VILNIUS GEDIMINAS TECHNICAL UNIVERSITY
FACULTY OF ENVIRONMENTAL ENGINEERING
DEPARTMENT OF ENVIRONMENTAL PROTECTION

Dovilė Vaitkutė

EVALUATION OF SEWAGE SLUDGE IMPACT ON PINE AND BIRCH DEVELOPMENT

NUOTEKŲ DUMBLO ĮTAKOS PUŠIES IR BERŽO AUGIMUI ĮVERTINIMAS

Master thesis

Environmental Management and Cleaner production, 62404T103
Environmental Engineering

Vilnius, 2009

VILNIAUS GEDIMINO TECHNIKOS UNIVERSITETAS
APLINKOS INŽINERIJOS FAKULTETAS
APLINKOS APSAUGOS KATEDRA

TVIRTINU

Katedros vedėjas

____

(Parašas)

Pranas Baltrėnas

(Vardas, pavardė)

____

(Data)

Technologijos mokslo sritis

Aplinkos inžinerijos mokslo kryptis

Aplinkos inžinerijos studijų kryptis

Aplinkos inžinerijos studijų programa, valstybinis kodas 62404T103
Aplinkos apsaugos vadybos ir švariosios gamybos specializacija

BAIGIAMOJO MAGISTRO DARBO
UŽDUOTIS
..2007 09 11..Nr. ....
Vilnius

Studentui ......Dovilei Vaitkutei……....………….............................…...
(Vardas, pavardė)

Baigiamojo darbo tema: ....Nuotekų dumblo įtakos pušies ir beržo augimui ertinimas...........

patvirtinta 2008 m. lapkričio 19 d. dekano potvarkiu Nr. 425 AP

Baigiamojo darbo užbaigimo terminas 2009 m. gegužės 25 d.

BAIGIAMOJO DARBO UŽDUOTIS:

Taruškų girininkijos kirtavietėje (Panevėžio raj.) 1998 metais buvo paskleistas sunkiaisiais metalais užterštas nuotekų dumblas. Po metų teritorijoje pasodinti pušies ir beržo sodinukai sudarė sąlygas nagrinėti šių medžių rūšių fitostabilizacines savybes ir įvertinti metalų patekimo į medžius kiekius. Be to, aplinkosauginiais ir ekonominiais tikslais labai svarbus medžių biomasės prieaugis.

Šio magistrinio darbo tikslas – įvertinti ant dirvožemio paskleisto nuotekų dumblo poveikį pušies ir beržo vystimuisi. Tikslui pasiekti numatyta paimti dirvožemio ir medžių ėminius, nustatyti svarbias medžiams augti dirvožemio savybes (pH, bendrąją anglį ir kt.), funkcinius medžių vystimosi bruožus ir sunkiųjų metalų koncentracijas dirvožemyje ir medžių audiniuose. Modeliavimo dalyje svarbu įvertinti ir palyginti su tyrimo rezultatais metalų patekimo į medžius tendencijas bei įtaką biomasės prieaugiui.

Darbo rezultatai turi būti pristatyti jaunųjų mokslininkų konferencijose ir paskelbti jų pranešimų medžiagoje. Remiantis gautais rezultatais ir jų statistiniu pagrindimu studentė privalo paruošti mokslinį straipsnį moksliniame žurnale, įtrauktame į ISI Master List duomenų bazę.

Baigiamojo darbo rengimo konsultantai:....prof. habil.dr. Donatas Butkus, doc. dr. Dainius Paliulis...........

(Moksl. laipsnis, vardas, pavardė)

Vadovas ....dr. Edita Baltrėnaitė...
(Parašas) (Moksl. laipsnis, vardas, pavardė)

Užduotį gavau

…………………………………..

(Parašas)

…Dovilė Vaitkutė.…………..

(Vardas, pavardė)

……2007 09 11…...............….....

(Data)

Vilnius Gediminas Technical University
Faculty of Environmental Engineering
Department of Environment Protection

ISBN ISSN
Copies No. ....
Date ..........-.....-.....

Master Degree Studies **Environmental Management and Clean Production** study programme
Master's Thesis

Title	**Evaluation of Sewage Sludge Impact on Pine and Birch Development**
Author	**Dovilė Vaitkutė**
Academic supervisor	**Dr Edita Baltrėnaitė**

Thesis language: English

Annotation

Sewage sludge could be used in forestry as enrichment of soil properties. It is also expected that sewage sludge which is rich in phosphorus, nitrogen and organic material can enhance the growth of tree seedlings in poor soils. Our study was performed in Taruskos experimental forest site in Panevezys region. The experimental site amended with industrial sewage sludge ten years ago was afforestated with birch and pine seedlings.

The aim of our thesis is to evaluate the influence of sewage sludge to trees development after long time period. To reach our goal we have assessed three aspects: i) amended with sewage sludge and control (untreated) soil properties, ii) biomass and morphological parameters (functional traits) of trees in control and amended with sewage sludge sites, iii) Cu, Cd and Pb concentrations in both soil and tree compartments. In modelling part was used Phyto DSS model for evaluation heavy metals accumulation rate in tree seedlings and biomass growth. In the end of our study we suggest tree functional traits which could be used to evaluate sewage sludge application positive and negative aspects. In addition, we excepted one tree specie which planting in contaminated site would be the most economically and environmentally relevant.

Thesis structure: Introduction; Heavy metals and tree growth; Methodology of soil and trees measurements; Results and analysis; Soil – tree system modeling; Conclusions and suggestions; references.
Thesis consist of: 76 p. text without appendixes, 44 pictures, 16 tables, 90 bibliographical entries. Appendixes included.

Keywords
sewage sludge, birch, pine, biomass, heavy metals, Phyto DSS model

Vilniaus Gedimino technikos universitetas
Aplinkos inžinerijos fakultetas
Aplinkos apsaugos katedra

ISBN ISSN
Egz. sk. ....
Data ..........-.....-.....

Magistrantūros studijų **Aplinkos apsaugos vadybos ir švariosios gamybos programos baigiamasis darbas**

Pavadinimas	**Nuotekų dumblo įtakos beržo ir pušies augimui vertinti**
Autorius	**Dovilė Vaitkutė**
Vadovas	**dr. Edita Baltrėnaitė**

Kalba: anglų

Anotacija

Tiriamojo darbo pagrindinis tikslas įvertinti pušų ir beržų augimo ypatumus augant nuotekų dumblu patręštoje vietoje. Atlikti beržų ir pušų medelių palyginamieji tyrimai nuotekų dumblu patręštoje vietoje su medeliais augusiais natūraliomis sąlygomis. Taip pat įvertintas dirvožemio bei medelių užterštumas sunkiaisiais metalais. Atliktas modeliavimas su Phyto DSS programa ir įvertintas sunkiųjų metalų kaupimąsis medžiuose bei biomasės prieaugis. Darbo gale pateiktos rekomendacijos kokius medelių funkcinius parametrus pasirinkti vertinant nuotekų dumblo panaudojimo teigiamasias bei neigiamasias savybes. Taip pat išskirta medžių rūšis, kurios auginimas užterštoje teritorijoje būtų efektyvesnis tiek aplinkosauginiu tiek ekonominiu požiūriu.
Darbą sudaro 8 dalys: Įvadas; Sunkieji metalai ir medžių augimas; Dirvožemio ir medžių tyrimų metodika; Rezultatai ir jų analizė; Dirvožemio – medžio sistemos modeliavimas, Išvados ir siūlymai; Literatūros sąrašas.
Darbo apimtis – 76 p. teksto be priedų, 44 iliustr., 16 lent., 90 bibliografiniai šaltinių.
Atskirai pridedami darbo priedai.

Prasminiai žodžiai

nuotekų dumblas, beržas, pušis, sunkieji metalai, biomasė, Phyto DSS modelis

Content

Abbreviations

ϕ_1 – the measured root absorption factor at concentration C_1 (mg L^{-1});

ϕ *(C)* – root absorption factor at soil solution concentration *C* (mg L^{-1});

ρ_z – bulk density of the soil (g·cm^{-2}) at depth *z*;

A – total area (ha);

B – above-ground dry biomass (kg);

c – cost of alternative technology (Lt/ha);

c_1 – cost of planting (Lt/ha);

c_2 – cost of production (Lt/ha);

C – the soluble metal concentration (mg·L^{-1}) in the soil solution.

C_r – the soluble metal concentration (mg L^{-1}) in the root xylem

I – interest rate (%);

FC – future cost (Lt);

HM – heavy metals;

K ($0 \leq K < 1$) is the HMs transportation to roots decay constant;

L – earnings from the land (Lt).

LE – loss of earnings off the land (Lt/yr);

LG – cost of legislation (Lt);

M – the metal concentration in the above-ground dry biomass (mg·kg^{-1});

Mz – change in contaminant metal concentration (mg·kg^{-1}) at depth *z*,

P_1 – the production of saleable biomass (t/ha);

P_2 – the production of bio-ore (t/ha);

*t*1 – time needed for conventional technology to remediate land (yr);

*t*2 – time taken for phytoextraction to remediate land (yr),

T – transpiration rate, or the total water use (L);

t – time period (days);

R_z – root density fraction (root mass at depth *z*)/(total root mass);

RP – the cost of loss of reputation/goodwill (Lt);

SRL – specific root length (m·g^{-1});

V_1 – the value of the biomass (Lt/t);

V_2 – the value of the bio-ore (Lt/t)

Introduction

Problem

Human induced pollution causes both local and global environmental problems. Expansive of industrial activity, intensive transport flow, development of urbanized territories make environmental problems more complex (AAA 2006).

Soil is one of the ecosystem media's which reflects the magnitude of pollution of the environment. The soil ability to accumulate and store heavy metals is very important. However, the increased pollution rate in environment could alter the change of soil properties, and the probability of heavy metals leaching to ground water or accumulate in the plants is getting bigger (Griffiths *et al.* 2005). This could lead to the further accumulation in the surface organisms. At the end concentrations of accumulated heavy metals could reach harmful and maybe even fatal limits.

In case of atmosphere pollution part of pollutants reach the ground in the way of wet deposition. They can accumulate gradually in the plants and in the soil; this is kind of pollution of which natural ecosystem could take care. However, environmental problems, e.g. associated with utilization of sewage sludge may imbalance natural system.

According to Lithuanian agreement with European Union Lithuania committed to implement in 1991 May 21 accepted directive No. 91/271/EEB of cities sewage sludge treatment. Thus sewage water should not exceed permissible limits and must to be treated according to requirements. Today most of Lithuanian agglomerations meet requirements and in to the surface water releases more clean sewage water then previously.

However, the utilization of formed solid waste during sewage water treatment raises a lot of problems and discussions. In 2005 in the sewage sludge agglomerations composed 65 700 t (of dry matter) of sewage sludge. The biggest part of sewage sludge, about 66% is stored in the sewage sludge storing fields, and about 25% of it is used in the agriculture (AAA 2006).

Thus it is important to look for new alternatives for the treatment of sewage sludge. Since it is high in nutrients it has a potential to replace artificial fertilizers.

