cfr. chapter 5 where an average pH of 6.8±0.3) and thus liming prior to the sowing was not necessary. In the current experimental site, Cd exceeded the legal criterion of the Flemish government (VLAREBO, 2009), while the concentrations of Zn were slightly below these legislation threshold values. No significant differences where found between the streshodl values of the different plots and was therefore set at 2.5±0.3 mg Cdand 350 mg Zn kg^{-1} dry soil. The concentrations in the $CaCl_2$-extract (0.42±0.11 for Cd and 19±5 for Zn) were 5-17 for Cd, and 4-15% for Zn of their concentrations measured in the *aqua regia* extract.

Table 6.2. Total concentration of N, P, Ca, Mg, K (mg kg^{-1}) (X_{tot}) and nutritive state of P, Ca, Mg, K and Na (mg kg^{-1}) (X_{nutr}) using a NH_4OAc-EDTA extraction for the different test plots (mean ± standard deviation, no significant differences were found between different treatment at α=0.05, n=3)

	N_{tot}	P_{tot}	P_{nutr}	Ca_{tot}	Ca_{nutr}	Mg_{tot}	Mg_{nutr}	K_{tot}	K_{nutr}	Na_{nutr}
Z1	683±131	656±70	71±20	1842±48	769±76	284±12	27±10	367±40	54±9	14±4
Z2	756±92	661±90	96±23	1941±297	940±268	289±9	35±7	370±7	62±10	23±14
Z3	919±145	739±73	119±18	2321±207	1267±78	251±25	44±7	299±44	58±10	20±11
Z4	799±137	665±62	89±16	2077±409	902±107	276±15	30±2	378±12	66±14	14±2
Z5	843±147	762±100	116±22	2164±167	1083±233	305±36	34±3	355±4	66±20	13±1
Z6	881±91	711±56	105±16	2001±144	924±112	262±34	36±2	340±38	57±9	14±1
Z7	955±3	775±85	137±22	2361±193	1091±65	266±3	43±12	364±54	88±46	23±10
General	827±133	707±790	103±26	2088±265	992±205	277±26	35±8	353±37	63±17	17±8

Table 6.3. Soil total and $CaCl_2$-extractable metal content (mg kg^{-1}) for the different test plots, together with the legal limits for remediation of agricultural soils according to the Flemish regulations (VLAREBO, 2009) (mean ± standard deviation, no significant differences were found between different treatment at α=0.05, n=6)

	Cd			**Zn**		
	Aqua regia	**$CaCl_2$**	**threshold**	***Aqua regia***	**$CaCl_2$**	**threshold**
Z1	5.0±1.2	0.46±0.10	2.4±0.2	270±30	22±6	342±13
Z2	5.0±1.4	0.38±0.13	2.4±0.3	279±55	17±6	345±21
Z3	6.5±2.2	0.42±0.15	2.7±0.1	339±105	18±6	361±5
Z4	5.1±1.9	0.37±0.11	2.7±0.2	275±65	16±4	344±15
Z5	5.7±1.7	0.43±0.09	2.4±0.3	298±85	18±5	360±16
Z6	5.5±0.4	0.45±0.09	2.5±0.3	292±28	21±6	349±17
Z7	4.7±0.7	0.42±0.06	2.5±0.2	273±25	21±2	349±12
General	5.3±1.5	0.42±0.11	2.5±0.3	289±62	19±5	350±15

6.3.2. Field experiment

The total fresh weight varied from 48±4 (Z5) to 68±12 ton ha^{-1} (Z4), with an average value of 53±10 ton ha^{-1}. The fresh biomass was mainly determined by the biomass of the stem and leaves (57±7%) (Figure 6.2). Biomass production was higher than measured in 2006 in Chapter 4. The lower productivity in 2006 might have been due to the relatively low fertilisation regime (50 kg ha^{-1} N) against 170 kg N ha^{-1} in the current setup. The dry matter content showed no significant difference between the different varieties (Figure 6.3). Only a small, but significant difference was found in the dry matter content of leaves and bract. Similar differences were observed for the dry mass yield. Z4 showed the highest dry matter yield (23±3 ton ha^{-1}).

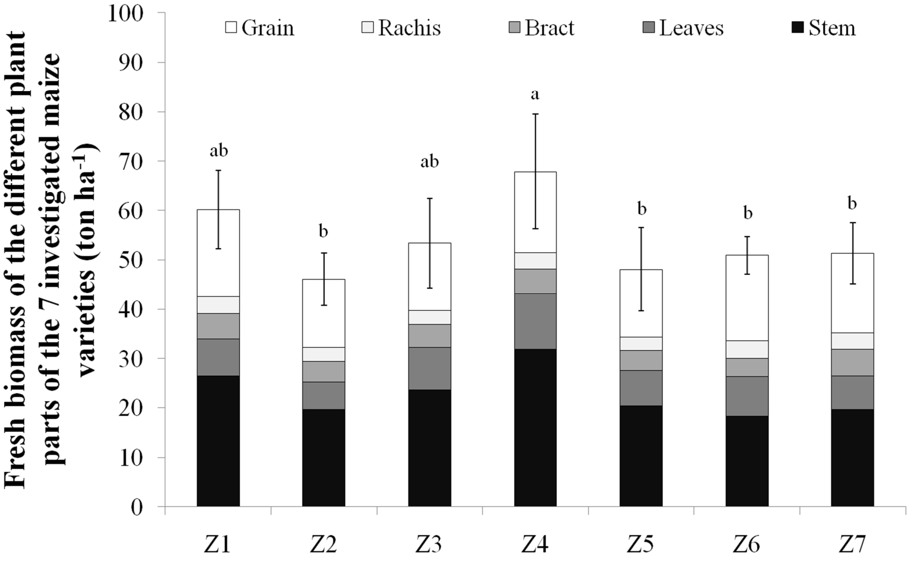

Figure 6.2. Fresh weight (ton ha^{-1}) of the different plant parts of the seven investigated maize varieties (Z1-Z7) (mean vales, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=6)

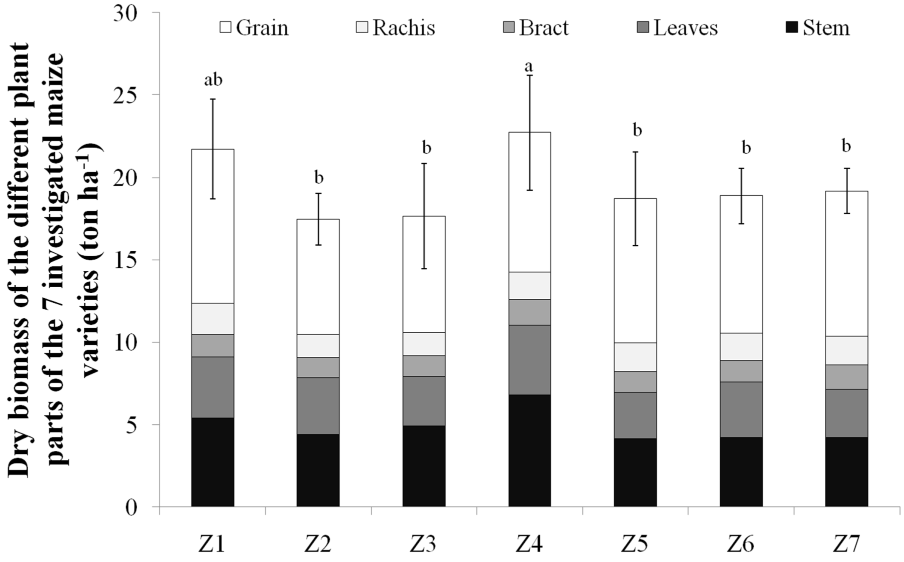

Figure 6.3. Dry weight (ton ha^{-1}) of the different plant parts of the seven investigated maize varieties (Z1-Z7) (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=6)

Table 6.4. Cd (mg kg^{-1} dry matter) and Zn (g kg^{-1} dry matter) in the different plant compartments (stem, leaves, bract, rachis and grain) and the total plant for the seven different varieties, European maximum levels for animal feed equal 1.14 for mg kg^{-1} Cd and 11.4 mg kg^{-1} for Pb (mean ± standard deviation, different letters represent different significance level of concentration per cultivar at α=0.05, n=6)

		Z1	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**
Cd	**Stem**	0.81±0.34a	1.7±1.3	0.85±0.45	0.93±0.32a	0.90±0.13a	0.89±0.09	1.1±0.6a
	Leaves	2.9±0.6a	3.5±0.4	2.2±0.9	2.7±0.9a	2.7±1.0a	3.5±0.6a	3.2±0.5a
	Bract	0.67±0.18b	0.68±0.22b	0.36±0.09c	0.43±0.09bc	0.53±0.13bc	0.71±0.11ab	0.88±0.16ab
	Rachis	0.33±0.33a	0.35±0.19a	0.07±0.05a	0.14±0.07a	0.20±0.13a	0.62±0.47a	0.41±0.29a
	Grain	0.21±0.03bc	0.27±0.02ab	0.21±0.01c	0.36±0.06a	0.30±0.16abc	0.034±0.023d	0.059±0.045d
	Total	0.89±0.21a	1.4±0.4a	0.81±0.24a	1.0±0.3a	0.99±0.19a	0.84±0.17a	0.87±0.17a
Zn	**Stem**	157±26a	398±92a	267±92a	244±79a	341±26a	299±22a	315±97a
	Leaves	47±74b	492±115ab	443±125b	509±126ab	542±137ab	709±136a	489±88ab.
	Bract	29±37ab	223±62bc	207±41bc	192±44c	266±66abc	277±26abc	314±49a
	Rachis	19±95a	105±16a	128±42a	93±11a	147±32a	223±130a	210±78a
	Grain	65±7a	51±3c	51±9bc	51±9c	57±8abc	64±5ab	64±5ab
	Total	211±19a	238±38a	198±47a	204±40a	254±40a	218±34a	217±34a

No significant differences in Cd and Zn concentrations were found between the different maize varieties (Table 6.4). The Cd and Zn concentrations were always highest in the leaves followed by the stem. The lowest Cd and Zn concentrations were always found in the grains. As metal concentrations and the biomass production for the different plant parts are known, the potential removal rate of Cd and Zn using energy maize can be calculated (Figure 6.4 and 6.5). For the stem and the leaves, no significant differences were found in the Cd and Zn removal rates although differences in concentrations and biomass were found.

For the stem the mean removal rate was 5.0±3 g Cd ha^{-1} year^{-1} and 1.5±0.4 kg Zn ha^{-1} year^{-1}. For the leaves, overall removal rates were 10±4 g Cd ha^{-1} year^{-1} and 1.7±0.6 kg Zn ha^{-1} year^{-1}. For the bract and the rachis, only significant differences were found for Cd by the bract. This removal rate ranged from 0.45±0.17 (Z3) to 1.3±0.3 (Z7) g Cd ha^{-1} year^{-1}. The removal rates of Zn by the bract was on average 0.33±0.11 kg Zn ha^{-1} year^{-1}, while the removal rate of the rachis was 0.57±0.53 g Cd, ha^{-1} year^{-1} and 0.27±0.17 kg Zn ha^{-1} year^{-1}. The potential metal removal rate through the grains ranged from 0.36±0.12 (Z3) to 1.1±0.8 (Z6) g Cd ha^{-1} year^{-1}, and 0.35±0.07 (Z2) to 0.62±0.16 (Z1) kg Zn ha^{-1} year^{-1}.

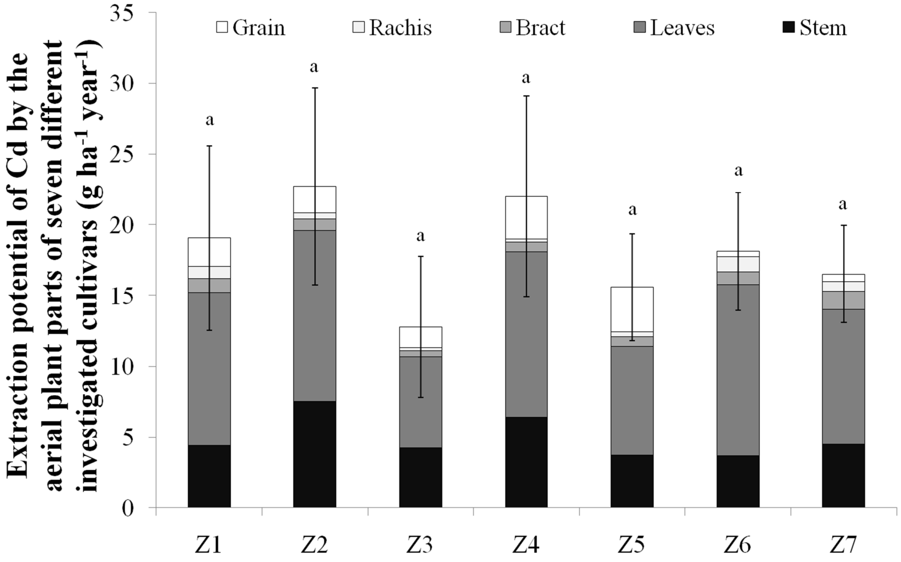

Figure 6.4. Extraction potential of Cd (g ha^{-1} year^{-1}) by the different aerial plant parts of the seven investigated maize varieties (Z1-Z7) (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=6)

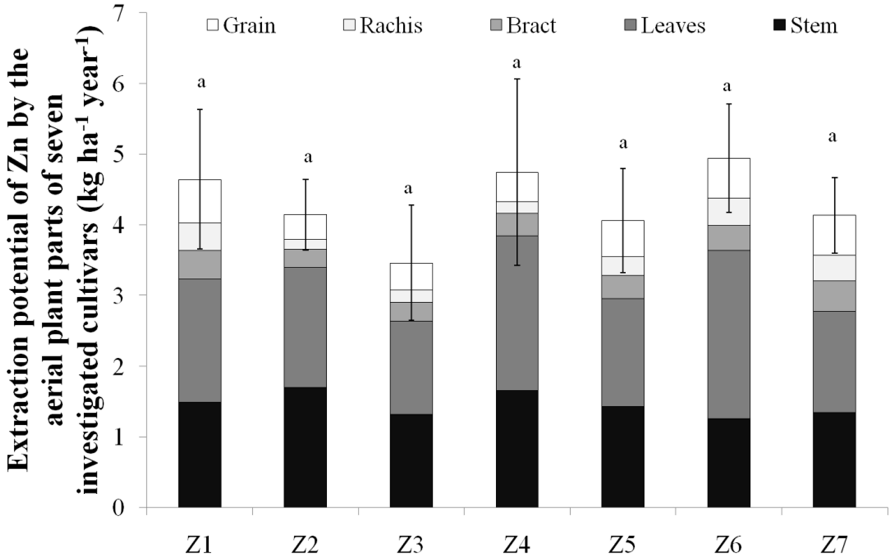

Figure 6.5. Extraction potential of Zn (kg ha^{-1} year^{-1}) by of the different aerial plant parts of the seven investigated maize varieties (Z1-Z7) (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n =6)

6.3.3. Biogas production potential

The dry matter content, N-concentration and C/N ratio are different, but similar. Although the Cd and Zn concentration significant differences were found between silage material originating from contaminated and non-contaminated land (Table 6.5), there was no difference in potential biogas production (Nm^3 ton^{-1} FM for maize grown on a metal contaminated site and Nm^3 ton^{-1} FM for energy maize grown on non-contaminated soil (Figure 6.6) .

Table 6.5. Dry matter content (DM), N-concentration, C/N-ration of the silage material (data from OWS, 2007), together with the Cd and Zn concentration (mean ± standard deviation, different letters represent different significance level of concentration per cultivar at α=0.05, n=3)

	DM (%)	**N** (g kg^{-1})	**C/N** (g kg^{-1})	**Cd** (g kg^{-1})	**Zn** (g kg^{-1})
Non-contaminated	39±1a	12±1a	41	0.081±0.042a	19±0a
Contaminated	45±1b	8.3±0.4b	58	0.96±0.29b	219±39b

This finding was investigated in further detail by measuring the biogas potential production of the different plant compartments from Z1 and Z6. The biogas production potential was mainly attributed to the grains, representing ±56% of the total biogas potential production. The bract and the rachis are representing respectively ~7 and ~9% of the total biogas potential production. When the biogas potentials of the different plant parts are added, it can be concluded that Z1 had a biogas potential of 209 and Z6 of 198 Nm^3 ton^{-1} FM.

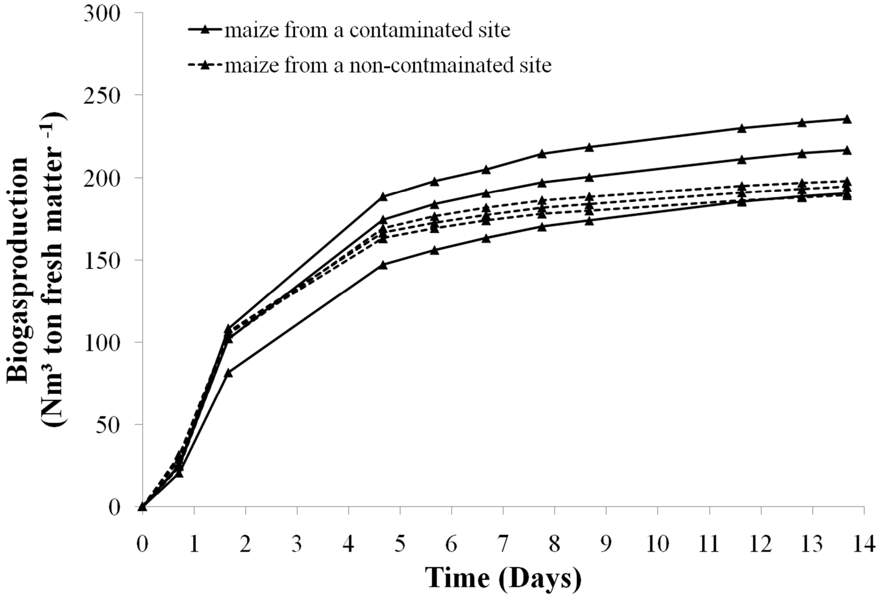

Figure 6.6. Biogas production potential of energy maize grown on a contaminated soil (solid line) and from maize growing on a non-contaminated site (dotted line) in Flanders (n=3)

6.3.4. Economic analysis

Reclamation of the soil is only economically viable if revenues from selling contaminated energy maize exceed costs of buying clean fodder maize. This depends on the relative yield of fodder and energy maize, their prices and the transport cost. In September 2008, farmers received a price (P) of € 30 ton^{-1} FM. The production cost of maize is approximately € 1200-1250 ha^{-1}. At fresh yields (mF and mE) of respectively 50 and 60 ton ha^{-1}, fodder and energy maize will therefore not be sold below their respective production cost of € 24-25 ton^{-1} FM and € 20-21 ton^{-1} FM. The farmer received in 2008 and 2009 a support (S) of maximum € 45 ha^{-1} for growing energy crops from the Agency for Agriculture and Fisheries (ALV). Transport costs (T) are assumed € 0.5 ton^{-1} km^{-1}. When 15 farmers convert from fodder to energy maize, 300 ha of energy maize will be established and 300 ha of fodder maize will need to be bought outside the contaminated area. Accordingly D_e=1.38 km and D_f= 8.8 km (Thewys *et al.*, 2010b). This results in a deterministic positive income change for the farmer of R= € 166.5 ha^{-1}. Detailed information and calculations can be found in Thewys *et al.* (2010b).

To estimate uncertainty intervals, these deterministic values are substituted by a range of values according to a predefined probability distribution, as explained in 6.2.5. In the base case, this results in a most likely effect on income of € 113.8 ha^{-1}, with Prob(R>0) = 82.6%. First, we analyzed the effect of relative yields of energy to fodder maize on the net income change. In the base case, energy maize (mE) has a minimum yield which is the same as the yield of fodder maize (mF). A 20% higher yield is most likely, and a maximum is 30% more yield, resulting in ratios mE/mF ranging from 1 over 1.2 to 1.3. In the pessimistic scenario, the relative yields of mE to mF (min.; average; max.) are less positive than in the base case, resulting in ratios mE/mF of 1; 1.1 and 1.2. Keeping mF at the value of the base case (50 ton ha^{-1}), the extra revenue R drops to € 12.5 ha^{-1}. In the optimistic scenario, the minimum yield of energy maize is at least 10% higher than fodder maize (ratios of 1.1; 1.2; 1.3). This has a positive impact on the extra revenue per hectare, which is now € 166.5 ha^{-1}. This shows that growing energy maize has a reasonable chance to be economically viable.

Second, we analysed the effect of price changes. The average extra income per hectare R (€ ha^{-1} year^{-1}) was calculated together with the probability of a positive extra income (Prob(R>0)), given that prices of energy and fodder maize are equal (P in € ton ^{1} FM), *ceteris paribus*. In the base case (*i.e.* at a most likely price of maize of € 30 ton^{-1} FM), R= € 113.8 ha^{-1} and Prob(R>0) = 82.6%. The minimum price is set at € 24 Mg^{-1} FM which is equal to the production cost per t fodder maize. When the farmer grows energy maize, he has a fair chance (75%-87%) to sustain and even increase his income, depending on the height of the prices (now assumed equal for energy and fodder maize), within the assumed range of € 24 to € 36 ton^{-1} FM.

In a following step the assumption that prices of fodder and energy maize are equal was discarded. The price range of energy maize and average R were calculated, given mF=50 and mE/mF as in the base case (1; 1.2; 1.3), for different price ranges of fodder maize, to have at least a 90% probability of a positive R (Prob(R>0) ≈ 90%). This was done for three price ranges of fodder maize, resulting in three price ranges for energy maize and three different R's (each with a probability of 90%). This means for example that when price ranges for fodder maize are € 24±10%, price ranges of energy maize should be € 25.5±10% to have a probability of 90% to at least maintain the level of income as before the switch, given mF=50 and mE/mF as in the base case (1; 1.2; 1.3). These prices result even in an extra income of € 151 ha^{-1}year^{-1}. Given price ranges of fodder maize of € 30±10%, price ranges of energy maize should be € 31.2±10%, resulting in an extra income of € 184 ha^{-1}year^{-1}.

6.4. Discussion

An average dry yield of 20±3 ton ha^{-1} (dry matter content of 36±6%) was obtained in 2006. This was an optimal yield, similar with that obtained in other screening experiments on non-contaminated soil in the Campine region (Geel) and elsewhere in Flanders (ILVO, 2009; Ghekiere *et al*., 2007). The average concentrations in the ensilaged plant were 0.96±0.29 mg Cd kg^{-1} and 219±39 mg Zn kg^{-1}. The metal concentrations in the aerial plant part were similar to previous findings of maize grown on metal contaminted soils with a full cropping stage of maize (Kurz *et al*., 1999; Lombi *et al*., 2001) especially when the experiments were conducted on soils with a similar contamination (Meers *et al*., 2010). The Cd and Zn concentrations were always highest in the leaves followed by the stem. The lowest Cd and Zn concentrations were always found in the grains. A similar compartmentalisation of Cd and Zn throughout the plant was reported earlier (Sękara *et al*., 2005).

Most of them, except Z2, complied to the European maximum permitted concentration for animal feed of Cd (1.14 mg kg^{-1} for Cd). Concentrations were similar to previous reported studies (Meers *et al*., 2010). Nevertheless, chances for exceeding Cd feed standards are limited, but real. Therefore, it is safer to target the harvested biomass for non-food purposes, as Flemish legal threshold values for metal concentrations in biomass aimed for bioenergy production by means of anaerobic digestion are less strict than for animal feed, at 6 mg Cd kg^{-1} Cd and 900 mg Zn kg^{-1}. This was also addressed in the previous chapter. Studies on the impact of anaerobic digestion of maize containing elevated concentration of trace elements are scarce.

The findings indicate that maize grown on this contaminated soil must preferably be used for biogas production through anaerobic digestion. The biogas potential varied between 180 and 220 Nm^3 ton^{-1} FM, which is the average energy productivity of energy maize under mesospheric conditions (Amon *et al*., 2004, cited from Walla and Schneeberger, 2008; 2007b). Nevertheless, the data are based on a batch test of 14 days. Long term experiments will be addressed in Chapter 7, along with a discussion on the concentration of the elements in the digestate itself.

Total metal removal by crops with a fast growth rate and metal tolerance can equal metal removal of hyperaccumulating plants (Vassilev *et al*. 2002; Meers *et al*., 2005b; Hernández-Allica *et al*., 2008). In this experiment energy maize revealed potential metal removal rates of 19±6 g Cd and 4.3±0.9 kg Zn ha^{-1} $year^{-1}$. Assuming constant extraction rates, this would correspond with an annual reduction in the 25 cm top soil layer of 0.005 mg Cd and 0.3 mg Zn kg^{-1} soil. Thus, to reduce Cd contents of the top soil layer from 5 (current Cd concentration in the field) to 2.5 mg Cd kg soil^{-1} (site specific remediation criteria according to VLAREBO (2009)), approximately 500 years would be needed. These are optimistic estimates as metal uptake efficiency of the crop may not maintain the same efficiency throughout the remediation period (Van Nevel *et al.*, 2007; Fässler *et al.*, 2010). The relative low concentrations of metals in different plant compartments however, are a positive aspect in the context of subsequent safe use of the produced biomass.

Besides the technical feasibility the economic feasibility of switching to energy maize is an important factor in the decision. The economic outlook for farmers who intend to use energy maize on contaminated soils seems to be positive, as the conversion would allow to maintain their average yearly income. Economic aspects are developed in more detail in Thewys *et al* (2010a,b). Research on optimising metal uptake, harvesting and valorising of energy maize will contribute to further optimise the land use. Also other potential energy crops needs integrated (metal uptake, conversion and valorisation study) in order to fully explore their cultivation on metal contaminated soils.

6.5. Conclusion

Under optimal field conditions the biomass productivity didn’t differ significantly with those on non-contaminated sites. Chances for exceeding Cd feed standards are limited, but real. Therefore, it is safer to target the harvested biomass for non-food purposes, such as bioenergy production by means of anaerobic digestion. The biogas production potential of energy maize grown on these soils did not differ from that of energy maize grown on an non-contaminated soil. Moreover, model calculations indicate that the average yearly income of the farmers can be preserved when they convert land use from fodder maize to energy maize. Therefore, the major drawback of energy maize as a phytoremediation crop (long remediation times) becomes less important and the use of energy maize can constitute as a safe and profitable alternative use of these moderately contaminated soils.

CHAPTER 7:

ANAEROBIC DIGESTION OF ENERGY MAIZE (*ZEA MAYS* L.) GROWN ON MODERATELY METAL-CONTAMINATED SOILS

Redrafted from Van Slycken, S., Meers, E., Witters, N., Cornelis, E.. Peene, A., Vangronsveld, J., Thewys, T. and Tack, F.M.G. (2011c). Anaerobic digestion of energy maize (*Zea mays* L.) grown on moderately metal-contaminated soils in the Campine region (Belgium). Bioresource Technology. Submitted for publication.

Abstract

Phytoattenuation is a new domain in phytoremediation research. In this approach the main objective is no longer the remediation of the contaminated land, but the establishment of an economic feasible alternative for using this land. For this purpose, energy maize (*Zea mays* L.) seems attractive since not only the biomass productivity is promising but also the agronomic and economic feasibility of shifting to this non-food crop is favorable. However, the question of the potential impact of the increased amounts of trace elements on biomass conversion remained. Lab scale tests showed that anaerobic digestion of maize containing elevated concentration of trace elements as Cd, Cu, Pb and Zn has a biogas production potential of 191±32 Nm^3 ton^{-1} FM. Semi-continuous test of 435 days delivered similar results (average 195±61 Nm^3 ton^{-1} FM). These results were similar to the biogas potential of maize from a non-contaminated site, which implies that the accumulation of metals in the reactor does not negatively affect biomethanisation processes. The concentrations of metals, expressed per dry matter, in the produced digestate were 3-4 times higher than the concentrations of the element in the input material. Based on this factor, a quality control can be made of the produced byproducts and different valorisation strategies can be considered in order to make energy maize for anaerobic digestion an option for alternative land use of metal contaminated agricultural soils.

7.1. Introduction

Energy maize is considered as a predominant crop for biogas production, not only because it has the highest yield potential between field crops in Central Europe but also because energy maize has a high energy production potential (Amon *et al*., 2007a; Braun *et al*., 2008). The produced biomass can be used for conversion into biogas through anaerobic digestion, which is the process of decomposition of organic matter into methane and CO_2 by a microbial consortium in an oxygen-free environment (Bridgewater, 2006). This technique can be employed to convert plant biomass, crops, crop residues, sludge, manure, *etc*. into methane rich biogas (Lehtomäki *et al*., 2007). This biogas can *e.g.* be used to produce heat or electricity or as a vehicle fuel and it therefore increases the share of renewables in electricity production and meets, among others, the targets of the Kyoto protocol (1997) (DEP, 2003). The biogas is mostly used in a gas engine with heat recovery, a Combined Heat and Power engine (CHP) (Thewys *et al*., 2010a). In the previous chapters it was concluded that energy maize on metal contaminated agricultural soils is not only showing good results concerning biomass productivity, but also the biomass treatment for biogas production is promising.

Concerning anaerobic digestion, the presence of metals can inactivate a wide range of enzymes by reacting with their sulphydryl groups or replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulner, 1972, cited from Chen *et al*., 2008; Shen and Kosaric 1994, cited from Sanchez *et al*., 1996). Studies on the impact of anaerobic digestion of maize containing elevated concentration of trace elements are however scarce. Most of the studies are focusing on potential detrimental effects as a lack of trace elements results in a decrease of biogas production (Zandvoort *et al*. 2006; Clemens, 2007 both cited in Probeheim *et al*., 2010). The previous chapter revealed no impact of the presence of the elevated concentration on the biogas production but effects on the long term were not included. The main objectives of this chapter are therefore (i) to investigate the biogas production potential of maize originating from a contaminated soil into more detail by using lab scale batch and a semi-continuous test and ii) to study the concentration and augmentation of nutrients and other trace elements in the digestate during a semi-continuous test.

7.2. Material and Methods

Samples of the maize grown on a metal-contaminated soil were collected from the silage of of the harvested maize of Chapter 6. As a reference, maize was collected by OWS (Organic Waste Systems, Ghent, Belgium) on a non-contaminated site in Flanders, similar to the one that was also used in Chapter 6.