There are many problems associated with the use of sewage sludge in agriculture because of its unknown risk to human health. The other possibility to use sewage sludge is by restoring abandoned soils and plant tree plantations. There is some discussion about a long term impact of sewage sludge on a biomass growth after sewage sludge use. The sewage sludge contains beneficial components for the soils (such as organic matter, phosphorus, nitrogen, calcium, magnesium, *ect.*) (Dahlia *et al*. 1997; Arcak *et al.* 2006). However, it is usually high in heavy metals especially Cd, Pb, Cu, Zn, typically originated from industry. At high concentrations heavy metals can be phytotoxic and cause reduced plant growth or plant death (Kabata-Pendias, Pendias 2001). Toxic metal ions present in the substrate may adversely affect plants by damaging roots, which leads to an inhibition of the transport of water and nutrients to upper parts of the plant (Kupčinskienė 2006). This situation can lead to economical problems because planting of trees requires investments and outcome could be lower biomass than expected (selling trees for biofuel).

Actuality of the work

The analysis of soil contamination shows the need to look for new approaches to clean it with less possible impact on natural environment. For example, trees are able to take up heavy metals and produce biomass. However, the plantations of trees used for heavy metals extractions (willow, poplar) usually differ a lot from local environment. Thus one of the possible options could be to plant native tree species for heavy metals phytostabilisation from contaminated soils.

The increase of tree biomass is relevant both environmentally and economically. Thus the determination of heavy metals concentrations and their impact on the trees development becomes important step towards the assessment of the cleaning up the contaminated sites.

Aim of the work

To evaluate sewage sludge application impact on pine tree (*Pinus sylvestris*) and birch tree (*Betula pendula*) development.

Objectives

1. To compare soil properties and tree functional traits (morphological parameter and biomass) in sewage sludge applied and control sites.
2. To estimate heavy metals contamination pattern in soil and tree.
3. To validate model of heavy metal uptake and biomass growth of pine and birch trees.

Novelty of the work

Generally researches of heavy metal uptake of tree seedlings were carried out in laboratory conditions (Arduini *et al.* 1994; Adalsteinsson *et al.* 1997; Balestrasse *et al.* 2003; Šottníková *et al*. 2003; Bojarczuk 2004; Máthé-Gáspár *et al.* 2005; An 2006; Lageard *et al.* 2008 *ect.*). In this study the work was done in field conditions. The heavy metals uptake by birch and pine was analysed in sewage sludge amended soil in Taruskos forestry in Panevezys region.

For more specific evaluation of trees development there were used tree functional traits which can show tree response to changes of environmental conditions. In this study such functional traits: root/shoot ratio; specific root length; root/shoot branching and length; tree biomass; stem length and diameter, were analysed and discussed.

Practical meaning of the work

In this work two native and widely spread tree species (*Betula pendula* and *Pinus sylvestris*) were analysed. Evaluation of development and uptake of heavy metal provide the information about trees phytostabilisation properties. Study results could suggest choosing tree specie with higher metal accumulation and bigger biomass.

In addition, study data could be of high interest during estimation of the forest biomass in contaminated areas.

Hypothesis

The sewage sludge highly contaminated with HMs inhibits a tree development.

1. Heavy metals and tree growth

1.1. Cd, Cu, Pb toxic effects to trees

The sewage sludge contains beneficial components for the soils (such as organic matter, phosphorus, nitrogen, calcium, magnesium, ect). However, it is usually high in heavy metals (HMs) especially Cd, Pb, Cu, Zn, typically originated from industry. At high concentrations HMs can be phytotoxic and cause reduced plant growth or plant death (Kabata-Pendias, Pendias 2001). Toxic metal ions present in the substrate may adversely affect plants by damaging roots, which leads to an inhibition of the transport of water and nutrients to surface parts of the plant (Kupčinskienė 2006).

HMs within sewage sludge are rarely present on their own as simple salts but are absorbed by or bonded strongly to the organic matter in the sludge, this reducing their availability to the plants (Fig 1.1). However, the soluble form of HMs can be taken up by plants, thorough the root, up taken by aerial parts as atmospheric deposition of metals.

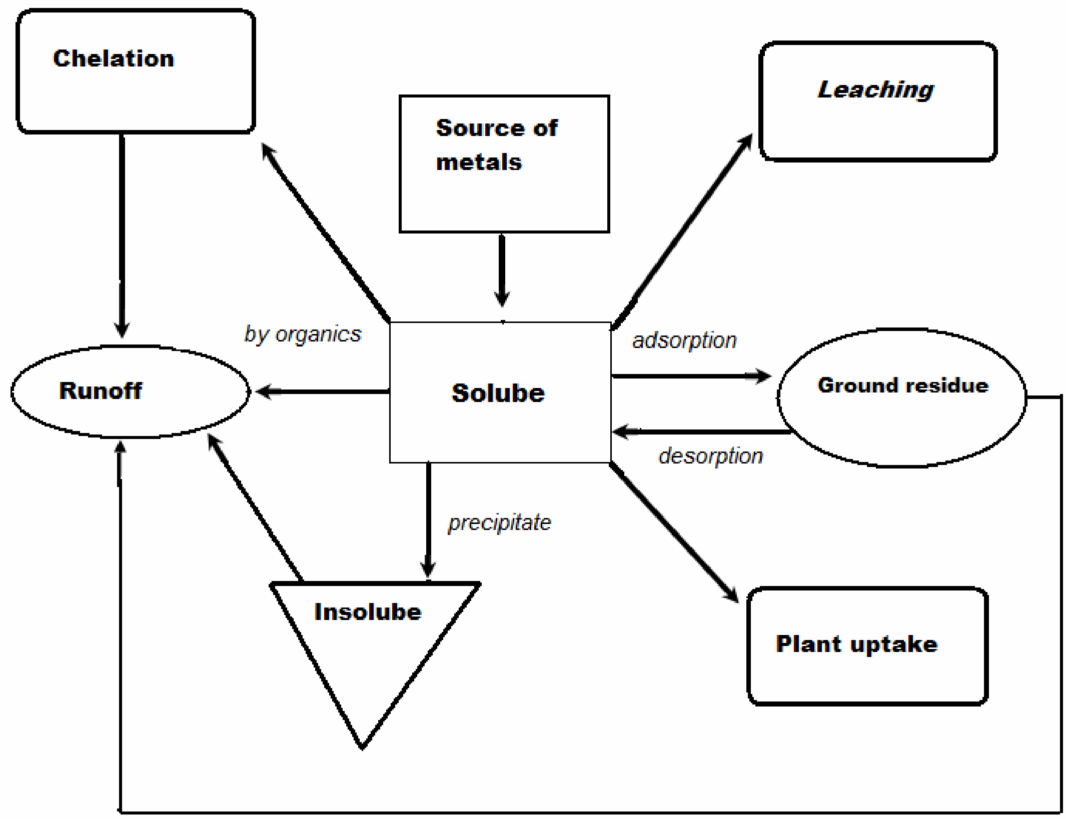


Fig 1.1 Heavy metals transformation and transportation way in soil (Water quality 2007)

Plants which are growing in contaminated with HMs soil accumulate them in different morphological parts differently. For example, Ag, Cr, Pb, Sn and V accumulate more in shoot (stems and leaves) compared to below ground biomass. The Cd, Co, Cu, Fe and Mo accumulate more in roots than in above ground biomass (stems and leaves). Ni, Mn and Zn are distributed more or less uniformly in root/shoot of the plant (Fig 1.2) (Prasad, Olivera Freitas 1999).

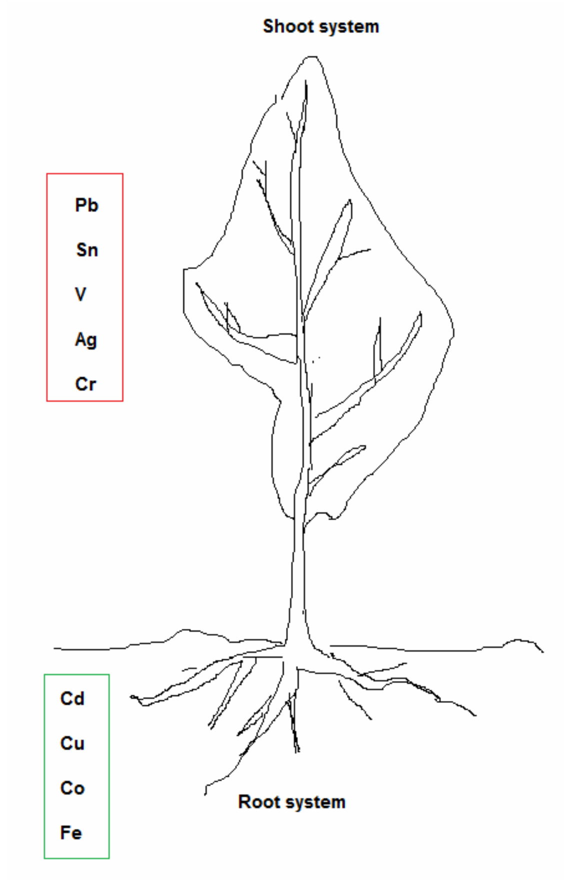


Fig 1.2 A generalized pattern of partitioning of metals in root and shoot system (Prasad, Olivera Freitas 1999)

Uptake of toxic metals, their translocation to plant parts and the plant resistance to them are depending on metal speciation which can be further modified by the metabolic processes of the plant.

HMs and Al primarily bind to the pectins in the cell walls and to the negatively charged cytoplasm-membrane surfaces due to their strong electrochemical potential. On binding, they displace cations such as Ca^{2+} and Mg^{2+} from the cell walls and membranes. Within the plant cells, heavy metals also bind to specific proteins, such as metallothioneins. The formed complexes are then transported to vacuoles or into and between cells. Many other bio-molecules, such as phytochelatins, organic acids, nicotianamine, or glutathione, could also be relevant to metal accumulation in trees (Brunner et al. 2008; Yruela 2005).

At the level of the whole plant, tissue, or cell, the excess of a metal usually is allocated to metabolically less active organs, tissues, or cell compartments. Tree roots in addition have symbiotically allied ectomycorrhizal fungi, which also can accumulate heavy metals in their cell walls and their vacuoles. The heavy metal ions bind to cell walls and polyphosphate granules, accumulating until reaching saturation (Brunner *et al*. 2008).

The level of adsorption of HMs as well as phytotoxicity will depend on the plant species. For example, *Betula* and *Salix* tree species are considered as metal tolerant populations of trees (Eltrop *et al.* 1991; Kahle 1993).

Other investigations showed that birch grown in metal polluted soil was affected and reduction of above – ground biomass was observed (Bojarczuk *et al.* 2002). Silver birch (*Betula pendula*), grown in polluted substrate was characterized by high biomass allocation to roots (60% vs. 30 to 40% in control substrate). The fertilization with sewage sludge as it consists mainly of nutritious organic material can accelerate their growth, and increase the biomass allocation to foliage (Bojarczuk *et al.* 2002).