7.2.1. Lab scale batch reactor tests for determination of the biogas production potential

At regular time intervals during the course of the long term semi-continuous experiment, the biogas production potential of the input material was determined. The batch tests for determination of the biogas production potential were based on ISO, 15985, ASTM D 5511-94 and DIN 38414 and consisted of adding a small amount of substrate (50-100 g fresh material) to a large amount of active inoculum (1 kg) which was stabilised prior to the start of the test. After thorough stirring, 2 L reactors were filled with the mixture. It was fermented at

52 °C during 14 days. The biogas production was measured by volumetric displacement of water. Produced volumes were converted into standard conditions of temperature and pressure (temperature=273 K, atmospheric pressure=1 013 hPa). By comparison with reference reactors with only inoculum, the net production of biogas was calculated in Nm^3 kg^{-1} of substrate. The Biogas Production Potential of the sample equals the net biogas production after digestion during 7 days, expressed in Nm^3 biogas ton^{-1} fresh sample.

7.2.2. Semi-continuous reactor tests for determination of the biogas production potentials and the metal concentration in the digestate

Experimental set-up

The semi-continuous batch test was performed in a reactor of 25 kg. This reactor was fed three times a week with a load of 2.5 kg per week. The amount of input was calculated based on the results of the small scale batch test in order to achieve a constant biogas production. On a daily basis the biogas production was measured by volumetric displacement of water and the volume was converted into standard conditions of temperature and pressure. On given intervals (after 0, 106, 234, 303, 435 days) samples of the digestate were analysed for their Cd, Cu, Pb and Zn content, as well as for nutrient content (N, P, K, Na and Ca). The concentration at day 0 equals the concentration of those metals after a continuous run of feeding the small scale reactor with the maize from the non-contaminated site. By comparing the results of day 0 and day 435 an estimation of the accumulation level of trace elements in digestate was made. On these samples nutrient levels were determined as well.

In order to estimate this accumulation, a sigmoid curve was fitted to the measured data. This sigmoid curve is described by following equation (Eq. 7.1.) (Tichopad *et al.*, 2002):

$$g(t, c_0, t', a, b,) = c_0 + \frac{a}{1+\exp\left(-\left(\frac{(t-t')}{b}\right)\right)} \qquad \text{(Eq. 7.1)}$$

With t the time after start of adding the contaminated material; c the concentration of the metal at time t; c_0 the concentration at time 0 while at time t' the inflection point of the curve is achieved. Parameter a, describes the differences between the concentration at steady state and the initial concentration, while parameter b describes the slope of the curve (Tichopad *et al.*, 2002; Spiess *et al.*, 2008).

Trace element and nutrient analysis

Samples of the input materials and the digestate were oven-dried at 50 °C (Dieser Memmert-Schrank, Germany). Dried samples were pulverised using a Culatti DCFH 48 grinder and sieved over a 1 mm sieve. Ground plant material was ashed at 450 °C and dissolved in nitric acid before element analysis. Metals, K, Na and Ca were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA) and inductively coupled mass spectrometry (ICP-MS, ELAN DRC-e, Perkin Elmer, Waltham, MA, USA) for analysis of Cd. Nitrogen concentration was determined using a Kjeldahl destruction (Van Ranst *et al.*, 1999). Phosphorus content was determined by colorimetry (Jenway 6400 spectrophotometer, Jenway, Essex, UK) using the method of Scheel on the *aqua regia* destruction (Van Ranst *et al.*, 1999).

7.2.3. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. For statistical analysis, normality was checked using a Kolmogorov-Smirnov test. Differences between the silage/digestate of maize from the contanminated and the non-contaminated site were tested using T-tests.

7.3. Results

7.3.1. Silage material

When comparing test silage material originating from contaminated and non-contaminated land, significant differences in moisture content were found. Although concentrations of Ca, Na and N were in the same order of magnitude, concentrations in the silage from the non-contaminated site were significantly higher (Table 7.1). When trace elements like Cd, Cu, Pb and Zn are considered, clear significant differences were observed between the reference silage and the silage of the maize grown on the contaminated soil (Table 7.2).

Table 7.1. The dry matter (DM) content (%) and the concentration of nutrients (N, P, K, Na and Ca) measured in the silage (S) and digestate (D) derived from energy maize from a non-contaminated site of OWS and from a contaminated area in Lommel (Belgium) (mean ± standard deviation, different letters represent different significance level at α=0.05, n=3)

		DM (%)	**N ($g\ kg^{-1}$)**	**P ($g\ kg^{-1}$)**	**K ($g\ kg^{-1}$)**	**Na ($g\ kg^{-1}$)**	**Ca ($g\ kg^{-1}$)**
S	Non-contaminated	31±1a	13±1a	1.8±0.1	33±1a	0.18±0.03a	2.9±0.1a
	Contaminated	24±5b	8.3±0.9b	1.7±0.3	8.4±1.4b	0.10±0.01b	2.5±0.1b
D	Non-contaminated	14±1a	28±1a	7.7±0.8	158±18a	0.40±0.01a	13±1a
	Contaminated	12±1a	26±1a	5.9±0.1	39±1b	0.31±0.02b	7.2±0.3b

Table 7.2. The concentration of Cd, Cu, Pb and Zn (mg kg^{-1}) measured in the silage (S) and digestate (D) derived from energy maize from a non-contaminated reference site of OWS and from a contaminated area in Lommel (Belgium) (mean ± standard deviation, different letters represent different significance level at α=0.05, n=3)

		Cd	**Cu**	**Pb**	**Zn**
S	Non-contaminated	0.081±0.042a	2.2±0.1a	1.2a	19±0a
	Contaminated	1.0±0.1b	5.9±0.6b	7.0±1.1b	257±34b
D	Non-contaminated	0.29±0.02a	11±1a	3.1±1.8a	116±70a
	Contaminated	3.2±0.10a	21±2b	2.0±1.0a	797±27b

7.3.2. Biogas production potentials

The calculated biogas production varied between 161 and 225 Nm^3 ton^{-1} FM with a mean value of 192 Nm^3 ton^{-1} FM. During the semi-continuous test, the biogas production was measured on a daily basis. Over the whole period on average 195 Nm^3 ton^{-1} FM was obtained with a minimum of 137 and a maximum of 258 Nm^3 ton^{-1} FM.

At several intervals (after 0, 106, 234, 303, 435 days) samples of the digestate were analysed for determination of Cd, Cu, Pb and Zn concentrations (Figure 7.1 for Cd, 7.2 for Zn, 7.3 for Cu and 7.4 for Pb). At the end of the semi-continuous batch experiment, not only the biogas production showed stable results but also the concentration of Cd, Zn and Pb. At t_{435} the concentrations of Cd, Cu, Pb and Zn were respectively 3.2±0.1, 21±2, 20±1 and 800±34 mg kg^{-1} (Table 7.2). This means that the concentrations for Cd, Cu, Pb and Zn were resp. 3.2±0.5, 3.6±0.5, 2.9±0.5 and 3.1±0.4 times higher than those from the input material.

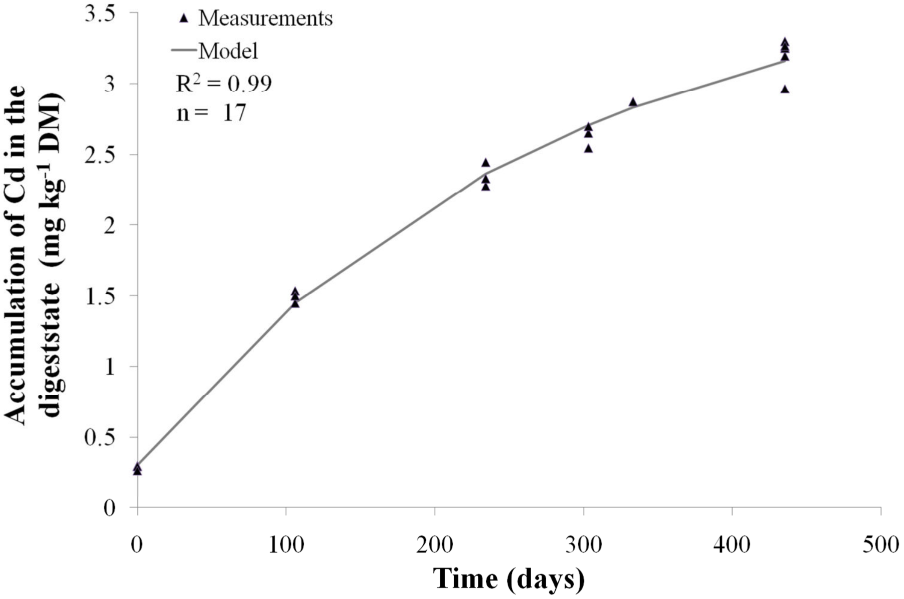

Figure 7.1. Evolution of the measured and the modulated concentration of Cd (mg kg^{-1} DM) at several intervals during the semi-continuous batch test

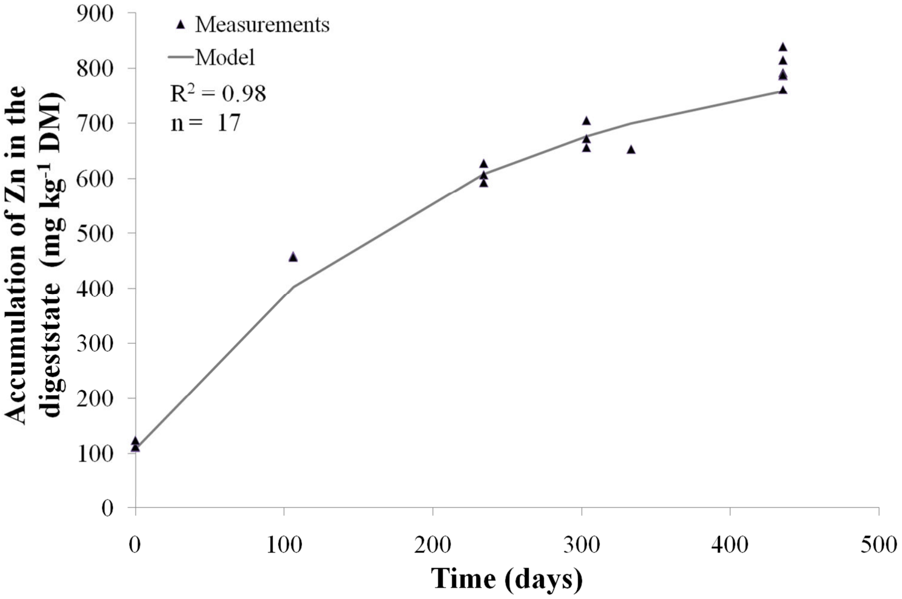

Figure 7.2. Evolution of the measured and the modulated concentration of Zn (mg kg^{-1} DM) at several intervals during the semi-continuous batch test

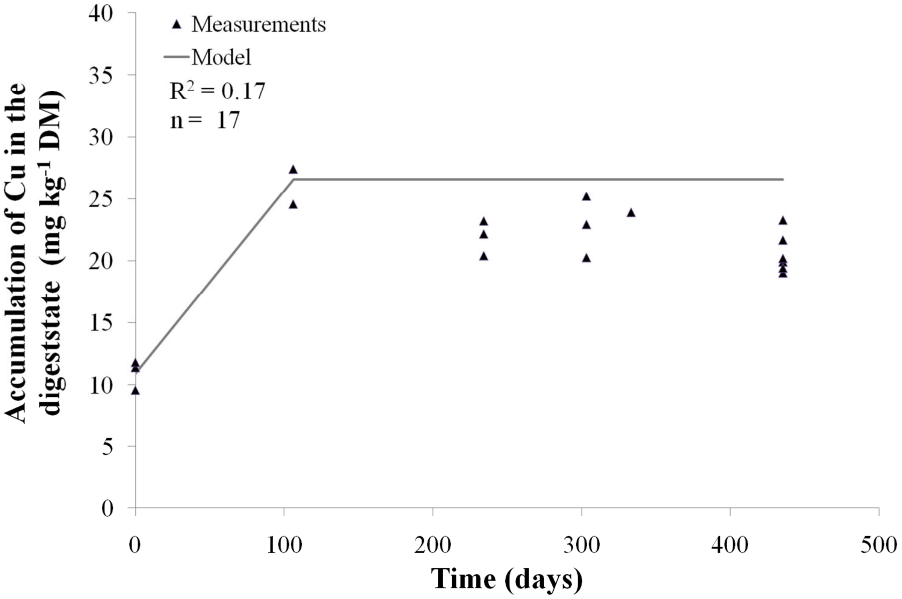

Figure 7.3. Evolution of the measured and the modulated concentration of Cu (mg kg^{-1} DM) at several intervals during the semi-continuous batch test

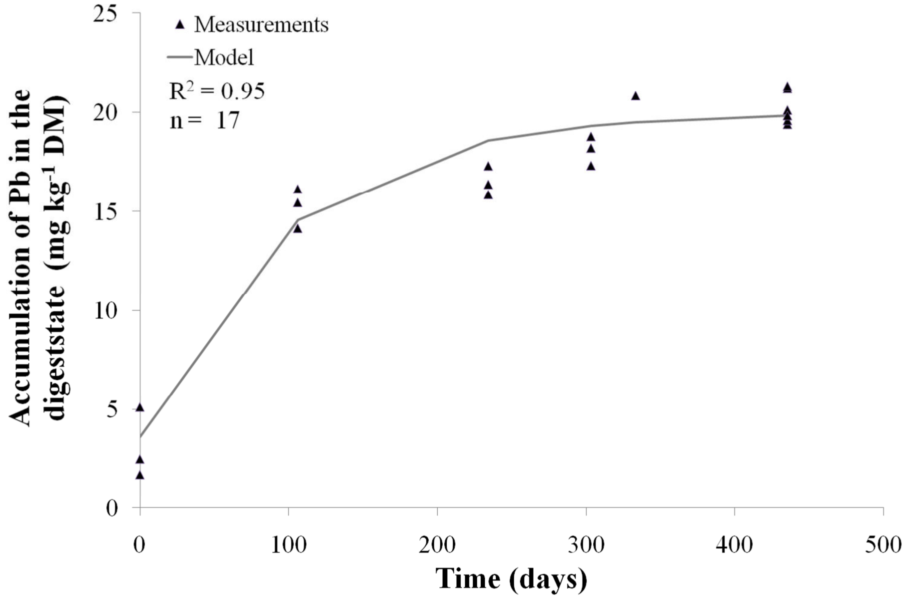

Figure 7.4. Evolution of the measured and the modulated concentration of Pb (mg kg^{-1} DM) at several intervals during the semi-continuous batch test

In order to get an estimation of the concentration at steady state a sigmoid curve was fitted to the measured data. According to the obtained modulated curves, the steady state concentration should be 3.9 mg kg^{-1} for Cd, 27 mg kg^{-1} for Cu, 20 mg kg^{-1} for Pb and 800 mg kg^{-1} for Zn. When using the fitted results, concentrations in the digestate are for Cd, Cu, Pb and Zn respectively 4.0±0.5, 4.6±0.4, 2.9±0.5 and 3.3±0.4 higher than the input.

When the concentrations of the nutrients (N, P, K, Na, Ca) in the digestate were compared at time t_0 and t_{435}, significant differences were observed (Table 7.1). Nevertheless, results were in the same order of magnitude. When comparing the concentration of the feed to the concentration in the digestate, the concentrations were, about 3 to 5 times higher, similarly as for the metals studied.

7.4. Discussion

Phytoattenuation is a new approach within the phytoremediation domain. In this approach, emphasis is no longer on the remediation of the contamination itself, but on the establishment of an economical feasible alternative for safe use of contaminated soils (Vangronsveld *et al.*, 2009; Meers *et al.*, 2010). Results from previous chapters showed very promising results of the possibilities of energy crops as potential phytoattenuation crop, from the technical and economical (Thewys *et al.*, 2010a,b) pont of view.

Nonetheless, the treatment of the biomass itself must also be safe and environmentally sound. Anaerobic digestion has been shown to be one of the most energy-efficient and environmentally beneficial technologies for bioenergy production (Berglund and Börjesson, 2006; Weiland, 2010). However, the presence of metals may inactivate a wide range of enzymes by binding to their sulphydryl groups or replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulner, 1972, cited from Chen *et al.*, 2008; Shen and Kosaric 1994, cited from Sanchez *et al.*, 1996). Studies on the impact of anaerobic digestion of maize containing elevated concentrations of trace elements are scarce. Most of these investigations focus on potential detrimental effects when deficiencies of trace elements results in a decreased biogas production (Zandvoort *et al.* 2006; Clemens, 2007 both cited in Probeheim *et al.*, 2010). For example much emphasis has been placed on the study of the impact of Mo, Ni and Co, which plays a crucial role in growth and metabolism of anaerobic microorganisms (Takashima and Speece, 1989; Goodwin *et al.*, 1990 both cited in Probeheim *et al.*, 2010).

The current study, aims to examine the impact of elevated concentrations of metals from phytoremediation biomass on the productivity of anaerobic microorganisms. The calculated biogas production potentials from the small scale batch test were 191±32 Nm^3 ton^{-1} FM. This was similar to those of the semi-continuous batch test (195±61 Nm^3 ton^{-1} FM). These values are similar to those reported in the previous chapter and to other experiments (Schittenhelm, 2008). When the total yield of the maize is known, energy yield per hectare can be calculated (Lehtomäki *et al.*, 2007). As the total dry matter yield of maize was 53±10 ton ha^{-1}, the total biogas yield can be estimated at approximately 10 000 m^3 ha^{-1}. This is also comparable with the total energy yields of maize after anaerobic digestion (58-60%) (Chynoweth *et al.*, 1993; Amon *et al.*, 2007a). The methane content in the biogas of the contaminated silage is 56±1% and is therefore not only comparable with the methane content in the reference silage

(59±1%) (used in Chapter 6), but also with those of other maize silages in bio-methanisation studies (Chynoweth *et al*., 1993; Amon *et al*., 2007a). Therefore it can be concluded that build-up of metals in the reactor did not result in an observed decrease of biogas production, suggesting that the increased concentrations had very limited to no effects on biomethanisation processes. Chynoweth *et al.* (1993), who analysed biogas production potential from waste feedstock, obtained similar results with small scale batch tests and with a semi-continuous test.

As metals are non-degradable, they will accumulate in the final digestate product (Braun *et al.,* 2008; Selling *et al*, 2008). When a biogas production of 200 Nm^3 ton^{-1} FM, with an input material containing 90% biodegradable volatile solids and 95% volatile solids is considered, a theoretical concentration factor – concentration in digestate over concentration in input- of 5.89 is assumed. When the calculated concentration of the digestate at steady state are applied, the concentration factors of Cd, Cu, Pb and Zn of respectively 4.0±0.5, 4.6±0.4, 2.9±0.5 and 3.2±0.4 higher than the concentration of the input material and are very close to the theoretical estimated concentration values. This is in line with an anaerobic digestion experiment performed by Hons *et al.* (1993), who reported increases of N and P concentrations of 4 to 5 times during anaerobic digestion of sorghum (*Sorhum bicolour* L.). It must however be mentioned that this concentration factor is only applicable when the energy maize is mono-digested. In practice also other types of digestion are possible, *e.g.* co-digestion with different types of manure and different mixtures, one step digestion, two step digestion, … (*e.g*. Gunaseelan, 1997; Faaij, 2006). This will automatically influence the final metal balance.

The quality analysis and characterisation of the digestate is crucial for the valorisation of the produced digestate. It is clear that in this study the most problematic elements are Cd, Cu, Pb and Zn. Metal and nutrient concentrations must be compared with the legal threshold values for usage. When the digestate is applied on the land, metals are returned to the land. The legal framework, varies between countries and states. As this research focused on the Campine region in Flanders, Belgium, metal concentrations in the digestate must be in accordance with the Flemish regulations for use as a soil amendment (VLAREA) in respect with values regarding (i) total concentrations of certain elements in the digestate, and (ii) total allowed soil application of these elements per year. The maximum allowable values in the digestate are 6 mg Cd, 375 mg Cu, 300 mg Pb and 900 mg Zn kg^{-1} dry matter. Maximum allowed application by digestate is 12 g Cd, 750 g Cu, 600 g Pb and 1800 g Zn ha^{-1} $year^{-1}$. However, transportation of significant amounts of non-essential elements such as Cd and Pb to other regions is not desirable. Further research is required towards techniques to treat digestate with elevated metal concentrations in a sustainable way or to manage the sequestration of metals in the different steps of the digestate production (Meers *et al*., 2010).

Application of Cu and Zn are less problematic when they are applied at suitable rates for plant growth, since they also represent essential plant nutrients (micro nutrients). However, Cd and Pb are non-essential and their presence in digestate or other types of fertilisers or soil amendments has no added benefits. For these non-essential elements, concentrations preferably remain at concentrations, which are not significantly higher than other types of fertilisers. In comparison to the abovementioned maximum allowable concentrations, none of the metals exceeded threshold limits: Pb and Cu remained at concentrations fifteen times lower than the threshold and Cd two times. Zn accumulation remained below the threshold limit yet approached it towards the end of the experiment.

As mentioned, the concentrations of Cd, Cu, Pb and Zn were higher in comparison to the digestate resulting from maize grown on non-contaminated land. Also in comparison to other soil fertilisers in Flanders (VLACO, 2010), the main conclusion remains the same, as it seems that also here concentrations of Cd, Cu, Pb and Zn were sometimes higher. The observed concentrations of these elements in the digestate from the maize of the contaminated site seem to fall within the range of metal concentrations measured in pure animal manures described by Nicholson *et al*. (1999). However, since the concentration factor during the anaerobic digestion is constant, good control of the usage constraints can be made.

7.5. Conclusion

Small scale batch tests already deliver a good estimate of the biogas production potential during longer experiments. These tests demonstrated a biogas production potential of 191±32 Nm^3 ton^{-1} during small scale tests and 195±61 Nm^3 ton^{-1} during a semi-continuous test of 435 days. The presence of slightly elevated concentrations of trace elements does not influence the potential of anaerobic digestion of maize. During the digestion process elements clearly accumulate in the digestate. Hereby it was found that (i) the accumulation factor (3-4) for maize from contaminated land and from non-contaminated land was similar; and (ii) a similar accumulation factor was observed for nutrients as for metals. Furthermore, the results are similar with those obtained for the biogas potential of maize from non-contaminated land. Using this factor, a simulation of the quality of the digestate can be made and different valorisation strategies can be foreseen.

CHAPTER 8:

AGRONOMIC OPTIMISATION OF ENERGY MAIZE (*ZEA MAYS* L.) AS AN ALTERNATIVE CROP ON METAL CONTAMINATED SOILS

Redrafted from Van Slycken S., Meers, E., Witters N., Peene, A. Vangronsveld J., Thewys T. and Tack, F.M.G. (2011e). Agronomic optimisation of energy maize (*Zea mays* L.) as an alternative crop on metal contaminate soils in the Campine region, Flanders (Belgium). Biomass and Bioenergy. Submitted for publication.

Abstract

Feasibility of maize for energy production depends on variety, location, climate, harvest time, as well as composition, conservation and pre-treatment of the biomass. In the context of energy crops on metal contaminated agricultural soils also metal accumulation must be taken into account. For exploring the impact of fertilisation, four N-regimes (0, 85, 170 and 255 kg N ha^{-1}) were tested on biomass production and metal concentration. Although fertilisation had a major impact on biomass production (32±5 ton FM ha^{-1} when no fertilisation was applied versus 63±15 ton FM ha^{-1} at the highest fertilisation rate), significant differences were only found between the total Cd and Zn extraction potential calculated between maize grown on a non-fertilised soil (13±1 g Cd and 3.3±0.4 kg Zn ha^{-1} $year^{-1}$) and those with fertilisation (17±3 g Cd and 5.2±0.9 kg Zn ha^{-1} $year^{-1}$). The impact of harvest time was tested by harvesting a cultivar 112, 122, 132 144, 154 and 164 days after sowing (DAS). Although no differences were found in total fresh biomass, the dry biomass increased significantly in time, due to the ripening of the grains. Concerning the metal concentration and extraction potential no significant effects was found. When the plant reaches a dry matter content of 37±2%, the total optimal energy yield was reached (12±1 10^3 Nm^3 ha^{-1}).

8.1. Introduction

The world in the 21st century is facing problems due to growing energy consumption and diminishing supplies of fossil fuels. This has led to research towards the use of renewable energy sources and the development of new technological processes of energy production (Vindis *et al.*, 2008). Within the context of the Kyoto protocol the EU committed to an 8% reduction in CO_2 equivalent emissions in 2008-2012 compared to 1990 levels (UN, 1998). Moreover, in 2007, the EU set a series of climate and energy targets to be met by 2020, including a reduction of greenhouse gas emissions by 20% as compared to 1990, the promotion of renewable energy, and an increase of its share to 20% by 2020 (COM(2008)030). On 23 January 2008, the Commission proposed a whole package of binding legislation on the 20-20-20 targets, amongst which the Directive 2009/28/EC, the Renewable Energy (RE) Directive (2009) which promotes the increase in renewable energy to 20% by 2020 and the increase in share of biofuels in transport to 10%. It is therefore essential to develop sustainable energy supply systems that aim at covering this demand for renewable resources (Faaij *et al*., 2006; Braun *et al*., 2008; Witters *et al*., 2011a).

Within this context at least 25% of all bioenergy can originate from biogas produced from wet organic materials such as animal manure, crop silages, wet organic food and feed residues, etc. (Lehtomäki *et al*., 2007; Holm-Nielsen *et al*., 2009). The most used input for biogas production is represented by animal manure, slurries from cattle and pig production units as well as from poultry, fish, fur, *etc*. Another agricultural substrate suitable for anaerobic digestion is represented by energy crops, of which the most common are grains, grasses and maize. Energy crops are very suitable as they not only have high biomass yields, but also high energy yields (Menzi, 2002, cited from Holm-Nielsen *et al*., 2011; Amon *et al*., 2007a; Braun *et al*., 2008). Maize (*Zea mays* L.) is the most dominant crop for the production of biogas as it is considered to have the highest yield potential out of field crops grown in Europe (McDonald *et al*. 1991 cited from Neuriter *et al*., 2005; Amon *et al.,* 2007a). The major advantages of producing biogas is the fact that it can (i) be produced when needed, (ii) easily be stored, (iii) be distributed through the existing natural gas infrastructure, (iv) be used in the same applications like the natural gas, (v) it regenerates less atmospheric pollutants and (vi) generates less carbon dioxide per unit energy (Chynoweth *et al*., 2001; Holm-Nielsen *et al*., 2009).

Previous chapters (Chapter 6) showed that the growth of energy maize on contaminated soil was similar as on non-contaminated soils in the same area as elsewhere in Flanders. Furthermore, the biogas production potential of energy maize grown on these soils does not differ from that of energy maize grown on a non-contaminated soil (Cfr. Chapter 6, 7). The metal will end up in the digestate with an accumulation factor of 3-4 (Cfr. Chapter 7). Therefore energy maize offers an economically feasible, risk based soil management approach for metal contaminated soils, with a restoration of the soil in time. Furthermore, by growing energy crops on marginal, poor or abandoned (degraded or polluted) land, the competition of land use for food or for biomass production can be avoided (Meers *et al*., 2011, Gold and Seuring, 2011; Ruttens *et al*., 2011). In order to optimise the potentials of energy maize the impact of variety, location, climate, harvest time, composition, conservation and pre-treatment of the biomass must be investigated (Amon *et al*., 2007a; Bruni *et al*., 2010). In the context of phytoremediation also the metal extraction plays a key role in evaluating its potentials.

The main objectives of this chapter were therefore optimising the biomass production, metal extraction and biogas production potential by investigating the impact of (i) fertilisation and (ii) harvesting time.

8.2. Material and Methods

8.2.1. Experimental setup

Impact of fertilisation

In order to study the impact of fertilisation, the experimental site (described in Chapter 5) was subdivided in 18 plots. Each plot was further subdivided into 4 subplots with a different fertilisation regime: 0, 85 170 and 255 kg N ha^{-1} (Figure 8.1). Fertilisation was performed with pig manure (Cfr. Table 6.1). The plot was planted with 6 commercially available *Z. mays* cultivars (Atletico, Fransisco, KWS1393, PR34B39, PR39F58, PR38H20), provided by KWS and Pioneer and referred to as Z1-Z6, making sure that for each cultivar and fertilisation regime, three replications were found.

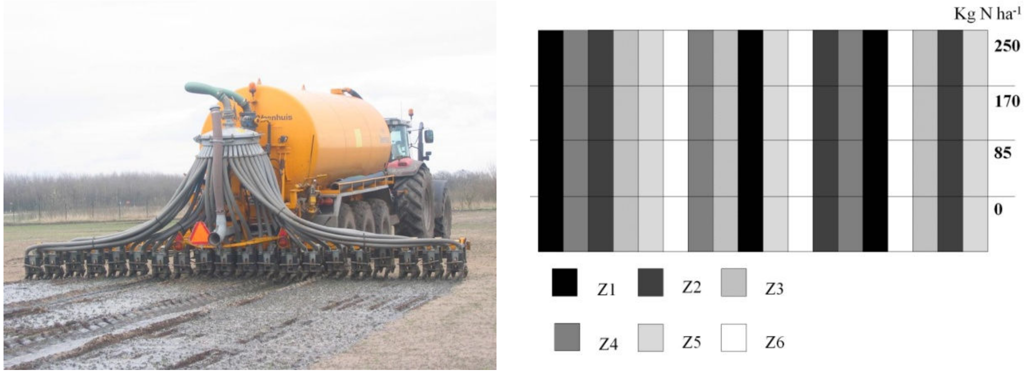

Figure 8.1. Fertilisation of the experimental plot, together with an overview of the experimental design (photo: Van Slycken, S.)

The cultivars were selected by the plant breeders for their capacity to adapt to the climatic conditions in Flanders. Their FAO-number varied, yet was always larger than 220, which is ideal for silage maize (Cfr. Chapter 5). This FAO-number represents the maturity date of the cultivar based on a uniform, standardized method (Jugenheimer, 1958, cited from Marton *et al*., 2004) and is therefore a measure of the greenness of the maize at a certain harvest date. The higher the number, the longer it will take a plant to achieve full ripeness (KWS, 2011). The crops were sown on May 15, 2007 at a density of 95000 seeds ha^{-1} and harvested in October 15, 2007, with attention for good agronomic practices.