Menon *et al.* (2007) research showed that willow is tolerant to toxic effects of pollutants, the fine root mass was significantly reduced by heavy metal (Zn, Cu, Pb and Cd) pollution in Norway spruce (*Picea abies*), poplar (*Populus tremula*) and birch (*Betula pendula*), but not in willow (*Salix viminalis*). Also the experiment on two Salix clones did not showed inhibition effects on growth for any of the heavy metal treatments (max. 41,4 $mg{\cdot}kg^{-1}$ Cd, 1914 $mg{\cdot}kg^{-1}$ Cr, 2422 $mg{\cdot}kg^{-1}$ Zn, 655 $mg{\cdot}kg^{-1}$ Pb) (Vandecasteele 2004).

Investigation of Iqbal and Shazia (2004) of Pb, Cd soil pollution on Lebbek Tree (*Albizia lebbeck*) and Leucaena (*Leucaena leucocephala*) tree seedlings showed untolerance, and their growth were significantly reduced at 500 and 700 $mg{\cdot}kg^{-1}$ of Pb and Cd as a compared to control.

The adverse effects of potentially toxic trace elements, like Cd or Zn, on xylem growth of spruce plants are discussed with regard to possible growth reductions in forest trees under field conditions (Hagemeyer *et al.* 1994).

The Cu, Cd and Pb are HMs known to have phytotoxic effects. Also they are known for the synergistic effects (Breckle, Kahle 1991; Arduinni et al. 1994; Kabata – Pendias, Pendias 2001).

Cu toxic effect to trees

Copper is an essential metal for normal plant growth and development, although it is also potentially toxic. Cu participates in numerous physiological processes and is an essential cofactor for many metalloproteins, however, problems arise when excess Cu is present in cells. Excess Cu inhibits plant growth and impairs important cellular processes (for example, photosynthetic electron transport) (Fig 1.3) (Yruela 2005).

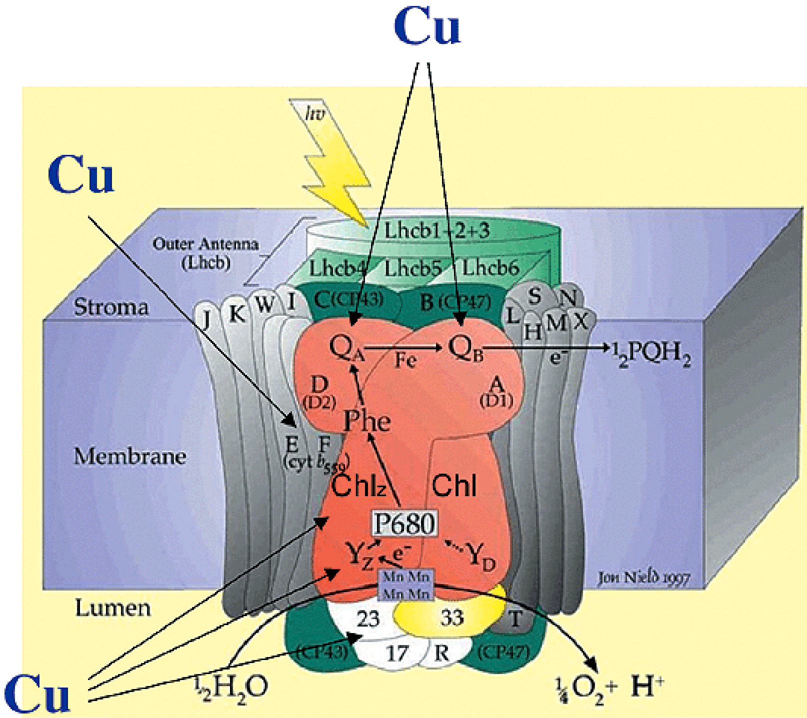


Fig 1.3 Scheme of toxic Cu action sites in photosystem II (Yruela 2005)

Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, amino oxidase, laccase, plastocyanin and polyphenol oxidase. At the cellular level, Cu also plays an essential role in signalling of transcription and protein trafficking machinery, oxidative phosphorylation and iron mobilization. Thus, plants require Cu as an essential micronutrient for normal growth and development; when this ion is not available plants develop specific deficiency symptoms, most of which affect young leaves and reproductive organs (Yruela 2005).

The redox properties that make Cu an essential element also contributes to its inherent toxicity. Redox cycling between Cu^{2+} and Cu^{+} can catalyze the production of highly toxic hydroxyl radicals, with subsequent damage to DNA, lipids, proteins and other biomolecules (Yruela 2005). Thus, at high concentrations, Cu can become extremely toxic causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth, for example, investigations on root elongation and development showed that the main root development inhibitor of poplar was copper (Cu>Pb>Al) (Bojarczuk 2004).

By using nutrient film technique (permit undisturbed growth of the roots during monitoring) there was determine the effect of Cu to birch root system. At uniform external Cu supply, average root length was affected by increased Cu concentration during the first four days while the next four days only the overall root length was significantly reduced. During the first four days in the split-root experiments with differentiated Cu supply, additional Cu primarily reduced root number on the

Cu-treated parts of the root system but at stronger Cu concentration the overall root length was also significantly reduced. In contrast, number and average root length of the part of the root system not exposed to Cu increased when 1, 2 and 5 $\mu g \cdot kg^{-1}$ Cu was added to the other side (Adalsteinsson *et al*. 1997).

The determined phytotoxic concentrations of Cu in the soil was 60 – 125 $mg \cdot kg^{-1}$ (Kabata – Pendias, Pendias 2001). The normal determined concentration in the tree seedlings were 1 – 10 mg/kg. Copper toxicity in plants usually begins when the tissue level exceeds 20 – 30 mg of Cu in 1 kg tissue (Pais and Jones 1997).

Cd toxic effect to trees

Cadmium can alter the uptake of minerals by plants through its effects on the availability of minerals from the soil, or through a reduction in the population of soil microbes, for example, Athar, Ahmad (2002) determined that Cd being the most toxic metal followed by Cu, Ni, Zn, Pb and Cr. Moreover, the presence of Cd in the soil resulted in the maximum inhibition (84.9%) in the number of free living *Azotobacter chroococcum* cells over the control. The phytotoxicity was apparently due to the susceptibility of the free living *Azotobacter chroococcum* cells to the toxic doses of heavy metals. Protein content decreased from 19.0 – 71.4% in metal exposed plants at metal concentrations equivalent to those found in polluted soil.

Stomatal opening, transpiration, and photosynthesis have been reported to be affected by Cd in nutrient solutions, but the metal is taken up into plants more readily from nutrient solutions than from soil. Chlorosis, leaf rolls and stunting are the main and easily visible symptoms of cadmium toxicity in plants. Chlorosis may appear to be Fe deficiency, phosphorous deficiency or reduce Mn transport. The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis (Benavides *et al*. 2005).

In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots. Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatments (Balestrasse *et al.* 2003). Cadmium treatments have been shown to reduce ATPase activity of the plasma membrane fraction of wheat and sunflower roots. Cadmium produces changes in the functionality of membranes by inducing lipid peroxidation, and disturbances in chloroplast metabolism by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation (Benavides *et al*. 2005).

As reported by Arduini *et al.* (1994) tap – root elongation of stone pine (*Pinus pinea*) and maritime pine (*Pinus pinaster*) was drastically reduced in the 5 $\mu m \cdot kg^{-1}$ Cd^{2+} and in the (Cd^{2+} +

Cu^{2+}) treatments. The other study revealed that Cd concentration 2.5 mg·kg^{-1} significantly decreased the biomass of shoots and roots of *Picea sitchensis* (Burton *et al.* 1986). The shoot growth was determined to be more sensitive to Cd than root growth in birch tree (Gussarsson *et al.* 1996; Österås 2004).

Growth parameters of six fast growing trees showed that the roots responded to Cd treatment more sensitively than the shoots. Cd-treatment suppressed rooting and root growth (length and biomass production) as well as its development in all tested species. Root systems of *Salix cinerea, Salix alba, and Populus* cv. *Robusta* were tolerant to Cd (Šottníková *et al*. 2003). Also research on determination of soil pollution showed significant increase in Cd also it's interaction with other metals (Máthé-Gáspár *et al.* 2005).

It is determined that for tree growth normal contents of Cd are 0.1 – 1.0 mg·kg^{-1} and usually the growth reduction starts when the level of Cd in a tissue reaches – 3 mg·kg^{-1} (Pais and Jones 1997).

However, plants grown in a greenhouse or a container take up more Cd than the same plants grown in soil with the same cadmium levels in the field. This is due to greater root development in a confined volume in containers and to the fact that all the roots are in contact with cadmium-contaminated soil. In the field, roots may grow down below the cadmium-contaminated level.

Pb toxic effects to trees

Significant increase in the Pb content of cultivated soils has been observed near industrial areas. Pb tends to accumulate in the surface ground layer and its concentration decreases with soil depth (de Abreu *et al.* 1998). It is easily taken up by plants from the soil and is accumulated in different organs. Pb is considered a general protoplasmic poison, which is cumulative, slow acting and slightly.

The Pb translocation from root to other above ground plant parts is usually repressed because of to the root endodermis which acts as barrier. At high Pb, or lethal, concentrations this barrier is broken and Pb easily is transported to other parts of the plant. Pb accumulation location is usually present in the intercellular spaces, cell walls and vacuoles (Sharma, Dubey 2005).

The Pb after entering inside the cells (even in small amounts) could be a cause of negative impact on physiological processes: hormonal status, membrane structure, water potential, electron transport and on enzyme activation. Pb decreases photosynthetic rate by distorting chloroplast ultrastructure, diminishing chlorophyll synthesis, obstructing electron transport and inhibiting activities of Calvin cycle enzymes. Pb also causes the imbalance of the minerals within the tissues, such as K, Ca, Mg, Mn, Zn, Cu, and Fe, by physically blocking the access of these ions to the absorption sites of the roots (Sharma, Dubey 2005).

Pb is harmful to the plants when its concentrations in the soil reaches 100 – 200 $mg \cdot kg^{-1}$ (Bergman 1986; Kabata–Pendias, Pendias 2001). Studies on Pb toxic effect on tree seedlings showed significant negative effects in smaller concentrations especially if it is combined with other metals, it showed synergistic effect. For example, root elongation rates of beech (*Fagus sylvatica*) seedlings were significantly reduced with 44 $mg \cdot kg^{-1}$ of Pb in plant – available Pb in the soil was about 30%, but only with 24 $mg \cdot kg^{-1}$ Pb when combined with 2 $mg \cdot kg^{-1}$ of Cd (Breckle, Kahle 1991). Decrease in birch biomass was observed when Pb concentration in soil reached 18 $mg \cdot kg^{-1}$ and Cd was 3.6 $mg \cdot kg^{-1}$.

The ability of lead to accumulate in trees can express atmospheric pollution quit precise, for example, Lageard with colleagues 2008 analysed of *Pinus sylvestris* growing around an isolated point source of atmospheric lead. Mean site records demonstrate patterns of lead-in-wood that can be related significant relation to documented pollution chronology and features of the climate site. These results indicated that assays of lead in the wood of *Pinus sylvestris* can be used to estimate the general scale and timing of atmospheric lead pollution episodes in areas where historic records are absent.