Sampling was performed on October 15, 2007. In each subplot 2 plant samples were collected. A plant sample was taken in each repetition plot of the cultivar. The plant sample consisted of all plant material in a sampled surface area of 1 m^2. The total plant was divided into its different plant organs (stem and leaves, bract, rachis, grain) for biomass determination and metal analysis. At each plot, soil samples were taking by the Belgium Soil Service for determination of the total N content and possibilities of N-leaching. As the metal distribution in the plant compartments is similar for the different cultivars (Cfr. Chapter 5, 6), concentrations in the plant parts were only evaluated for cultivar Z1.

Impact of harvest time

For the purpose of this experiment, the experimental plot of Chapter 5 was used. In accordance with the impact of fertilisation, only cultivar Z1 was used. At six different intervals in time, September 3, 2007 (112 days after sowing (DAS)); September 13, 2007 (122 DAS); September 23, 2007 (132 DAS); October 5, 2007 (144 DAS); October 15, 2007 (154 DAS) and October 25, 2007(164 DAS), a plant sample was taken in each repetition plot of the cultivar. The sample consisted of all plant material in a sampled surface area of 1 m^2. The total plant was divided into its different plant organs (bottom 0-30 cm part of the stem and leaves (shoot$_{\text{bottom}}$), rest part of stem and leaves (shoot$_{\text{rest}}$), bract, rachis, grain) for biomass determination and metal analysis.

8.2.2. Plant analysis

All different plant samples were weighed for determination of fresh weight biomass production, washed with distilled water, and oven-dried at 50 °C (Dieser Memmert-Schrank, Germany). The individual plant compartments were pulverised using a Culatti DCFH 48 grinder and sieved over a 1 mm sieve. Plant samples were ashed at 450 °C. The ash was dissolved in nitric acid (Van Ranst *et al.*, 1999) before Cd and Zn analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA) and inductively coupled plasma mass spectrometry (ICP-MS; ELAN DRC-e, PerkinElmer, Waltham, MA, USA) for the determination of the Cd content in the grains. Total Nitrogen content in al plant parts was measured by the Kjeldahl method (Van Ranst *et al.*, 1999). With observed dry biomass and metal concentration in the different plant compartments, the average metal content of the whole plant was calculated.

8.2.3. Batch tests for determination of biogas production potential

A subset of 150 g of each plant sample (bottom 0-30 cm part of the stem and leaves ($\text{shoot}_{\text{bottom}}$), rest part of stem and leaves ($\text{shoot}_{\text{rest}}$), bract, rachis, grain) taken at September 3, October 5 2007 and October 15, 2007 were transported to Organic Waste Systems (OWS, Ghent, Belgium) for determination of the biogas production potential.

The test is based on ISO, 15985, ASTM D 5511-94 and DIN 38414 and consisted of adding a small amount of substrate (50-100 g fresh material) to a large amount of active inoculum (1 kg) which was stabilised prior to the start of the test. After thorough stirring, 2 L reactors were filled with the mixture. It was fermented at 52 °C during 14 d. The biogas production was measured by volumetric displacement of water. Produced volumes were converted into standard conditions of temperature and pressure (temperature=273 K, atmospheric pressure=1 013 hPa). By comparison with reference reactors with only inoculum, the net production of biogas was calculated in $\text{Nm}^3\ \text{kg}^{-1}$ of substrate. The Biogas Production Potential of the sample equals the net biogas production after digestion during 7 days, expressed in Nm^3 biogas ton^{-1} fresh sample

8.2.4. Statistical analysis

Descriptive statistics were performed using the SPSS 11.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. For statistical analysis, normality was checked using a Kolmogorov-Smirnov test. For the impact of fertilisation, significance of observed differences was assessed by using an two factor-ANOVA combined with a Tukey HSD test (when homoscedasticity was found) or with a Dunnet C test (when no homoscedasticity was found), while the assessment of significance of observed differences on the impact of harvest time ANOVA, combined with a Tukey HSD test, was used.

8.3. Results

8.3.1. Impact of fertilisation on growth and metal uptake

For the determination of the total biomass, interaction was found between the chosen cultivar and the fertilisation regime. Therefore the impact of the fertilisation on the total biomass was studied for each cultivar seperately. Also the differences between the cultivars was studied for each fertilisation regime separately. For each cultivar, a significant difference was found between the total fresh biomass of maize grown a fertilised soil with the total fresh biomass of maize grown on a non-fertilised soil. When no fertilisation was applied, no significant differences were found between the total fresh biomass of the different cultivars, and was on average 32±5 ton FM ha^{-1}. Between fertilisation (85, 170 and 255 kg N ha^{-1}), no differences were found in the total fresh biomass of each cultivar. Hereby the total fresh biomass of Z4 was always significantly higher then the total fresh biomass of the other cultivars (Figure 8.2).

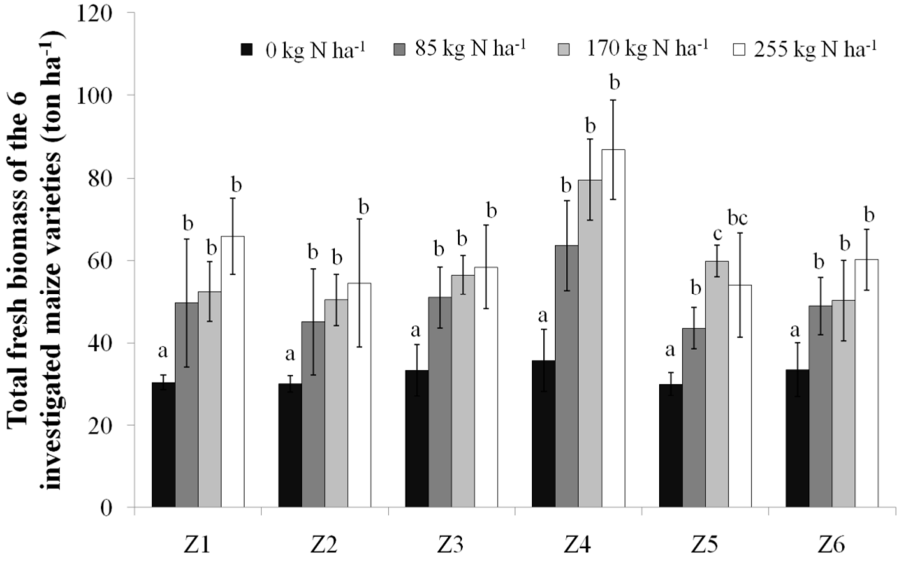

Figure 8.2. Total fresh weight (ton ha^{-1}) of the six investigated maize varieties (Z1-Z6) under the different fertilisation regimes (mean values, flags denote the standard deviation of the total fresh weight, different letters represent different significance level at α=0.05 between the total fresh biomass of each cultivar, n=6)

In contrast to the productivity levels, the concentration of Cd in the different plant parts (Table 8.1), showed no significant differences between the different N application rates. For Zn, differences could be found for some plant parts, mostly when comparing the fertilisation of 0 and 85 kg N ha^{-1} with those of 170 and 225 kg N ha^{-1}. The N concentration in the different plant augmented with increasing N-application, but was only significantly different between the lowest (0 kg N ha^{-1}) and highest (255 kg N ha^{-1}) N-application rate (Table 8.1).

Table 8.1. Cd, Zn concentrations (mg kg^{-1} dry matter) and N concentrations (g kg^{-1} dry matter) in the different plant compartments of variety Z1 (stem, leaves, bract, rachis, grain and total plant) under different N application regimes (mean ± standard deviation, different letters represent different significance level of concentration per cultivar at α=0.05, n=6)

	Cd			
	0	**85**	**170**	**255**
Stem	0.71±0.10a	0.87±0.12a	0.52±0.10b	0.43±0.12b
Leaves	3.3±0.9a	2.6±0.2a	3.0±0.6a	2.8±0.3a
Bract	0.89±0.21a	0.70±0.11a	0.54±0.12a	0.61±0.15a
Rachis	0.33±0.2a	0.31±0.1a	0.27±0.3a	0.25±0.1a
Grain*	19±9a	29±15a	23±7a	18±3a
Total	1.2±0.1a	0.96±0.03a	0.77±0.19a	0.79±0.06a

	Zn			
	0	**85**	**170**	**255**
Stem	352±60a	421±133a	336±95a	275±94a
Leaves	531±80a	540±122a	648±49a	595±95a
Bract	371±17a	332±85ab	231±75ab	269±21b
Rachis	205±83a	156±12a	108±22a	109±21a
Grain*	40±3a	47±6a	52±6a	48±6a
Total	298±39a	249±6a	276±6a	244±44a

	N			
	0	**85**	**170**	**255**
Stem	2.3±0.1a	2.9±0.2a	3.3±0.5a	5.3±1.3b
Leaves	6.7±0.1a	7.3±0.1a	13±1b	15±5b
Bract	3.8±1.1a	6.5±0.5b	6.1±2.5ab	7.0±0.6b
Rachis	4.6±0.6a	5.0±2.0a	8.2±1.4ab	8.9±0.9b
Grain*	14±5a	13±3a	18±6ab	24±5b
Total	7.0±0.9a	9.1±1.0ab	13±4abc	13±1c

*** Concentrations in µg kg^{-1} dry matter**

Multiplying the measured biomass production of shoots, cobs and grains by the observed concentrations, results in the total metal removal capacity of the crop per hectare. Hereby no significant differences were found between the the total Cd and Zn extraction potential calculated with maize grown at N-applicate of 85, 170 and 255 kg N ha^{-1} (on average 17±3 g Cd and 5.2±0.9 kg Zn ha^{-1} year^{-1}), but they were all significalty different with the extraction potentials of maize gronw on the non-fertilised soil (13±1 g Cd and 3.3±0.4 kg Zn ha^{-1} year^{-1}) (Fig. 8.3 and 8.4).

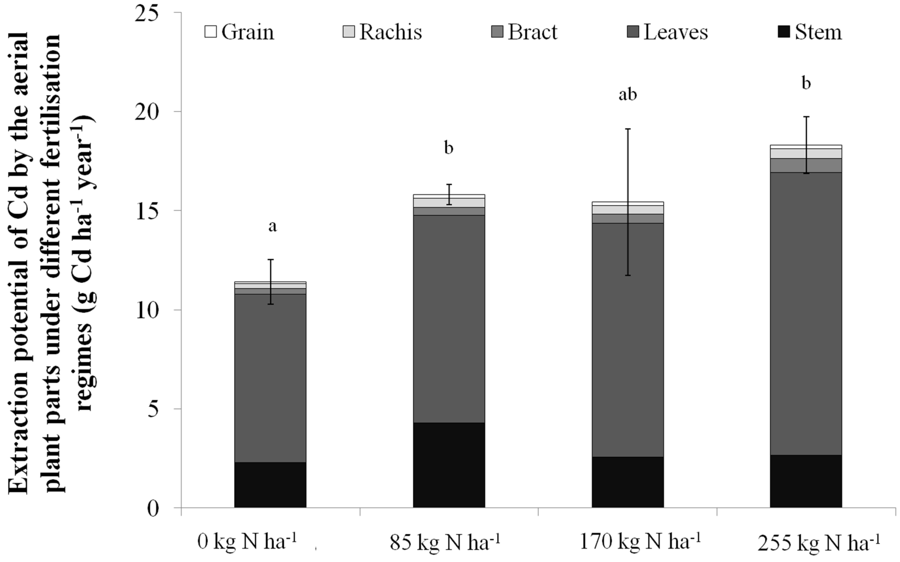

Figure 8.3. Extraction potential of Cd (g ha^{-1} year^{-1}) by of the different aerial plant parts of maize grown under different fertilisation regimes (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=6)

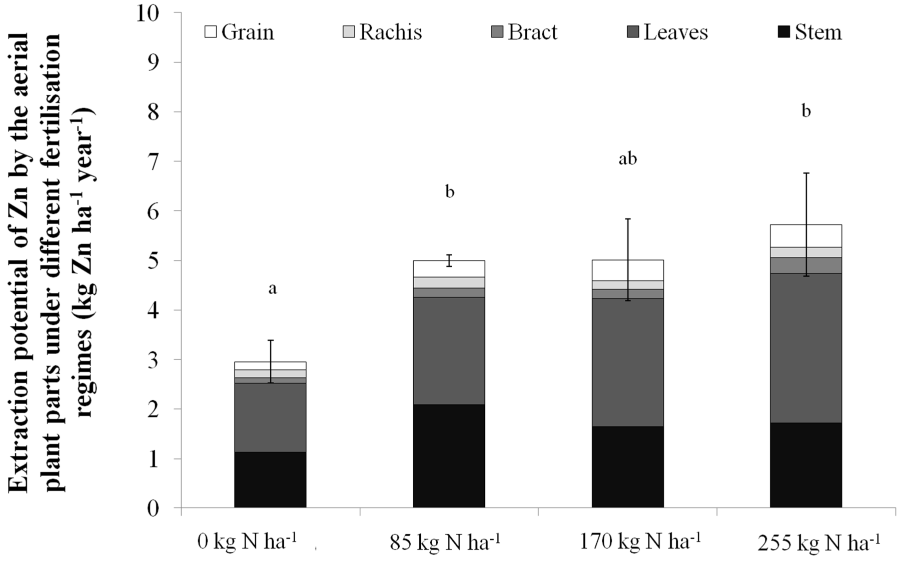

Figure 8. 4. Extraction potential of Zn (kg ha^{-1} year^{-1}) by of the different aerial plant parts of maize grown under different fertilisation regimes (mean values, flags denote the standard deviation of the total biomass, different letters represent different significance level at α=0.05 of the total biomass, n=6)

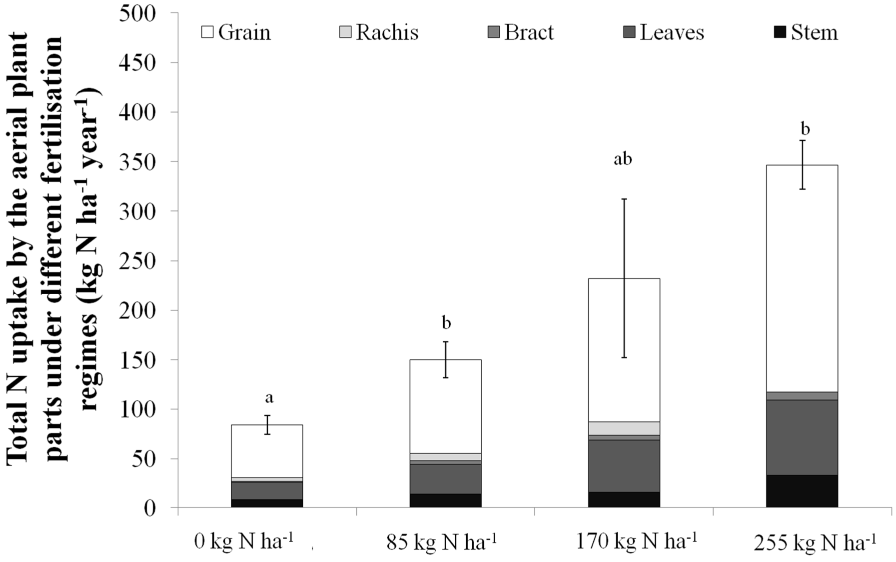

Figure 8.5. Total N uptake (kg ha^{-1} year^{-1}) of the different aerial plant parts under different fertilisation regimes (mean values, flags denote the standard deviation of the total extraction potential, different letters represent different significance level at α=0.05 of the total extraction potential, n=6)

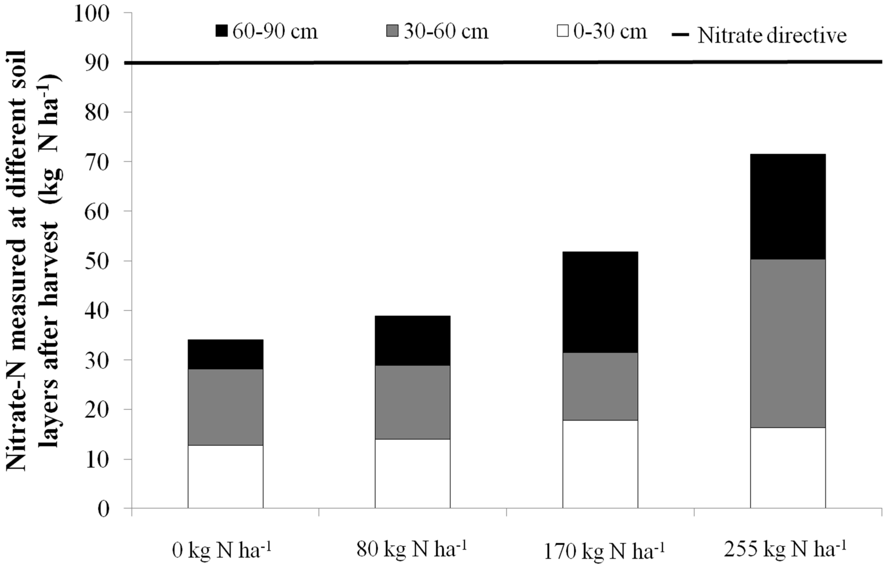

Figure 8.6. Results of the determination of N-nitrate (kg N ha^{-1}) performed by Belgian Soil Service at different soil depths

Between the total N uptake of the plant under different fertilisation rates (Figure 8.5), also significant differences were found. As for the total Cd and Zn uptake in the total plant, the total N uptake of maize grown on a soil with no fertilisation (77±9 kg N ha^{-1}) was significantly different with maize grown on a soil with fertilisation. Between the 3 different fertilisation regimes, the total N uptake was significantly different between the lowest fertilisation rate (163±18 kg N ha^{-1}) and the highest (314±24 kg ha^{-1}). In order to fully analyse the nitrate residue, after the harvest soil samples were taken by the Belgian Soil Service at 3 different soils depths (0-30, 30-60, 60-90) and analysed its N-Nitrate content (Fig. 8.6). The nitrate residue augmented at higher application rates of N, but remained in accordance with the nitrate directive of 90 kg NO_3-N ha^{-1}.

8.3.2. Impact of harvesting time

The fresh biomass of maize showed almost no significant differences between the harvesting periods (Figure 8.7). During October there was a decrease in the total biomass, were the total biomass of the last harvesting time (56±3 ton FM ha^{-1}) is significantly different with the first harvest (73±8 ton FM ha^{-1}). This change was mainly due to a decrease in biomass of the stem and leaves and the bract. At October 15 (154 DAS) the total fresh biomass of Z1 (60±8 ton FM ha^{-1}) was similar to the measured fresh biomass of Z1 of the fertilisation plot with 175 kg N ha^{-1} (53±7 ton FM ha^{-1}).

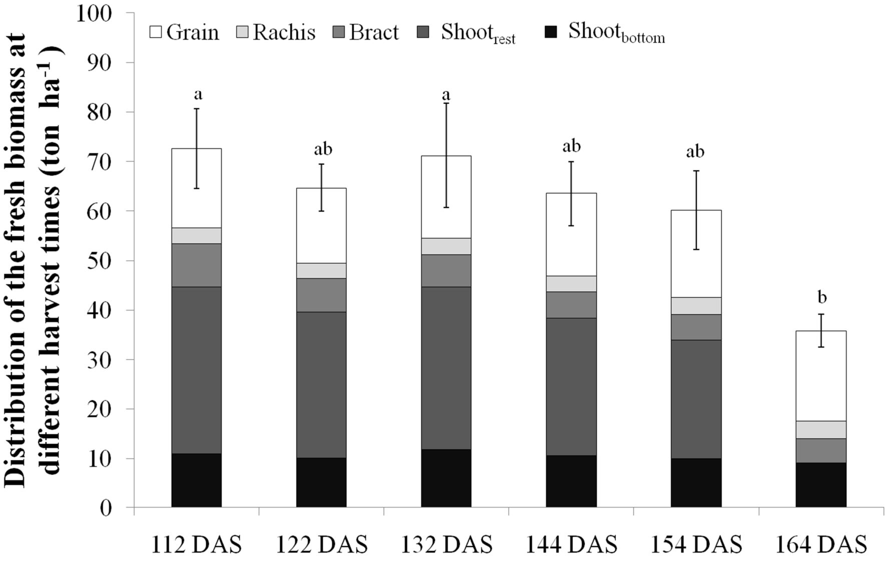

Figure 8.7. Mean fresh weight (ton ha^{-1}) of the different plant parts of maize grown on a soil where 170 kg N ha^{-1} was applied, taken at six different periods throughout the growing season (mean values, flags denote the standard deviation of total fresh biomass, different letters represent different significance level at α=0.05 of the total biomass, DAS=days after sowing, n=6)

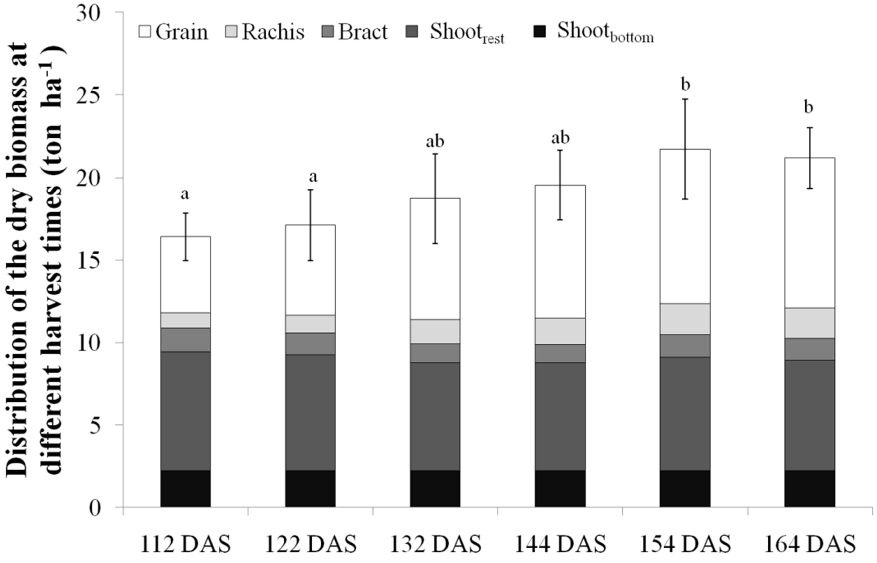

Figure 8.8. Mean dry weight (ton ha^{-1}) of the different plant parts of maize grown on a soil where 170 kg N ha^{-1} was applied, taken at six different periods throughout the growing season (mean values, flags denote the standard deviation of total dry weight, different letters represent different significance level at α=0.05 of the total biomass, DAS=days after sowing, n=6)

The total dry biomass on the other hand increased significantly in time (Figure 8.8) and ranged from 16±1 ton DM ha^{-1}in the beginning of September (112 DAS) to 21±2 ton DM ha^{-1} at the end of the experiment (164 DAS). Under normal condition the bottom part of the plant, up to 30 cm from the soil (shoot$_{\text{bottom}}$), remains in the field. If this part of the plant is not taken into account, biomass productivity reached levels between 14±1 (112 DAS) and 19±2 ton DM ha^{-1} (164 DAS). In contrast to the fresh material, the dry biomass of the stem and leaves and bract did not change significantly over time. Grain and rachis on the other hand increased significantly over time. The total dry matter of the plant varied between 23±2% at the first harvesting time to 38±2 at the latest harvest sampling period. The full harvest of samples at October 15 (154 DAS) was ideal for early to medium ripening varieties (FAO 240-390) as the dry matter content of the total plant was between 35-39%. Therefore no further sampling dates were scheduled.

The biogas production potential of the whole crop varied from 102±1 Nm³ ton^{-1} FM at 112 DAS to 120±20 (132 DAS) and 194±1 Nm³ ton^{-1} FM (154 DAS) (Figure 8.9). The biogas production potential measured after 154 DAS was in accordance with the energy productivity level of energy maize under mesophilic conditions and is similar to other measurements of the ensilaged maize. This means that at the beginning of September maize showed a biogas production potential of 7.4± 0.1 10^{3} Nm³ ha^{-1} and increased to more than 12±1 10^{3} Nm³ ha^{-1} (Figure 8.10).

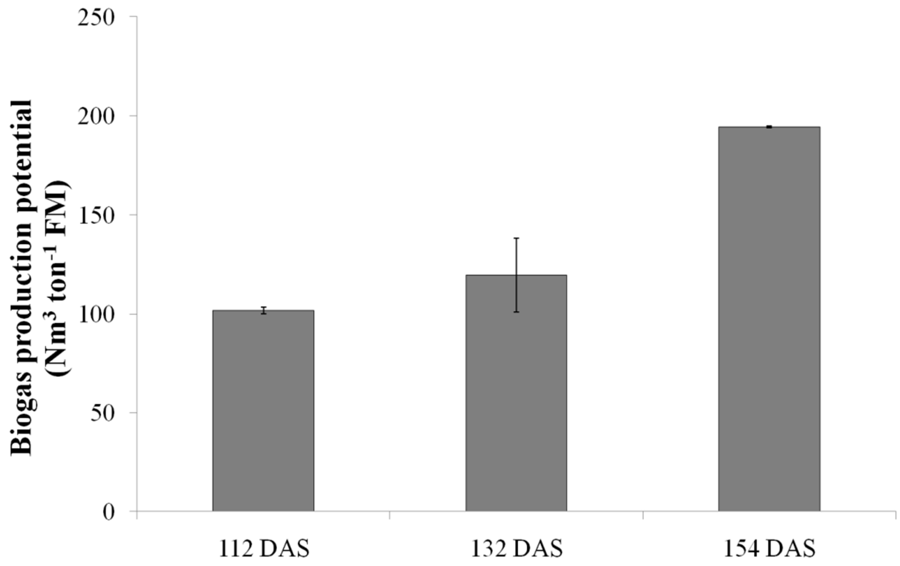

Figure 8.9. Biogas production potential of the total plant (Nm^3 ton^{-1} FM), determined at three different periods throughout the growing season (mean values, flags denote standard deviation, DAS=days after sowing, n=2)

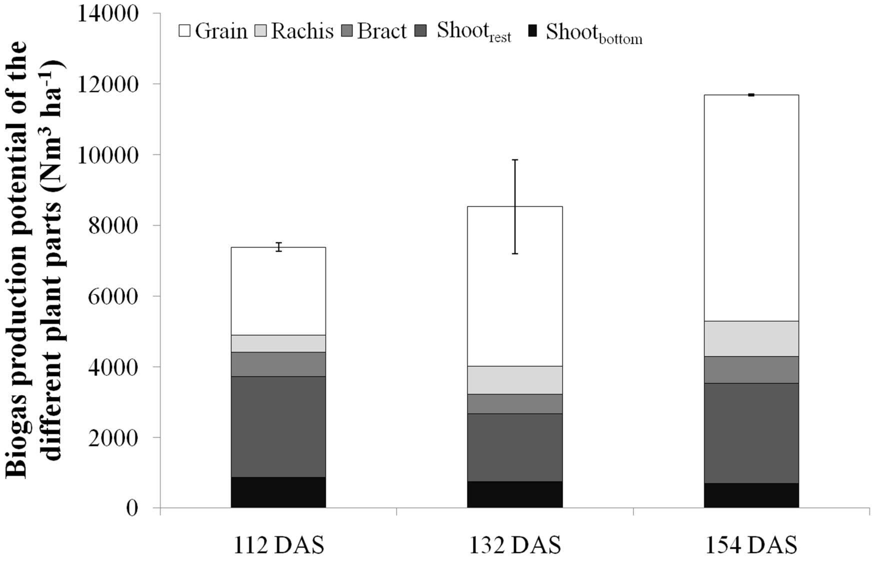

Figure 8.10. Mean biogas production potential of the different plant parts together with the standard deviation of the biogas production potential of the total plant, determined at 3 different periods throughout the growing season (mean values, flags denote standard deviation of the biogas production potential of the total plant, DAS=days after sowing, n=2)

No great differences were observed between the harvesting time and metal concentration in the different plant compartments (Table 8.2). Cd and Zn concentrations in the grain were highest at the latest harvesting period. Also, the concentrations of the bract at the first harvesting period were significantly lower in comparison to the other periods. Furthermore, measurements of the latest harvest (154 DAS) were showing high deviation levels in comparison to the other measurements.

Table 8.2. Cd, Zn and concentrations (mg kg^{-1} dry matter) in the different plant compartments determined at three different harvesting periods: 112, 132 and 154 DAS (days after sowing) (mean ± standard deviation, different letters represent different significance level of concentration per cultivar at α=0.05, n=6)

	Cd		
	112 DAS	**132 DAS**	**154 DAS**
Shoot$_{bott}$	2.9±2.1a	1.2±0.3a	1.1±0.4a
Shoot$_{rest}$	6.1±5.6a	1.8±0.4a	1.7±0.5a
Bract	0.47±0.14a	0.53±0.12a	0.67±0.18a
Rachis	0.31±0.18a	0.35±0.20a	0.33±0.33a
Grain	0.080±0.080a	0.096±0.047a	0.21±0.03b

	Zn		
	112 DAS	**132 DAS**	**154 DAS**
Shoot$_{bott}$	245±115a	348±82a	306±25a
Shoot$_{rest}$	247±154a	416±75a	358±31a
Bract	157±25a	262±64b	289±37b
Rachis	129±58a	161±42a	193±95a
Grain	71±25ab	91±13b	65±7a

Multiplying the measured biomass production of shoots, cobs and grains by the observed concentrations, results in the total metal removal capacity of the crop per hectare. The total extraction potential after 122 DAS was 14±1 g Cd and 4.5±0.4 kg Zn ha^{-1} year^{-1}. At optimum extraction potentials of 17±4 g Cd and 4.7±0.7 kg Zn ha^{-1} year^{-1} were achieved (Figure 8.11 and 8.12). Although the extraction potentials increased, no significant differences could be found.