In conclusion, the investigation on different kind of crops showed, that the concentration of metals in the soil that reduces the growth of shoots and roots by 50% in mg Pb kg^{-1} dry soil and mg Cu kg^{-1} dry soil were in the range of 519 to >1280 (285–445), and 48–232 (<40–110), respectively (An 2006). It was determined that Cu is more toxic than Pb to the plants, and that root growth is more sensitive to the toxicity endpoint than shoot growth in Cu or Pb amended soils.

1.2. Heavy metals in sewage sludge

Sewage sludge consists of the by-products of waste water (municipal and industrial) treatment. It contains both compounds of potential environmental value (including organic matter, nitrogen, phosphorus and potassium, and to lesser extent, calcium sulphur and magnesium), and pollutants (including heavy metals, organic compounds, pharmaceuticals and pathogens). The content of sludge depends on the original pollution load of treated water, and also the treatment process applied to the waste water and sludge (European Commission 2001).

For example, treatments carried out on sludge can greatly influence its content of nitrogen and phosphorus (Table 1.1).

Table 1.1 Contents of nitrogen and phosphorus in sludge after different treatments and in other urban wastes and animal manures: DM – dry matter (European Commission 2001)

	Total Nitrogen (% of DM)	**Phosphorus (% of DM)**
Urban Sludge		**0.9 – 5.2**
Liquid	**1 – 7**	
Semi-solid	**2 – 5**	
Solid	**1 – 3.5**	
Composted	**1.5 – 3**	**0.2 – 1.5**
Urban compost	**0.96**	**0.39**
Green wastes composting	**1.0 – 2.4**	**0.04 – 0.44**
Animal manure	**4 – 7**	**0.91 – 3.3**

As it showed above sewage sludge contains organic matter, major and minor nutrients, and valuable trace elements. Such sludge can be used to stabilise soil and other waste prone erosion and to provide the initial stimulation to help recover disturbed land (Sewage sludge for land restoration 2004). Sewage sludge addition will promote plant growth which will over the time provide a stable source of organic matter (Lazdiņa1 *et al.* 2007).

The safe disposal of the sewage sludge is one of the major environmental concerns throughout the world. Disposal alternatives that have been tried include soil application, dumping at sea, landfilling and incineration (Sanchez Monedero *et al.* 2004). Landfilling and land application of the sewage sludge are suggested to be the most economically feasible sludge disposal methods (Metcalf and Eddy 2003).

The sewage sludge applications induced a significant accumulation of organic carbon and nitrogen in the surface soil layer. After a last application, a fast decrease in organic carbon and nitrogen took place to reach 7.5 and 0.58 g m^{-2} respectively, in 1998. The C values of the whole soil organic matter increased continuously in both the control and the Sewage sludge amended soils, which indicates an increasing incorporation of maize C from 1974 to 1993 (Parat *et al.* 2007). Changes in soil C are known to influence soil physical properties (Denef *et al.* 2001) and so it could be said that the soils amended with sludge are more physically stable than the unamended soil (Sort and Alcaniz 2001).

Sewage sludge use in forestry

The use of sewage in forestry is considered more than in agriculture because the application of sewage sludge to forest soils offers several advantages compared with application to agriculture soils, such as a) less chance of human contact with the bioresidue; b) elimination of pollutants from the human food chain; and c) flexibility in the time of application associated with perennial crops (Egiarte *et al*. 2005; Moffat, 2006)

Organic waste materials, e.g., composted sewage sludge appear stimulating for increasing the microbial biomass and activity of the grey forest soil of the forest nursery as well as for the germination and size of pine seedlings (Selivanovskaja, Latypova 2006).

The 2-year results of sewage sludge application to apple trees study demonstrated that sewage sludge applied to apple trees did not cause toxicity in the leaves, but there is always risk that long-term sewage sludge application could result in the accumulation of some heavy metals in the soil and their entry into plants in quantities above the maximum permitted concentrations (Bozkurt, Yarilgaç 2003).

Liquid and cake sewage sludge's have been used to increase the fertility of infertile forestry sites in Britain for many years. A substantial research programme supports the practice, demonstrating the use of sewage sludge can improve foliar nutrition and forestry productivity without deleterious environmental consequences, provided that the amounts applied are appropriate to the needs of the trees (Bayes *et al*. 1991).

Taylor and Moffat (2006) estimate that 75 000 hectares of forestry in Great Britain could benefit from the nutritional effects of sewage sludge application. Sludge application may be carried out during different periods of tree growth. When considering re-afforestation, land spreading may be carried out prior to planting. In intensive forestry production sludge application may also be performed just after sowing, thinning or clear felling. In established forests, sludge application could potentially occur all year round, if good practices are observed and local conditions are acceptable (European Commission 2001).

During the investigation of sewage sludge use to recultivate the woodcutting area near Panevėžys town, there was determined that optional norm of wastewater sludge in replanted woodcutting areas of peaty forests of this region is 300 $t \cdot ha^{-1}$. Also it can be increased to 400 $t \cdot ha^{-1}$ and more in the cases when: the content of hazardous chemical elements in sludge is correspondingly lower; the thickness of undisturbed peaty forest soil layer is greater then 0.5 m (Katinas *et al.* 2002). However, the investigation of technogenic pollutants from Panevėžys town industrial waste water sludge influence to the seedlings showed that this sludge has good influence on seedlings growth rate, but during the first year the excess of toxic elements and their mobile forms depressed their rooting and uptake of some biophilic trace elements. However, the visual analysis of birch and pine trees after 7 years in site treated with industrial sewage sludge did not revealed any negative effects for both tree species (Baltrėnaitė, Butkus 2007).

The other researches (Selivanovskaja, Latypova 2006) of sewage sludge influence on growth of pine showed that even at very high application rates of 150 and 175 $t \cdot ha^{-1}$, no toxicity symptoms were exhibited in the pine seedling yields and plant behaviour. The rates of composted sewage sludge from 75 to 100 $t \cdot ha^{-1}$ can be recommended to guarantee the best conditions for pine seedlings and the least effect on soil microbial population.

The metal concentrations in the sewage sludge depend on several factors such as: (a) sewage origin, (b) sewage treatment processes, and (c) sludge treatment processes (Hue and Ranjith 1994)

The main origins of them in sludge are domestic effluent, road runoff and industry (Human health and environmental impacts… 2007). In Table 1.2, there are shown the main heavy metals found in sewage sludge also it summarizes the average of these metals in member states sewage sludge and compares them with limits imposed by the European Commission.

Table 1.2 – Average content of heavy metals in sewage sludge in member states: DM – dry matter (European commission 2001)

Heavy metals	**EC Directive 86/278/EEC, mg kg^{-1} DM**	**Range in the member states, mg kg^{-1} DM**
Cd	**20 – 40**	**0.4 – 3.8**
Cr	1000 – 1750	16 – 275
Cu	**1000 – 1750**	**39 – 641**
Hg	16 – 25	0.3 – 3
Ni	300 – 400	9 – 90
Pb	**750 – 1200**	**13 – 221**
Zn	2500 – 4000	142 – 2000

Part of heavy metals by amending soil with sewage sludge is accumulating in soil and the other part is going down to groundwater. Anyway in many times by investigating migration of heavy metals there weren't determination deeper migration than 0.5 m down. More than 90 % of heavy metals stays in 0 – 15 cm layer, about 1 % are migrating deeper than tillage layer, and till 10 % are accumulating in the plants (Torri and Lavado 2007).

Different studies of sewage sludge application on soil showed that after many years the concentration of metals such as Cd, Cu, Pb, Zn and others increases in the soil (Dahlia *et al.* 1997; Arcak *et al.* 2006), but these concentrations did not reach the limit concentration. However, other research about heavy metals (Cu, Ni and Zn) leaching after sewage sludge application showed that after single addition of metal – enriched sewage sludge, the risk of heavy metal pollution of groundwater appears to be low (Montse and Joan 2006), but there is always a risk that after longer sludge application the soil contamination could be bigger.

During the investigation of sewage sludge from Vilnius waste water treatment plant to the soil using it as fertilizer, there was not determine any changes in heavy metal concentrations after first year fertilization (10t/ha). After second fertilization or fertilizing with single norm of 20t/ha, there was determined in the soil tillage layer that Zn concentration was bigger about 2 times, Cr – 2-5, Cu – 2 – 4 times. But still these concentrations did not exceed the limits (Gasiūnas 1997).

According to heavy metal concentrations in sewage sludge Lithuanian requirements for sewage sludge application to land restoration and fertilization – LAND 20-2005, are classified in three categories (Table 1.3).

Table 1.3 Sewage sludge classification according to heavy metal concentrations

The category of Sewage sludge	The concentration of heavy metals, mg·kg^{-1}						
	Pb	**Cd**	**Cr**	**Cu**	**Ni**	**Zn**	**Hg**
I	<140	<1,5	<140	<75	<50	<300	<1,0
II	140–750	1,5–20	140–400	75–1000	50–300	300–2500	1,0–8,0
III	>750	>20	>400	>1000	>300	>2500	>8,0

In Torri and Lavado (2007) study, two types of sewage sludge were used: sewage sludge and sewage sludge containing 30% DM of its own incinerated ash, at rates equivalent to a field application of 150 t DM ha^{-1}. Pots were maintained at 80% of field capacity through daily irrigation with distilled water. Soil samples were obtained on days 1, 60, 270 and 360. Results showed that sludge application increased the less available forms of Cd, Cu and Pb. This is also minimizing the leaching of HMs in to deeper soil layers and ground water; however it could repress the extraction of HMs from soil.

In conclusion, the sewage sludge used as fertilizers in forestry is still an object of discussions. The deforested soils usually are lack of nutrients and mainly acidic, especially exploited peat areas (Gradeckas *et al*. 1998). Sewage sludge could be used in forestry as enrichment of soil properties. It is also expected that sewage sludge which is rich in phosphorus, nitrogen and organic material can enhance the growth of tree seedlings in poor soils.

1.3. Heavy metals in the soil

Soil properties and heavy metals

Soil naturally contains heavy metals, originating from the parent rock, but anthropogenic impact on environment can add heavy metals in the soil, and these bigger concentrations could cause toxic effects to plants, animals and humans. Overload concentrations of heavy metals can be accumulated in plants, or could be leached to the ground or surface water.

Soil has an inherent potential to resist (stability) and recover from (resilience) environmental stresses. Although both of these factors are well-recognized as key components of soil quality, only soil stability has been the subject of considerable research (Griffiths *et al.* 2005). If heavy metals are well stored in the soil environment their toxic and harmful effect could be avoided. But this process depends on many factors starting from soil texture, pH, Eh (redox potential), cation exchange capacity (CEC) *ect*. These factors in different conditions may cause mobility of the heavy metals.

Soil texture

Soil texture type depends on particles sizes and percentage of clay, sand and silt in fraction, for determination of soil texture it is used soil texture triangle (Fig 1.4).

Quantity of HMs differs a lot in coarse (sandy) and in fine (clay loam and clay) solid. The main reason of this difference is different soil mineralogical composition or different quantity of sandy and clay particles (Mazvila 2001).

Usually in sands dominate quartz and coarse particles, these fractions have a very small quantity of HMs. In loamy clay and clay there are minerals and clay particles, in which there is big quantity of HMs. These facts confirms by data of investigations made in 1993 – 1997 by the Center of Agrochemical Investigations (Table 1.4).

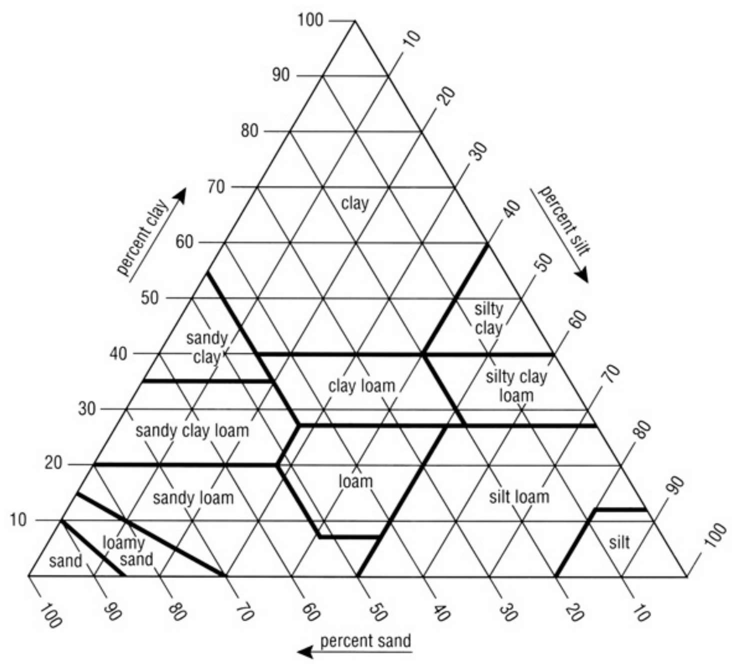


Fig 1.4 The soil texture triangle (Williams 2008)

The biggest dependence on soil texture was determined of Cr, Ni, Cu and Zn. In soil of medium weight texture humus layer there were found Cr 1.8 – 2.3, Cu 1.8 – 2.2 times respectively

3.8, 3.8 times more than in sandy soil. The biggest differences between quantities of HMs are between fine and heavy texture soils, which are very different and according physical clay particles (<0.01 mm) number. In humus layer of sandy soils physical particles of clay are not more than 20%, whereas in soil with heavy texture they consists more than 40%. In sandy loam there were determined Cd, Pb – 1.1, Cu – 1.2 times more than in light loam (Mazvila 2001).

Table 1.4 The concentration of Cu, Pb, Cd and Zn in the soils with different texture mg·kg^{-1} ± SD (dry material) in Lithuania (Mazvila 2001)

Depth, cm	**Cd**	**Pb**	**Cu**	**Zn**
Sand				
0 – 20	0.35±0.15	8.8±2.5	3.3±1.6	17.8±8.6
20 – 40	0.35±0.16	8.0±2.5	3.1±1.5	16.4±8.0
Sandy loam				
0 – 20	0.42±0.17	11.1±2.5	6.1±2.6	27.1±9.5
20 – 40	0.43±0.18	10.5±2.7	6.0±2.5	25.7±9.0
Light loam				
0 – 20	0.48±0.21	12.6±2.3	7.3±2.7	30.1±9.3
20-40	0.49±0.22	12.0±2.4	7.8±3.2	29.7±10.0
Medium, clay loam and clay				
0 – 20	0.62±0.27	15.8±3	12.4±4.8	47.2±11.5
20 – 40	0.64±0.3	15.1±2.9	13.7±4.4	48.7±13.4
Peat, muck peat				
0 – 20	**0.73±0.35**	**15±6.1**	**10.8±7.4**	23.1±11.5
20 – 40	**0.68±0.27**	**12.8±4.2**	**10.7±7.8**	20.2±11

In peaty soils there are found Zn – one fifth less, Pb – 1.3, Cu and Cd – 1.6 times more that in average in country's mineral soils.

It is important to know the variation of natural born metals in different locations or of different texture of soils and genesis. As data for topsoils of Lithuania shows, the average concentrations of HMs are greater in loamy and clayed soils than in sandy soils (Tables 1.5).

Table 1.5 The concentration of Cu, Zn, Cd and Pb in the topsoil of Lithuania (average $mg \cdot kg^{-1}$ (dry material)) (Kadūnas *et. al.* 1999)

Soil texture	Cu	Zn	Cd	Pb
Sand	6.5	22.2	3.5	15.5
Sandy loam	9.6	28.9	5.0	14.9
Loam-clay	11.4	35.7	6.4	15.3
Peat	10.6	39.9	3.6	36.2

Other research on the soils in Lithuania has shown that in the humus layer (0-20cm) there are the following average quantities of heavy metals: Cd – 0.46, Pb – 11.9, Cu – 6.9, Zn – 28.5, Mn – 253, Fe – 8.21 $mg \cdot kg^{-1}$ (Soils of Lithuania 2000).

Soil moisture content

As it is known the soil moisture has influence on the transportation of the soil solution through the roots. Lower moister content can also indicate suppression of the diffusion and the mass flow in soil and to plant (Robinson *et al*. 2003).

Soil moisture regime alos strongly affects the over-all mobility of metals in soils as a result of its influence on metal redistribution among solid-phase components. However, the moisture effects on metal reactivity are influenced strongly by soil properties and the nature of the metal (Han *et al.* 2001).

Soil organic matter

The mobility of HMs can be reduced by one of the main soil organic compound, called humus acid. It creates quit strong and long lasting bounds with metal ions, and these derivatives are called chelates. For example, the residence time of Pb in soils rich in organic matter has been calculated as hundreds of thousands of years (Manceau *et al.* 1996).

High HM concentrations in organic matter slow down its decomposition by reducing the activity of soil invertebrates and micro-organisms. On sites with low to moderate contamination, the concentrations of contaminants are likely to be insufficient to significantly influence organic matter decomposition. As contaminated litter decomposes, heavy metals are released back into the soil. If organic matter and plant cover can be built up to a point where natural decay of vegetation produces further organic matter, normal cycling is established, thus reducing the risk of toxicity and of released heavy metals being leached to controlled or potable waters (Hutchings 2002).

During the investigation of heavy metals distribution in Lithuanian soils there was determine that, for example, when the amount of humus was around 2%, Cd concentration in average was 0.4, Pb – 10.1 and Cu – 5.2, when humus amount is 2.1 – 4% - 0.46, 12, 6.9, and when humus amount reaches 4.1 – 10% – 0.52, 13.8 and 8.7 $mg \cdot kg^{-1}$ respectively (Mazvila 2001).

The incorporation of soil amendments such as composted organic matter and crushed limestone can be used to control the mobility and reduce the availability to plants (phytoavailability) of contaminants to levels that can be tolerated by trees. This form of reclamation will also increase nutrient availability and thus aid tree growth and survival. Soil amendments can also be used to optimize soil carbon: nitrogen ratios to promote the microbial degradation of organic contaminants. For example, study of sewage sludge influence to organic carbon amount in tillage soil, revealed that sludge-derived organic matter contributed to the formation of macroaggregates through the binding of pre – existing microaggregates. These macroagreggates were thus responsible for the accumulation of trace metals in the coarsest fraction as well as for the protection of maize-derived organic matter against biodegradation. After sludge application ceased, the disaggregation of macroaggregates occurred simultaneously with high losses in Cu and Pb (Parat *et al* 2007).

Soil pH

Soil pH is one of the soil quality measures. Soil pH is the most commonly-used index of plant root-zone acidity or alkalinity. Soil pH is important to plants because it influences the chemical form of many elements in the soil, and it influences soil microbial processes.

The pH of soil or more precisely the pH of the soil solution is very important because soil solution carries in it nutrients such as nitrogen (N), potassium (K), and phosphorus (P) that plants need in specific amounts to grow, thrive, and fight off diseases.

If the pH of the soil solution is increased above 5.5, N (in the form of nitrate) is made available to plants. P, on the other hand, is available to plants when soil pH is between 6.0 and 7.0 (Miller, Hills 2000) (Fig 1.5).

As it is known, the low soil pH is mostly caused by free hydronium ions in the soil solution, which replace other positive ions. During this process many elements which are important to the growth of plants, like Ca, Mg and K are washed away (Kupčinskienė 2006).

The soil pH helps to evaluate the soil fertility, nutrients assimilation to the plants and toxic effect of heavy metals. For example, toxic effect of aluminium (acidic, pH <5.5) cause the thickness and shortness of root fibres, leading to decrease assimilation of nutrients from the soil, and resulting in slow the development of plant. These effects can be smoothed by fertilization or adding microelements to the soil.

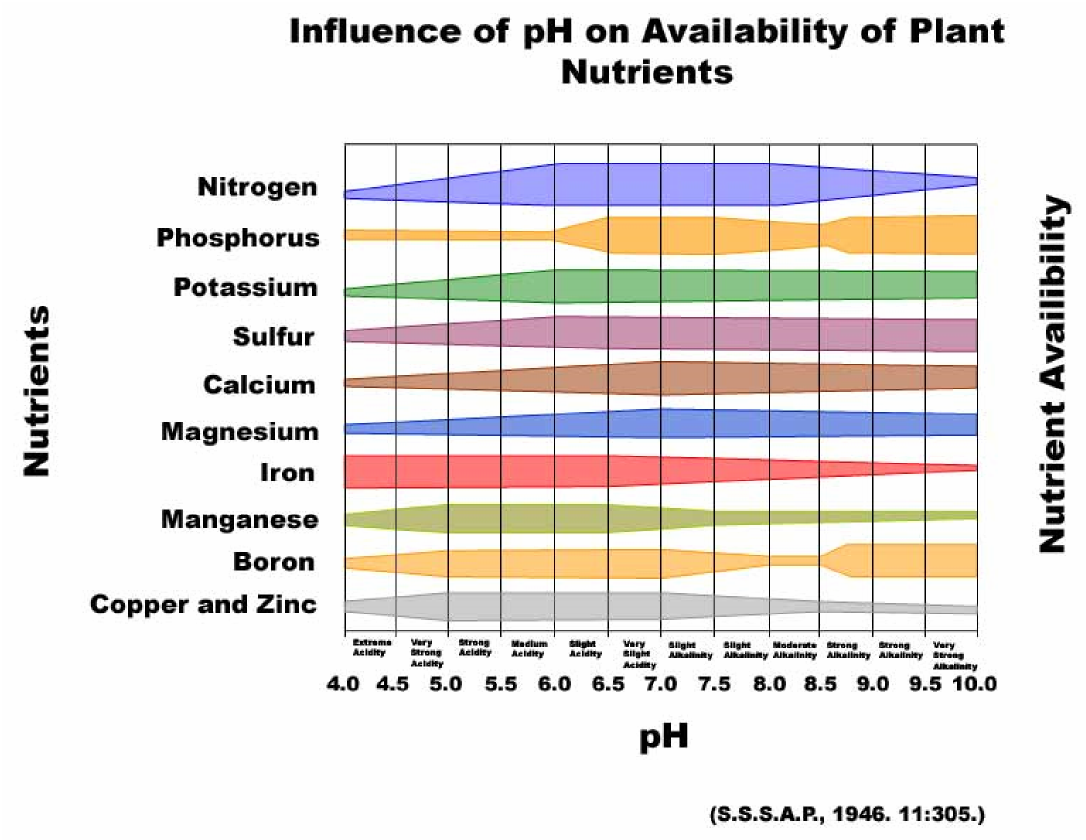


Fig. 1.5 Nutrients and minerals availability in different pH values (Hart 2008)

What is more, it is also known that some metals or elements can change biochemical processes in the tree. For example, research on birch (*Betula pendula*) seedlings has shown that high concentrations of aluminium in the substrate diminish assimilation of Ca, K, P, K and Zn by plants, causing strong inhibition of root and shoot development (Göransson, Eldhuset 1995; Bojarczuk *et al.* 2002). According to investigation on beech (*Fagus sylvatica*) at $\geq$1.0 mg·kg^{-1} Al significantly increases the concentrations of starch in both the shoots and the roots, and at $\geq$ 0.5 mg·kg^{-1} the roots contained more of total phenols than untreated seedlings. Increased concentration of starch and phenols may cause reduction of shoot growth (Påhlsson 1991).