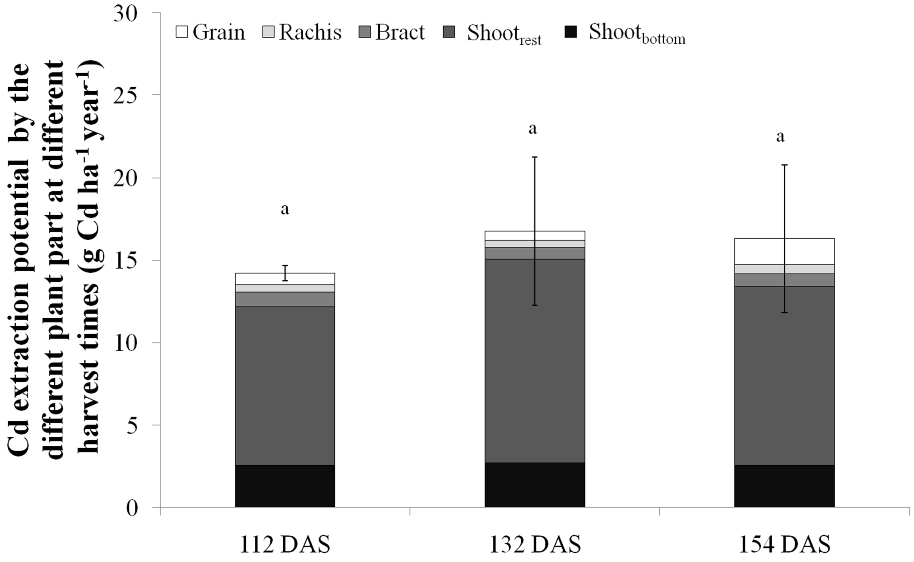

Figure 8.11. Extraction potentials of Cd (g ha^{-1} year^{-1}) of the different aerial plant parts in the harvesting periods: 112, 132 and 154 DAS (days after sowing) (mean values, flags denote the standard deviation of the total extraction potential, different letters represent different significance level at α=0.05 of the total extraction potential, n=6)

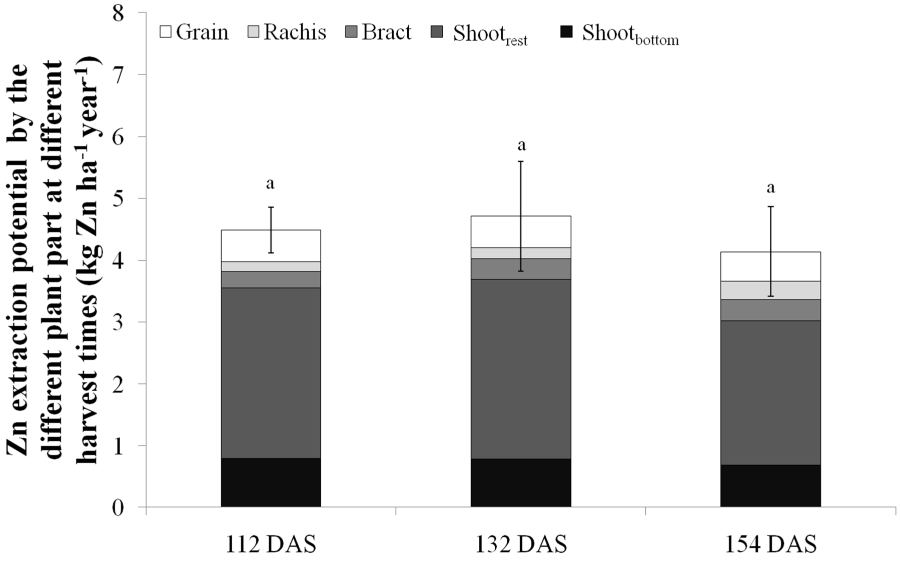

Figure 8.12. Extraction potentials of Zn (kg ha^{-1} year^{-1}) of the different aerial plant parts in the harvesting periods: 112, 132 and 154 DAS (days after sowing) (mean values, flags denote the standard deviation of the total extraction potential, different letters represent different significance level at α=0.05 of the total extraction potential, n=6)

For the different plant parts, and the total plant no significant differences were found between the harvesting time and the total extraction potential of Cd and Zn. The lower part of the stem and leaves ($shoot_{bottom}$) showed an extraction potential of 2.6±0.8 g Cd and 0.75±0.2 kg Zn ha^{-1} $year^{-1}$. With extraction potentials of 11±3 g Cd and 2.6±0.5 kg Zn ha^{-1} $year^{-1}$ the upper part of the stem and leaves showed the highest extraction potential of all plant parts. The extraction potentials for the bract were respectively 0.8±0.3 g Cd and 0.33±0.10 kg Zn ha^{-1} $year^{-1}$, while those for the rachis were 0.5±0.4 g Cd and 0.22±0.13 kg Zn ha^{-1} $year^{-1}$. At 112 DAS the extraction potential of the grain were 0.68±0.51 g Cd and 0.50±0.14 kg Zn ha^{-1} $year^{-1}$ while at 154 DAS, the extraction potentials increased to 1.7±0.5 g Cd and 0.50±0.11 kg Zn ha^{-1} $year^{-1}$ (Figure 8.11 and 8.12).

8.4. Discussion

The feasibility of maize for energy production depends on variety, location, climate, harvest time, as well as composition, conservation and pre-treatment of the biomass (Amon *et al.*, 2007a; Bruni *et al.*, 2010). In the context of energy corps on metal contaminated agricultural soils also metal extraction must be taken into account. As the impact of variety is low, which is already discussed in previous chapter 5 and 6, it will not be handled during this discussion.

When the optimal N application rate is reached, the N must be available at the appropriate growth stage of the plant. Furthermore attention must be paid to the fact that at the end of the growing season not too much N remains in the soil, and thus preventing a N-leach (Van de Ven *et al.*, 2008). In Flanders, it is therefore stated that between 1 October and 15 November the total NO_3-N in the soil profile of 0-90 cm should not exceed 90 kg NO_3-N ha^{-1}. This residual NO_3-N is the result of i) the remaining NO_3-N from the fertilisation that was not taken up by the plant, ii) the nitrate derived from mineralisation of soluble organic material in the soil and iii) organic material derived from plant or manure residues. If this amount is too high, a considerable risk excists for leaching in the following winter (El-Sadek *et al.*, 2001). Although no increase in biomass production was found for the soils with fertilisation (85 170 and 255 kg N ha^{-1}) (Figure.8.2), the total N concentration increased and thus also the total N uptake (Figure. 8.5). Atlhough the total N extraction was higher then the N input, the remaining NO_3-N increased but were still in accordance with the nitrate directive (90 kg N-NO_3-N ha^{-1}) (Figure 8.6). If a drought or an extreme wet period occurs at such high application dose, the leaching risks may become higher. The current study focussed on the impact of N. However currently legilaslation is strengthened and the uptake and possible leaching of other elements (e.g. phosphorus, potassium) are also important and needs to be taken into account in future field experiment in Flanders, together with the impact of high fertilisation inputs on the long term (Finke *et al.*, 1999, Van de Ven *et al.*, 2008, Ver Elst, 2010).

Although the adjustment of N fertilisation and harvesting period, influenced the biomass productivity, the higher N application (23±1 ton DM at 35% DM) and a later harvesting (21±2 ton DM at 38±2% DM) was not significantly different with the current commonly used agronomic practices (N application 170 kg N ha^{-1} and harvested when the total biomass had a DM content of 36±4%) where a yield of 22±3 ton DM ha^{-1} was measured. Also, these yields were in line with the previous research (20±3 ton DM ha^{-1} measured in Chapter 6) and other researches of the same cultivar on non-contaminated sites in the Campine region and elsewhere in Flanders (ILVO, 2009; Ghekiere *et al.*, 2007). Concerning the metal extraction potential the adjustments did not significantly influence the Cd and Zn extraction potential (18±1 g Cd and 5.8±1.0 kg Zn ha^{-1} year^{-1} at highest fertilisation regime versus 17±4 g Cd and 4.7±0.7 kg Zn ha^{-1} year^{-1} in the current commonly used 170 kh N ha^{-1}). Therefore the corresponding remediation periods, remains in the same order of magnitude as found in the previous chapters (19±6 g Cd and 4.3±0.9 kg Zn ha^{-1} year^{-1} calculated in Chapter 6).

Besides optimal biomass production, the conversion of maize into energy is also important. Experiments on the biogas production potential of maize from metal contaminated soils, showed no significant difference with energy maize from non-contaminated soils on short term experiments (Chapter 6, 7) but also during semi-continous tests (Chapter 7). Together with the growth of the plant (Figure 8.7 and 8.8), the specific methane yield of the maize plant parts evolved (Figure 8.9). Maize is hereby optimally harvested, when the product from specific methane yield and biomass yield per hectare reaches a maximum (Amon *et al.*, 2007b). During the growth of the plant the crude proteins, fibres and cellulose content declines, while hemi-cellulose, and starch content increases (Amon *et al.*, 2004, cited from Walla and Schneeberger, 2008). This optimum harvesting period depends on the variety as it is stated that the biomass yield of late ripening maize varieties (FAO 600) increased until full ripeness, with a total dry matter content of more than 43%. The early and medium ripening (240-390) varieties only showed an increasing biomass yield until wax ripeness and need to be harvested when the plant reaches a dry matter content of 35-39% (Amon *et al*., 2004, cited from Walla and Schneeberger, 2008; 2007b).

In the current experiment early ripening varieties were used and confirmed this statement. A general increase of the biogas production potential was observed. After 112 DAS, maize showed a dry matter content of 22±2% and a biogas production potential of 7.4±0.1 10^3 Nm³ ha^{-1}. The biogas production was mainly attributed to the stem and leaves (up to 50%). During the ripening stages the biogas production of the stems remained the same, while those of the other plant parts augmented. After 154 DAS the contribution of the grain to the total biogas production potential augmented from 30% to 50% (Figure 8.10). The total biogas production potential reached hereby a level of 12±1 10^3 Nm³ ha^{-1}, which is more than 60% higher. The plants showed to have an ideal dry matter content of 37±2% (Amon *et al*., 2004, cited from Walla and Schneeberger, 2008; 2007b).

It must be stated that for this experiment and for the purpose of phytoremediation, the whole plant was harvested. In practice, the bottom stem and leaves (0-30 cm) ($shoot_{bottom}$) remains on the field. For the early harvesting period, a decrease of 13% was observed if these plant parts were not taken into account. This is due to the fact that in the beginning of the experiment the biogas production is mainly attributed by the digestion capacity of the stem and leaves. However, at the end of the experiment (154 DAS), this plant part only represents 6% of the total biogas production potential and doesn't influence the main conclusion of this research: even without these plant parts, the total biogas production potentials were still in accordance with previous studies of energy maizefrom non-contamited soils in general (Amon *et al*., 2007b; Schittelhelm, 2008; Bruni *et al*., 2010; Oslaj *et al*., 2011, Chapter 6 and 7).

Thewys *et al*. (2010a,b) and results of Chapter 6 showed that besides the technical, also the economic feasabily for farmers to use energy maize on metal contamined (*i.c*. Cd and Zn) are positive. Chapter 5 stipulated that as in the grains low metal concentrations are observed, chances for exceeding the European threshold values for animal feed are minimised. Therefore maize grains could be valorised in the food marked. When this option is followed, two remarks should be made: (i) Although current state-of-the-art agro-technology is moving towards the development of harvesting hoppers which can harvest and separate both plant compartments, current agronomic practices for maize, do not yet allow simultaneous harvest and isolation of grains from the shoot biomass. (ii) For achieving a gradual reduction of metals in the soil, one has to remove all the aerial biomass from the environment. When only these plant parts are used for anaerobic digestion, the total biogas production potential drops with at least 50% and becomes insufficient for using it in a mono-digester.

In order to provide continuous operation throughout the year the harvested plants need to be preserved, which is usually done by ensiling. Due to compression of the material and air-tight sealing anaerobic conditions are established. This promotes the growth of autochthonous lactic acid bacteria, which convert free soluble carbohydrates into lactic acid, methanol, alcohols, formic acid, H^+ and CO_2. The resulting decrease in pH, prevents the growth of undesirable microorganisms. These products are important precursors for methane formation (McDonald *et al*., 1991 from Neureiter *et al*., 2005; Madigan *et al*., 2000, cited from Amon *et al*., 2007b). Amon *et al*., (2007a) showed that this will even increase the biogas production potential. This could be attributed to pre-decomposition of crude fibre in course of the silaging process, which improves the availability of nutrients for the methanogenic metabolism.

8.5. Conclusion

The main goal of this chapter was to find optimal agronomic conditions for the use of energy maize on metal contaminated soils in the Campine region and its impact on the extraction potential of Cd and Zn. The application of N increased the total fresh biomass of maize and its corresponding Cd and Zn extraction potential significantly. Nevertheless, there was no influence between the commonly used (170 kg N ha^{-1}) and a higher N-application (255 kg N ha^{-1}) rate on the biomass production potential and on the Cd and Zn extraction potential. Also the optimal harvest period for productivity and treatment are in line with the commonly employed harvesting dates. Changing the harvest date did not influence the metal concentration and extraction potential of the plant compartments significantly. Therefore standard agronomic practices can be applied in order to achieve optimal results of the cultivation of energy maize on metal contaminated land.

CHAPTER 9:

GENERAL CONCLUSION AND FUTURE PERSPECTIVES

In the 19th and 20th century (up to 1970s), historic atmospheric deposition of trace metals from metal refinery activities has resulted in elevated concentrations in agricultural soils in the Campine region. As a consequence, a surface area of at least 700 km^2 is now contaminated with several toxic metals, including cadmium (Cd), zinc (Zn) and lead (Pb). Previous studies also reported health problems in the vicinity of the smelters with increased risks for renal dysfunction, osteoporosis, lung cancer as well as and other health-related issues due to a higher exposure of cadmium. Furthermore, the soils in the region are characterised by a sandy texture and relatively low pH, which entails an enhanced risk for uptake of these metals in crops or leaching to the groundwater. Conventional soil remediation approaches tend to be overly expensive considering the moderate levels of pollution and extended areas that need treatment. Phytoremediation involves the use of plants and their associated microorganisms for the stabilisation, degradation and/or removal of pollutants from the environment. The purpose of this research was to assess the feasibility of non-food crops, and more specific rapeseed for plant oil production (biodiesel), willow under short rotation coppice and energy maize for biogas production by anaerobic digestion, not only as a phytoremediation option, but also as an alternative approach for metal contaminated agricultural soil.

In the field experiments, rapeseed exhibited a total shoot biomass of 5.8±2.0 ton DM ha^{-1}. However, the seed yield itself was low (0.5±0.3 ton DM ha^{-1}), probably due to suboptimal fertilisation. For rapeseed, not only N fertilisation but also application of S is important in order to achieve proper seed yields. This was confirmed when the observations were compared with another field experiment of rapeseed on non-contaminated sites in the area. In that experiment the seed yield was approximately 3 ton ha^{-1} under optimal fertilisation. This could not be confirmed in own experiments, as during subsequent experiments the growth of rapeseed failed entirely. Observations from the field experiment suggested that 27±14 g Cd ha^{-1} and 2.0±1.0 kg Zn ha^{-1} may be removed from the soil through harvest of the rapeseed. Farmers in the studied region are not yet familiar with the cultivation of rapeseed. Also the fact that rapeseed should be used in a rotation scheme in order to decrease possible crop failures contributes to the potential unsuitability of this crop as an appropriate alternative cropping system for metal contaminated agricultural soils in the Campine region.