Also lower pH of the soil may cause greater mobility of toxic elements. Which could be leached to the ground water or be uptaken and accumulated by plants (Kabata – Pendias; Pendias, 2001).

Mobility of trace elements in soil media (Kabata – Pendias; Pendias 2001):

1. Oxidizing and acid, pH<3: a) very mobile – Cd, Co, Cu, Ni, and Zn; b) mobile – Hg, Mn, Re, and V.
2. Oxidizing in the absence of abundant Fe-rich particulates, pH >5: a) very mobile – Cd and Zn; b) mobile – Mo, Re, Se, Sr, Te, and V.

3. Oxidizing with abundant Fe-rich particulates, pH>5: a) very mobile – none; b) mobile – Cd and Zn.
4. Reducing in the absence of hydrogen sulfide, pH>5: a) very mobile – none; b) mobile – Cd, Cu, Fe, Mn, Pb, Sr, and Zn.
5. Reducing with hydrogen sulfide, pH>5: a) very mobile – none; b) mobile – Mn and Sr.

For example, for using sludge in agriculture and for other fertilization there are set limits of trace and heavy metals according to soil pH (Table 1.6).

Table 1.6 Limits of concentration of various elements in sludge (use in agriculture) regulations 1989 apply (Sniffer 2007)

Element	**Limit According to pH of soil**			
	5.0<5.5	5.5<6.0	6.0-7.0	>7
Zinc	200	250	300	450
Copper	80	100	135	200
Nickel	50	60	75	110
For pH 5 and above				
Lead	300			
Cadmium	3			

However, the maximum permissible loads are based on the assumption that field pH values do not change very rapidly or do so over periods of several years rather than weeks or months. On the contrary, data from pot experiments have suggested that there may be significant pH fluctuations some weeks or months after sewage sludge has been added to soil (Hooda and Alloway, 1993; Antoniadis *et al.* 2008). It is not clear whether such pH changes occur in the field, and what effect they may have on heavy metal dynamics, because most of the field experiments measure heavy metal availability with a frequency of several months or years.

1.4 Tree functional traits to evaluate metal toxicity

The several indicators, so called functional traits, could be used to show the changes of morphological parameters of trees affected of changing environment. The functional traits of the plants can show the adaptation to environment. Also it could show the responses of plant to environmental disturbances such as contamination soil with heavy metals, decreasing moisture content or nutrient resource availability. The plant growth reduction as itself could be the basic indicator of unfavourable environmental conditions to plants. However, sometimes it is difficult to evaluate whole plant response to the environmental disturbance particularly for trees. In those cases separate parameters for different parts of the plant are used (Table 1.7). Their variation could inform about possible changes in soil properties or about its contamination. Moreover, these parameters also used to determine the plant relative growth rate (RGR).

Table 1.7. Examples of parameters used in plant growth analysis and the units in which they are expressed (Ryser 1996; Landjeva *et al.* 2003; Mahmood *et al*. 2007; Singh and Agrawal 2007)

Parameter	Unit
specific root length (SRL)	$m \cdot g^{-1}$(root)
root mass ratio (RMR)	$g \cdot g^{-1}$ (plant)
stem mass ratio (SMR)	$g \cdot g^{-1}$ (plant)
leaf mass ratio (LMR)	$g \cdot g^{-1}$ (plant)

The other parameters used to determine plant development process are the leaf area, root or shoot length, root or shoot number (branching), stem length. These are more commonly used to evaluate the toxicity of HMs in soil. The most common functional traits are discussed further.

Root:shoot ratio/root mass ratio (RMR)

Root:shoot ratio shows if plant develops healthy, however, the increased ratio is good if it came from greater root size and not from a decrease in shoot weight. For most trees under normal conditions, the root/shoot ratio is 1:5 to 1:6; the top is 5 to 6 times heavier than the roots (Harris 1992). An increase in the root/shoot ratio, on the other hand, would indicate that a plant was probably growing under less favourable conditions. However, in other case the equal root and shoot biomasses are considered as plants good development.

In the case of soil contaminated with HMs the decrease of ratio could be related with root biomass inhibition. For example, the root/shoot ratio or RMR could decrease because of the high Cd accumulation and toxicity in roots. Cd inhibits (stunts) the further development of root system. The RMR or root/shoot ratio in non – wooden plants is usually reduced in the response to high Cu exposure (Landjeva *et al.* 2003; Mahmood *et al*. 2007)

Specific root length (SRL)

The specific root length (SRL) (for fine roots, <2mm) can be used successfully as an indicator of nutrient availability to trees in experimental conditions or as indicator of changing soil properties resulting from environmental changes (Ostonen *et al.* 2007). Specific root length (SRL) defines the carbon allocation to root system. The lower values of SRL indicate the plant decreased ability to uptake water and nutrients (Hartikainen *et al.* 2001).

According Eissenstat (1991), roots with high SRL have lower tissue density than those of low SRL. The morphological responses to nutrient rich environment, such as increases in SRL, related with increased surface area of absorptive tissue (Ryser 1996).

Stem diameter, stem length (height)

The changes in stem diameter or stem length can indicate bad environmental conditions as water shortage, lack of nutrients, or disability to get useful materials in response to soil contamination. For example, study of sewage sludge fertilization (HMs did not exceed limits) showed that stem diameter and stem length with fertilization of soil increased comparing with control sites (Nóbrega *et al.* 2006).

Root, shoot length and branching

The effect of reduced root elongation and branching are used to assess HMs toxicity (Ryser, Emerson 2007). It is known that shoot elongation is found to be less sensitive to HMs than root elongation (Landjeva *et al.* 2003; Cheung *et al.* 2004, Singh and Agrawal 2007). Also in the study with crops was determined that root growth is more sensitive to the toxicity endpoint than shoot growth in the soils amended with Cu or Pb (An 2006).

There are done many investigations on root and shoot growth inhibition in exposure of HMs. For example, Pb produced highly significant effects on shoot, root lengths and seedling dry biomass of *Lythrum salicaria* (Juseph *et al.* 2002). It is also defined that Cd can affect root metabolism, which shows sensitivity to Cd^{2+} toxicity by a reduction in lateral root size (Wójcik and Tukendorf 1999).

Review of chapter 1

The analysis of literature related with heavy metals (HMs) and their influence to the plants development suggested excepting several main metals. There were chosen three main HMs (Pb, Cu and Cd), which have the mainly influence to tree and other plants development and as the main issue that they act with each other synergistically (Breckle, Kahle 1991; Arduinni *et al.* 1994; Kabata – Pendias, Pendias 2001). However, the bigger part of the investigations was made in the artificial environment (greenhouses, laboratories) or in the conditions where the plants were treated with already known metal concentrations (Arduini *et al.* 1994; Adalsteinsson *et al.* 1997; Balestrasse *et al.* 2003; Šottníková *et al*. 2003; Bojarczuk 2004; Máthé-Gáspár *et al.* 2005; An 2006; Lageard *et al.* 2008 *ect.*). There are not enough of investigations which could show the development of the trees and plants in the natural conditions in highly polluted environment. Moreover, the majority of these studies are done on cultivated species that are usually herbs or shrubs while only a few researches look at tree species.

The sewage sludge use in plant growth is also reviewed. It is discussed about importance of continuing further investigations of sewage sludge disposal as it is not much known about the release of HMs from it. In other cases it is suggested to dispose sewage sludge not in agricultural areas but in forestry that is considered to reduce unwanted impact on human health. The different studies were done to evaluate the impact of sewage sludge on environment, especially soil and vegetation (Bayes *et al*. 1991; Gasiūnas 1997; Gradeckas *et al*. 1998; Dahlia *et al.* 1997; Katinas *et al.* 2002; Bozkurt, Yarilgaç 2003; Arcak *et al.* 2006; Montse and Joan 2006; Taylor and Moffat 2006; Selivanovskaja, Latypova 2006; Torri and Lavado 2007). The biggest attention was paid to HMs distribution to ground water, accumulation in plants. Moreover, the good sides of sewage sludge use were highlighted as it could enrich soil with organic matter, nutrients and could replace the artificial fertilizers.

The soil properties are also important to the migration of HMs in soil such as: soil texture, organic carbon content, pH, exchangable acidity, soil moisture (Påhlsson 1991; Hooda and Alloway, 1993; Göransson, Eldhuset 1995; Manceau *et al.* 1996; Miller, Hills 2000; Kabata – Pendias; Pendias, 2001; Mazvila 2001; Bojarczuk *et al.* 2002; Hutchings 2002; Kupčinskienė 2006; Parat *et al* 2007; Antoniadis *et al.* 2008). These properties are also very important to HMs transportation through roots to plants above ground tissues.

2. Methodology of soil and trees measurements

2.1 Site description

Soil samples were taken in experimental site of Gitėnai forest near Panevėžys town (Figure 2.1), where 300 t/ha of the industrial sewage sludge was spread on 2 ha site of woodcutting area in 1998. In 1999 the tree seedlings of birch (*Betula pendula*) and pine (*Pinus sylvestris*) were planted. The surface layer of the soil (0.2 – 0.5 cm) is a peat and forest litter layer, deeper layer, 0.3 – 0.5 cm, consist of gravely sand with pebble, and layer laying in 0.8 – 1 m depth consist of fine sand with interlayer of aleurite in lower part (Katinas *et al* 2002) (Fig 2.2).

The average annual precipitation of this region reaches 500 – 600 mm, dominant winds – south-west. In surface water and ground water are met rather high Fe and Mn concentrations (0.42 – 0.67 and 0.02 – 0.06 mg/l, respectively), and this is specific to whole Panevėžys region

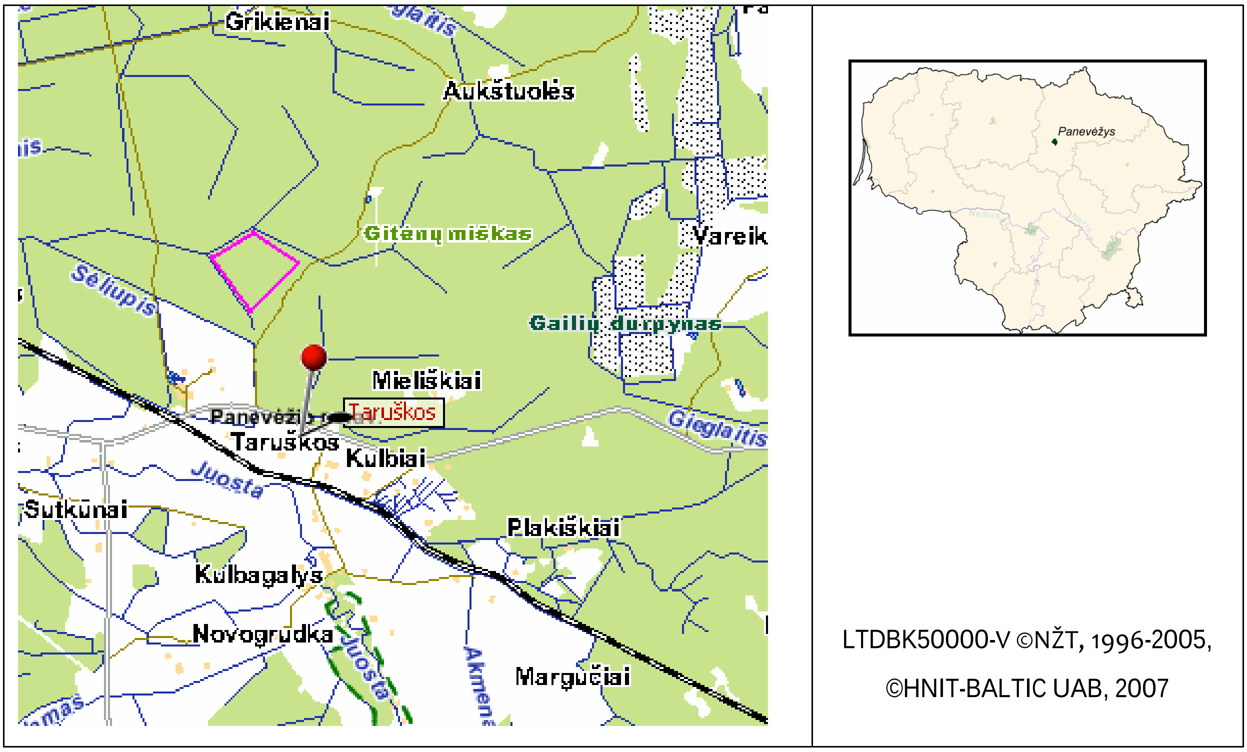


Fig 2.1 Experimental site in the Gilėnai forest of the Taruškos forestry located in Panevėžys region M 1:75000 (55° 44’ N; 024° 33 E)

The obvious geochemical barriers were two – the surface one related to the peat layer and the lower one to Fe – Mn hydroxides

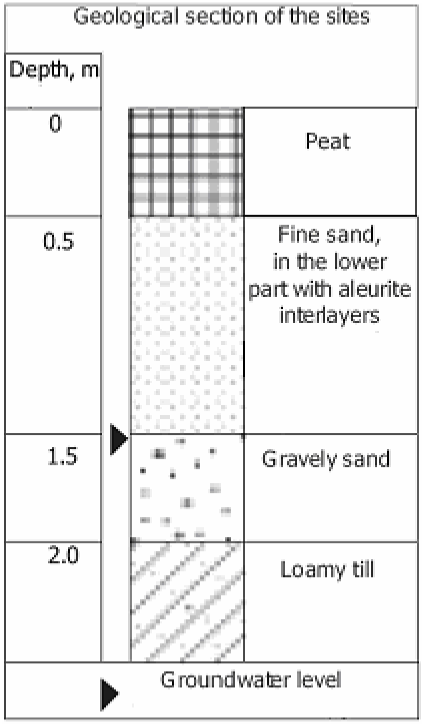


Fig 2.2 Soil geological composition of Taruškos experimental field (Katinas *et al*. 2002)

Table 2.1 shows the concentrations of HMs in industrial sludge of Panevėžys town which was spread in 1999 in Taruškos forestry in Gitėnai forest experimental site and background concentrations of HMs in that site soil.

Table 2.1 Concentrations of HMs in Panevežys industrial sewage sludge and background concentrations in experimental site (Katinas *et al*. 2002)

Heavy metals	Concentration in the industrial sewage sludge, mg·kg^{-1}	Background concentration of experimental site, mg·kg^{-1}
Cu	291	3.1
Pb	1456	11 – 15
Cd	6.2	2.2 – 5

n.d. - not determined

Sewage sludge according to Lithuanian regulation LAND 20 – 2005 is of II sludge category and can be used in forestry or in agriculture only every three year. High concentrations of Cu and Pb in sewage sludge were due to high performance of industry of Panevėžys Town, typically electroplating, refrigerator manufacturing.

Soil sampling

The soil samples were taken in 2007 May. 50x50m area sites were chosen of sewage sludge applied site (S1) and the wood – cutting site near experimental area, which was chosen as control site (C2). In this area 3 plots of 5x10m diameters were randomly chosen. Five samples of soil from each plot were taken and mixed according to depths (0-10, 10-20, 20-30, 30-40 cm) for composite samples (Fig 2.3). In both control and sewage sludge amended site 24 composite soil samples were taken.

Fig 2.3 Soil sampling site

Samples were transported in 4^{o}C to the laboratory and air-dried. For further analysis, soil samples were oven – dried in the 105^{0}C temperature and sieved through 2 mm sieve (*Retsch*, *As 200*).

Tree seedlings sampling

Betula pendula and *Pinus sylvestris* seedlings of 10 year-old were sampled from both sewage sludge amended and control sites (one birch and pine seedling per plot).

For further analysis tree seedlings were air-dried and divided into separate compartments: leaves or needles, shoots, stem and roots (coarse >2mm and fine <2mm). Each compartment was weighted (±0.05 g) and measured with meter (±1 mm). Due to not exactly equality of the trees height, for further analysis of trees biomass (to compare biomass of trees from different investigation sites) was chosen relative measurement, $g \cdot m^{-1}$. The other masses of tree compartments were also recalculated per 1 meter length of shoots, stem and roots, $g \cdot m^{-1}$.

2.1 Determination of soil properties

Important soil properties which affect the partitioning of metals include pH, carbon content, hydrous oxides, redox status and soil moisture status. In this study it is chosen parameter like soil pH, exchangeable acidity, soil moisture, total carbon content (ICP forest manual 2006).

Pretreatment of soil samples

Our soil samples were air dried before chemical analysis. The clods of the soil greater than 2 mm were crushed (not ground) taking care that crushing of original particles is minimized. The mineral soil samples were sieved above a 2 mm sieve (*Retsch*, *As 200*). Further grinding was done only for the analysis of total carbon.

Determination of moisture content

15 g of each sample of soil was weighed (±0.01g), and then these samples were placed in oven to dry at 105°C. The soil samples were left in the oven for 7 h. After that, they were cooled (covered) and weighed again. To represent our results we used following equation:

$$w = \left(\frac{m_2 - m_3}{m_3 - m_1}\right) 100\% \tag{2.1}$$

where: w – moisture content (%); m_1 is the mass of container (in g); m_2 is the mass of container and wet soil (in g); m_3 is the mass of container and dry soil (in g).

Soil pH determination

Naturally dried soil samplings were sieved through 2 mm diameter sieve (*Retsch*, *As 200*). For one soil pH determination method 30 g (±0.2) of each soil sample was weighed. Each soil sample was prepared in duplicate.

For soil:$CaCl_2$ method 15 g of soil was diluted with 75 ml of 0.01 M $CaCl_2 \cdot H_2O$ solution. Calcium chloride solution was prepared by diluting 1.47g $CaCl_2$ salt in 1 litre of distilled water. $CaCl_2$ and soil suspension was shaken for 60 min, using the mechanical shaker *Gerhardt*, *Rotoshake RS 12*. Mixed suspensions were left for 1 h to settle. Soil pH was measured with pH meter – pH 538 WTV. The measurement was repeated for 3 times for each sample solution.

Total carbon determination

Air dried and sieved through 2 mm sieve soil was milled and homogenized. Then there was 100 mg soil which was from mineral layer. The total carbon content was measured in apparatus (TOC-V by SHIMADZU) (Fig. 2.4), in 900°C temperature.

Fig 2.4 Total organic carbon analyzer – TOC – V CSN by Shimadzu (speciation.net)

During the ignition process the apparatus measures CO_2 gasses and the total carbon content was recalculated using 2.2 equation:

$$w_{C,t} = 1000 \times \frac{m_2}{m_1} \times 0.2727 \qquad (2.2)$$

where: $w_{C,t}$ – total carbon content (mg/kg) on basis of air-dried soil; m_1 – mass (mg) of test portion; m_2 – mass (mg) of released CO_2; 0.2727 – conversion factor for CO_2 to C.

Exchangeable acidity (ExA and free H^+)

The exchangeable acidity was determined in 0,1 mol/l $BaCl_2$ solution. After two hour shaking the extract of soil was titrated with a 0.05 mol/l NaOH solution up to pH = 7.8. The pH of solution was measured with pH meter – pH 538 WTV. The measurement was repeated for three times and using duplicates of one soil sample.

Also the free H+ acidity is realised using a method in which sodium fluoride is added to the soil extract before the titration; the Al ions are bind and only H^+ acidity is detected.

The total exchangeable acidity on basis of air-dried soil is given by:

$$E_A = \frac{(V_a - V_b) \cdot c_{NaOH} \cdot 100 \cdot V}{V_S \cdot m} \qquad (2.3)$$

where: E_A – total exchangeable acidity (cmol/kg) of the soil on basis of air-dried soil; V_A – volume NaOH (ml) used for the test sample; V_B – volume NaOH (ml) used for the blank; $cNaOH$ – concentration of NaOH (mol/l); V_S – volume (ml) pipetted for analysis; m – mass (g) of the laboratory sample; V – final volume (ml) of the extract.

2.2 Heavy metal analysis

Total and mobile Cd, Cu and Pb in the soil

Bioavailable Cd, Cu and Pb were measured in extraction of neutral salt 0.01 M $CaCl_2$, at 1:10 ratio (5g of soil). The solution was mixed and shaken for 16h, at room temperature, 20^{o}C. Total concentration of metals was determined after mineralization process. Each soil sample, weighing 0.5g, within 10 ml of HNO_3 and 2 ml of HCl solution was digested for 31 min in *Milestone* ETHOS digester (Fig. 2.5).

Fig 2.5 *Milestone* ETHOS digester (speciation.net)

The solution was then poured into 50 ml flask and diluted with distilled water to reach the mark of 50 ml.

Fig 2.6 *210 VGP* atomic absorption spectrophotometer of the company *Buck Scientific* (speciation.net)

Total and mobile heavy metal concentrations in the solutions were analysed using the *210 VGP* atomic absorption spectrophotometer of the company *Buck Scientific* (Fig. 2.6), applying the flame method – flame atomic absorption spectroscopy (FAAS).