In the short rotation coppice (SRC) field experiments, large differences were found in biomass production between eight different investigated commercial available clones after four years of growth. Zwarte Driebast exhibited the highest productivity level (~ 12 ton DM ha^{-1} $year^{-1}$), followed by Loden (~ 4.3 ton DM ha^{-1} $year^{-1}$). Yields of these clones were highly in contrast with the lowest biomass producing clones (Inger, 1.5 ton DM ha^{-1} $year^{-1}$). Harvesting the woody biomass itself resulted in the removal of 72 g Cd and 2.0 kg Zn ha^{-1} $year^{-1}$. Under normal conditions short rotation coppice is harvested in winter. However, as the leaves contain high amount of metals, it was concluded that when SRC is harvested with leaves, the extraction potential can be increased by 40%. When choosing for short rotation coppice, a multi-clonal plantation is preferred, as it creates a buffer against diseases or other unforeseen failures of a clone. For a final conclusion on selecting the optimal clones, regrowth and productivity levels during subsequent rotations should also be taken into account. Although data suggest promising results for the applicability of short rotation coppice, the conclusions are based on a selection of willow clones originating from Swedish research experience and on the experience of other Belgian or Dutch breeding programs. Therefore it is suggested to further investigate the potentials of other clones, including their re-growht, and to breed/select clones that are not only specific for SRC, but also for phytoremediation and for the Campine soil characteristics. Our study revealed that for the moment the current wood price does not guarantee a sufficient income to provide for a suitable farming alternative, therefore hampering the potential of SRC as an alternative cultivation. This needs to be addressed if short rotation coppice is to become a viable alternative as a risk based option for metal contaminated soils.

The use of energy maize was investigated as a third non-food crop. Under optimal fertilisation, energy maize showed an average yield of 53±10 ton FM or 20±3 ton DM ha^{-1}. The observed productivity levels were similar to those obtained with the same cultivars on non-contaminated sites elsewhere in the region and in Flanders. Economic studies indicated that the average annual income of the farmers can be maintained when they convert land use from fodder maize to energy maize. As maize is a metal excluder, it not only exhibited the lowest plant concentrations of all crops tested, but also the lowest extraction potential for Cd, but highest Zn (19±6 g Cd and 4.3±0.9 kg Zn ha^{-1} $year^{-1}$). Energy maize is considered as a highly suited crop for biogas production because of its high yield combined with a high energy production potential. Maize from a contaminated soil shows to have a biogas production potential 191±32 Nm^3 ton^{-1} FM, based on lab scale digestion tests. No significant difference in biogas production potential between energy maize from a contaminated site and a non-contaminated site was observed. These biogas production potentials were also maintained during a semi-continuous test of 435 days (on average 195±61 Nm^3 ton^{-1} FM). The digestate produced from a mono-digestion showed concentrations of elements that were on average 3-4 times higher in comparison with the input material, which is similar to the upconcentration observed for maize from a non-contaminated site. The obtained upconcentration factor can therefore be used for assessing the quality and characterisation of the digestate and subsequently comparing it with legal threshold values and limitations for usage. It can therefore be concluded that due to the positive biomass treatment opportunities and the associated economic perspectives, the major drawback of energy maize as a phytoremediation crop (long remediation times) becomes less important and the use of energy maize can constitute a safe and profitable alternative of these moderately contaminated soils. As anaerobic digestion gives promising results, treatment of phytoremediation biomass with higher metal accumulation values should also be considered for anaerobic digestion during short and long term experiments.

When evaluating the potentials of rapeseed (*Brassica napus* L.), willow (*Salix* spp.) under short rotation coppice and energy maize (*Zea mays*) as a phytoremediation strategy for the Campine region, rapeseed exhibited the most problems for cultivation, while the others can achieve productivity levels which are similar to non-contaminated sites. Treatment of the different types of harvested biomass for energy production is technically feasible. Nevertheless the net environmental costs for energy production and the total environmental pressure of each crop should also be taken into account For each treatment option the fate of elements can be calculated and therefore quality, characteristics and treatment of the new formed products can be estimated.

The current study furthermore concludes that short term soil remediation is unrealistic. However, if economic viability of energy crop production can be ascertained, it can allow for a long term safe alternative use of the contaminated agricultural land. In this way, remediation is reduced to a secondary objective with sustainable risk-based land use as the primary objective. In this context the application of non-food crops for risk-based soil remediation has been adopted as ‘phytoattenuation’, setting it apart from ‘conventional’ phytoextraction where the active extraction is the primary focus.

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Curriculum Vitae

Personalia

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Education

2005- 2006	Master in Environmental Sanitation Ghent University, Belgium Master thesis: Influence of heavy metals on the light response of beans (*Phaseolus vulgaris* L.). Promoters: Prof. dr. eng. F. Tack and Prof. dr. eng. R. Samson
1999-2005	Master in Bioscience Engineering, option Land and Forestry Management Ghent University, Belgium Master thesis: Respiration of stem and branches by beech (*Fagus sylvatica* L.) under greenhouse and field conditions Promoters: Prof. dr. R. Lemeur and Prof. dr. eng. R. Samson
1993-1999	Science-mathematics (8h), College Paters Jozefieten, Melle, Belgium

Professional activities

2006-2011 Scientific staff member at the Laboratory of Analytical Chemistry and Applied Ecochemistry (Ecochem)

Phd research:

Risk-based management of metal contaminated agricultural soils in the Campine region

Promoters: Prof. dr. eng. F. Tack and Prof. dr. eng. E. Meers

Involved in following projects:

- Energy crops on agricultural soils enriched heavy metals. Research on possibilities for alternative land use using phytoextraction and energy crops. (Translated from Dutch: *Energiegewassen op landbouwgronden aangerijkt met zware metalen: Onderzoek naar functionaliteitsherstel door middel van fytoextractie en/of biomassateelt als volwaardig alternatief voor klassieke landbouw*) (IWT-CLO project)

- Remediation of difuse contaminated soils with energy crops for the production of biofuels. (Translated from Dutch: *Sanering van diffuus verontreinigde bodems gekoppeld aan de productie van biobrandstoffen* (MIP-project)

- Risk based soil remediation in the Campine region, base don the reduction of the mobility and availability of heavy metals in soils. (Translated from Dutch: *Risicogebaseerde bodemsanering in de Kempen, gestoeld op een reductie van de mobiliteit en de biobeschikbaarheid van zware metalen in de bodem* (IWT-TETRA-project)

- Hydraulic control of contaminated ground water using willows and poplars. (Translated from Dutch: *Hydraulische barrières met wilg en populier voor matig verontreinigd grondwater* (Soil sanitation project ABO)

2011-curent: Contract manager Soil at AIB-Vinçotte Envirnment

Publications

A1 publications

Submitted

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DeJonghe, W., Meers, E., Ruttens, A., Guisson, R., Van Ginniken, L. **Van Slycken, S.**, Witters, N., Peene, A., Tack, F.M.G., Vangronsveld, J., Thewys, T., Kegels, J., Beeckman, E., Smis, J., De schepper, S., Fastenaekels, H. and Diels, L. Phytoremediation for heavy metal contaminated soils and combined bio-energy production. Innovations for Sustainable Production Conference (I-SUP), 22-25 April 2008, Bruges, Belgium.

Meers, E., **Van Slycken, S.**, Ruttens, A., Meiresonne, L., Vangronsveld, T., Thewys, T. and Tack, F.M.G. The use of energy crops for phytoremediation allows economic valorisation of moderately contaminated land during site decontamination. International Conference on the Biogeochemistry of Trace Elements (ICOBTE), 15-19 July 2007, Beijing, China.

Van Slycken, S., Meers, E., Ruttens, A., Meiresonne, L., Witters, N., Tack, F.M.G., Vangronsveld, J. and Thewys, T. *Korte Omloop Houtteelt op bodems uit de Kempen aangerijkt met zware metalen* Starters in het bosonderzoek 2007: jonge wetenschappers stellen hun resultaten voor, 4 March 2007, Brussels, Belgium.

Van Slycken, S., Saveyn, A., Samson, R. and Lemeur, R. (2005). *Stam- en takrespiratie bij beuk (Fagus sylvatica L.) in groeikamer- en veldomstandigheden*. Starters in het Bosonderzoek, 22 March 2005, Brussels, Belgium.

Conferences, workshops and seminars

- Active participation: oral

19-21 October 2010 - Contaminated Site Management in Europe (CSME), Ghent, Belgium.

26-29 September 2010 - 7th International Conference on Phytotechnologies, Parma, Italy.

8-18 June 2010 - Sustainable Approaches to remediation of Contaminated Land in Europe (SARCLE), Ghent, Belgium.

27-29 October 2009 - Contaminated Site Management in Europe, Contaminated Site Management in Europe (CSME), Ghent, Belgium.

19 March 2009 - Startersdag in bosonderzoek, Brussels, Belgium.

10-12 March 2009 - Integrated Assessment of Agriculture and Sustainable Development; Setting the Agenda for Science and Policy (AgSAP 2009), Egmond aan Zee, the Netherlands.

15 September 2008 - 14th PhD symposium on applied biological sciences, Ghent, Belgium.

- Active participation: poster

16 December 2010 - Studiedag Houtige biomassa in de landbouw, Antwerp, Belgium.

27-29 October 2009 - Contaminated Site Management in Europe (CSME), Ghent, Belgium.

27 November 2008 - Powerlink-kick-off, Oostende, Belgium. 1 November 2008, Young Botanists' Day, Flora and vegetation of Belgium and neighbouring regions, 2nd circular, Brussels, Belgium.

23 June 2008 - BeNeKempen Symposium, Turnhout, Belgium.

11 February 2008 - Demo-dag korteomloophout Rijkevelde, Belgium.

20 September 2007 - Umicore dag, Ghent, Belgium.

4 March 2007 - Starters in het Bosonderzoek, Brussels, Belgium.

25 January 2007 - BeNeKempen Symposium, Mol, Belgium.

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Tutorship/begeleiding van thesissen

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Jan Van den Ouwelant, J. (2007-2008). *Fytoremediatie van zware metalen door korteomloophout met populier (Populus spp.)*. Master in Bioscience Engineering, option Land and Forestry Management, option Soil and Water. Promoters: Prof. dr. eng. F. Tack and Prof. dr. eng. E. Meers, Tutor: eng. Stijn Van Slycken. 117p.

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De Wilde, C., Temmerman, A., Schouppe, J., Smet S., and Vandegehuchte M. (2007-2008) *Diffuse verontreiniging met zware metalen in de bodem: welke opties voor gebruik blijven open?* Bachelor project. Promoter: Prof. dr. eng. F. Tack, Tutor: eng. Stijn Van Slycken.

Review activities

Science of the Total Environment

Educational support

14 January 2011 - *Duurzaam beheer van metaalverontreinigde landbouwgronden in de Kempen*. IVPV-contactday, Ghent University.

2 December 2010 - Assisting Lab-work practical course: Determination of Phosphate and Nitrate in water, Ghent University.

26 November-3 December 2010 - Assisting Lab-work practical course: Determination of Phosphate and Nitrate in water, Ghent University.

17-26 March 2010 - Assisting Lab-work practical course: Determination of Phosphate and Nitrate in water, Ghent University.

14 December 2007 - *Fytoremediatie op metaalaangerijkte gronden in de Kempen*. IVPV-contactday, Ghent University.

5 April 2007 - Assisting practical courses of Soil contamination and remediation, Ghent University.

8 December 2006 - *Fytoremediatie op metaalaangerijkte gronden in de Kempen*. IVPV-contactday, Ghent University.

Institutional contributions

24 February 2011 - Presentation Tempus project. Sustainable management of metal-contaminated soils in the Campine region Phytoremediation and bio-energy production, Ghent University.

1 October 2010 - Presentation for visit of Prof. Prasad, M.N.: Sustainable management of metal-contaminated soils in the Campine region Phytoremediation and bio-energy production, Ghent University.

25 June 2010 - Coöperation in Visit of inhabitants Campine region to the Hasselt University, on the use of metal contaminated backyard in the Campine region. (Translated from Dutch: *Uitnodiging bezoek Labo's Universiteit Hasselt/PHL Vastleggen van schadelijke metalen in Kempense tuintjes. Diepenbeek. Vragenronde/participatie tussen particulieren en wetenschappers).*

18 September 2010 - Short rotation coppice on metal contaminated Agricultural soils in the Campine region (Translated from Dutch: *Korteomloophout op landbouwgronden aangerijkt met zware metalen*). Open house of the Faculty Bio-science engineering, celebtrating its 90th anniversary. Ghent University.

14 March 2009 - Energy crops on agricultural soils enriched heavy metals. Research on possibilities for alternative land use using phytoextraction and energy crops (Translated from Dutch: *Energiegewassen op landbouwgronden aangerijkt met zware metalen: onderzoek naar functionaliteitsherstel door middel van fytoextractie en/of biomassateelt als volwaardig alternatief voor klassieke landbouw*). Open house of the Faculty Bio-science engineering, Ghent University.

22 March 2007 - Remediation of difuse contaminated soils with energy crops for the production of biofuels (Translated from Dutch: *Sanering van diffuus verontreinigde bodems gekoppeld aan de productie van biobrandstoffen*). Vlaanderendag 2007, Mol.

17 January 2007 - Presentation visit: Phytoremedation on metal contaminated soils in the Campine region (Translated from Dutch: *Fytoremediatie op metaal-aangerijkte gronden in de Kempen*). Hogeschool Kortrijk.

Organisation of institutional activities

9 June 2011 - Risk based management of metal contaminated soils (Translated from Dutch: *Risicobeheer en aanpak van metaalverontreinigde bodems*). Symposium organized by University College, Dept. PHL-BIO, Hasselt University (Centre for Environmental Sciences) and Ghent University (Laboratory of Analytical Chemistry and Applied Ecochemistry).

24 September 2010 – Phytomanagement of metal contaminated soils in the Campine region (Translated from Ducth: *Phytomanagement van metaalverontreinigde gronden in de Kempen*). Symposium organized by Laboratory of Analytical Chemistry and Applied Ecochemistry in cooperation with Centre for Environmental Sanitaton (Ghent Univeriesty), Centre for Environmental Sciences (Hasselt University) and Institute for Land and Forest Management (INBO), www.phytomanagement.ugent.be.

12 November 2009 Demosntration of the harvest of Short rotation coppice in Lommel (the Campine region) Organized by the Laboratory of Analytical Chemistry and Applied Ecochemistry in cooperation with the Institute for Land and Forest Management (INBO) and the Centre for Environmental Sciences (Hasselt University).

6 October 2007 - Field visit on short rotation coppice on metal contaminated soil in Lommel, by Centre Populiculture de Hainaut. Organisation of the Institute for Land and Forest Management (INBO).

5 April 2007 - Field visit on the use of energy crops on metal contaminated soil in Lommel of Wageningen Univeriesty and *Actief Bodembeheer de Kempen* (ABdK). Organised by the Centre for Environmental Sciences (Hasselt University) and Institute for Land and Forest Management (INBO).

14 February 2007 - Field visit on the use of energy crops on metal contaminated soils in Lommel and visit to Bio Oil Exploitations.