Determination of heavy metals in tree

The parts of the trees (roots, shoots, stem, needles / leaves) were digested in 400 ^{o}C temperature. 0.5 g of wooden ashes sample were digested with a mixture of HNO_3 (65 %) and HCl (37 %) at the microwave digester *Milestone ETHOS* (Soon, Abboud 1993). The solution was poured in flasks of 50 ml and diluted with deionised water to the mark of 50 ml. Each 0.5 g sample of the needles / leaves ashes was mixed with 65% of HNO3 and 37% of HCl. Placed into the *Milestone* ETHOS digester and heated for 20 min. The solution was then poured into a 50 ml flask and diluted with distilled water to reach the mark of 50 ml.

2.3 The determination of tree parameters (functional traits) and biomass

2.3.1 Sample preparation and measurement of tree parameters

Preparation of tree leaves and needles. Leaves and needles were separated from the tree seedlings and dried in the air. Before and after drying they were weighted, ±0.05 g. Prepared tree samples were left to dry and then separated in to different parts.

Preparation of roots. Before chemical analysis (determination of HMs in the roots), roots were dried in the air. Further they were cleaned with brush and the photos were taken to compare their structure and development with each

Root system development was assessed on scale of 0 – 5: 0 – no roots; 1 – very poor development (1 – 20 % roots in medium); 2 – poor development (21 – 40%), 3 – moderate development (41 – 60 %); 4 – strong development (61 – 80%); 5 – very strong development (81 – 100%) (Bojarczuk 2004).

Roots were divided in to main and fine roots. The length of main roots was estimated by using meter. Also the number of roots was estimated and used as the parameter of roots development (branching).

Measuring Circumference. Circumference is measured at 1.3 m above the ground. In our case, because of small trees we have chosen alternative way of estimating circumference. We measured it at 0.3 m height, ±0.02 cm.

Measuring the tree length. Tree length was measured from the root crown neck till the end of the tree stem by using a meter (±0.02 cm).

*Crown height w*as measured from the first bigger than 1 cm diameter branch of the tree till the end of the stem also by using meter (±0.02 cm).

Measuring of shoots length and number. Branches were divided in two categories: main and secondary. The main branches – are branched off of the stem and secondary which are branched off of the main branch.

Number of branches was chosen for further studies as comparison with roots system. Also all branches were measured with meter (±0.02 cm).

Diameter at breast height (DBH). Commonly diameter at breast height (1.3 metres), i.e. DBH, is measured (this is often referred to as DBHOB to indicate that the measurement is taken over-bark) (Fig 2.7).

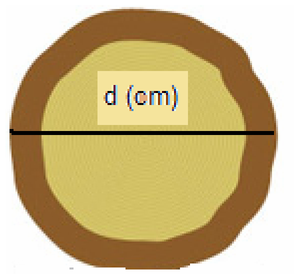


Fig 2.7 Tree stem diameter measurement

However, our tree seedlings are small so we measured at a height of 30 cm (d*30*), ±0.02 cm.

2.3.2 Stem and crown biomass estimation

Tree stem weight. Each stem of the tree seedlings were air dried and cut in to smaller pieces. All pieces were weighted and the total biomass of the stem was determined, ±0.05 g.

Crown weight estimation. All separated shoots (branches) from the stem were firstly divided in to primary and secondary shoots and weighted, ±0.05 g.

2.4 Statistical analysis

Statistical analysis was made by Statistica 7.0 program and by Microsoft Excel Office program. There was made comparison analysis of data by using t-test ($p<0.05$).

For each soil analysis there was analysed 12 samples (6 in one site amended with sewage sludge and 6 in control site). Each soil sample was measured in duplicates (in total 24 samples). And the analysis of one sample was replied three times.

There were investigated 6 tree seedlings of each two tree species, 3 in sewage sludge amended site and 3 in control site. All samples of different parts of tree seedlings were measured in duplicates and analysis was replied for three times.

The final results were averaged and the Standard Deviation was estimated.

Review of chapter 2

To evaluate sewage sludge impact on growth of trees we have assessed four aspects: a) compared control and sewage sludge amended soil properties, b) biomass of trees in control and sewage sludge amended sites, c tree morphological parameters and, d) Cu, Cd and Pb concentrations in both soil and tree compartments.

Soil properties methodology was used from ICP forest manual (2006) which is based on ISO standards for soil quality evaluation. The basic soil properties were measured as pH, exchangeable acidity, soil moisture, total carbon content.

The morphological parameters for evaluation of seedlings growth considered as most important (D'Aoust *et al.* 1994) such as stem height, diameter, and dry mass was considered. For deeper analysis the root, shoot measuring were also considered.

For determination of total HMs in soil were extracted with $HCl:HNO_3$ solution and measured with flame atomic absorption spectroscopy (FAAS). To measure the mobile forms of HMs in the soil was chosen method with $CaCl_2$ extraction.

The determination of HMs in the tree seedlings was based on Soon, Abboud (1993) extraction methodology. The measurement was also proceeding with flame atomic absorption spectrophotometer (FAAS).

3. Results and analysis

3.1 Soil analysis

Soil pH – comparison of two methods in different soil types

Soil pH is normally measured in a soil:water. The presence of soluble salts in a soil sample may affect pH, and for that reason, some analysts prefer to measure it in a mixture of soil and 0.01 M $CaCl_2$. The excess salt in this solution masks the effects of differential soluble salt concentrations in individual samples (Eckert, Sims 1995). When the soil is diluted with water, most of the H^+ ions tend to remain attracted to the soil particles and are not released into the soil solution. The addition of small amounts of calcium chloride provides Ca^{2+} ions to replace some of the H^+ ions on the soil particles, forcing the hydrogen ions into the solution and making their concentration in the bulk solution closer to that found in the field (Eutech Instruments Pte Ltd. 1997).

The proceed study of two pH methods revealed that the highest value of the soil pH was determined in tillage soil for both methods (pH(w) = 7.21; pH($CaCl_2$) = 5.99) and the lowest in site amended with sewage sludge (Panevėžys region; pH(w) = 5.60 and pH($CaCl_2$) = 4.80) (Fig 3.1).

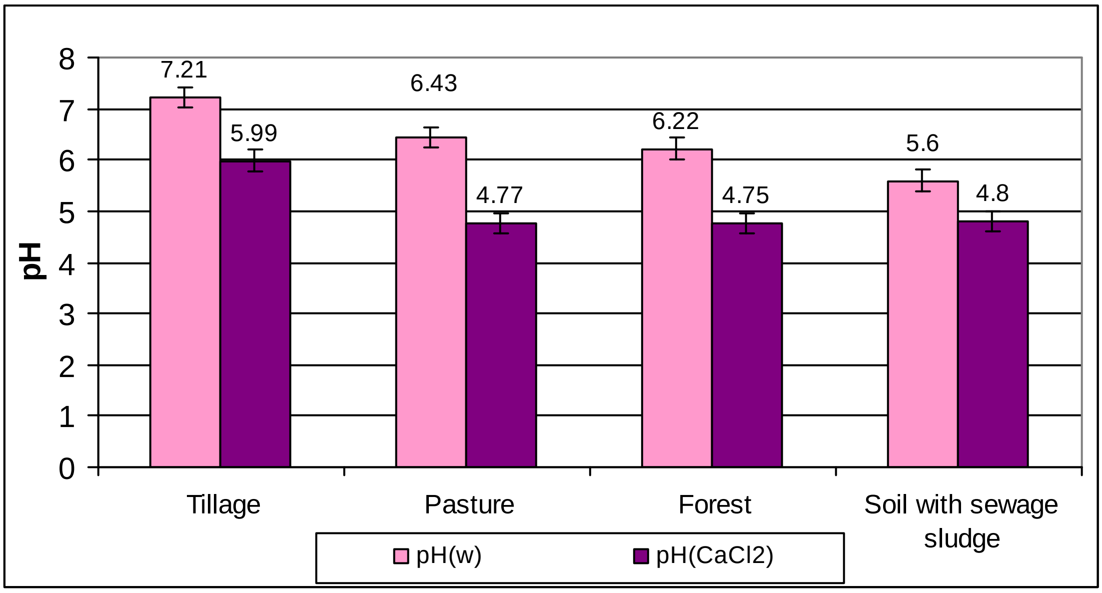


Fig 3.1 pH values in different land use soil – comparison of two methods

The statistical analysis of the data showed that soil pH(w) values were significantly higher than soil pH($CaCl_2$) values (Table 1). The biggest difference between methods was determined comparing pH values in pasture (1.66 ± 0.74) soil samplings, the smallest difference (0.8±0.15) was determined in soil with sewage sludge. The reason could be that soil amended with sewage sludge was peaty. Peat is known to be high in organic and low in inorganic components, so less in salt, that resists high pH variations (Parent and Tremblay 2003).

Table 3.1 Estimated difference between different pH determination methods.

Land use type	Difference between methods ± SD diff.	n – number of samples
Tillage	1.22 ± 0.17*	14
Pasture	1.66 ± 0.74*	48
Forest	1.47 ± 0.27*	36
Soil with s. sludge	0.8 ± 0.15*	18
Total	**1.41 ± 0.58***	**116**
* t-test is significant at $p<0.05$		

As a rough guide, the pH in $CaCl_2$ is usually 0.8 unit pH lower than in water, but can be as much as 2.0 pH units lower on grey sands. In Parent and Tremblay (2003) research in peaty soil difference was determined between 0.49 – 0.8. In agricultural land use difference between two pH values was determined 0.6 (Kissel and Vendrell 2006). It could be made some prediction, than difference between two methods depends on soil type, smaller pH difference is determined in soil with more organic content, and the bigger in sandy soils.

Soil pH determination method using water is simple and could be use *in - situ* while method with $CaCl_2$ is more precise and let us to avoid high variation of results during some period. The linear regression equations could be used for recalculation of pH values (Table 3).

Table 3.2 Linear regression between pH value of two methods and correlation coefficient

Land use type	Simple linear regression equation, n – number of samples	Correlation coefficient
Tillage	pH(w) = 2.8707 + 0.72520 * pH($CaCl_2$), n=14	r = 0,84*
Pasture	pH(w) = 8.1643 – 0.3640 * pH($CaCl_2$), n=48	r = -0,27
Forest	pH(w) = 2.4078 + 0.80397 * pH($CaCl_2$), n=36	r = 0,72*
Soil with s. sludge	pH(w) = 1.7240 + 0.80667 * pH($CaCl_2$), n=18	r = 0,99*
All	pH(w) = 3.4488 + 0.58655 * pH($CaCl_2$), n=116	r = 0,54*
* significant at $p<0.05$		

The estimated correlation coefficients were high and significant for most soil types, except pasture. It could be influenced of soil type variation (sandy and loam) in the different plots also in depths of pasture land.

Soil properties

Soil moisture content in site amended with sewage sludge significantly varied from 3.49 ± 0.44 % in surface soil layer (0 – 10 cm) to 6.79 ± 0.32 % in deeper soil layer (20 – 30 cm), $p<0.05$. In the control site moisture content varied in opposite way in surface layer – 1.87 ± 0.79 % and in deeper – 0.50 ± 0.14 %, $p<0.05$. The values were significant only in the deeper soil layer between sites, $p<0.05$.

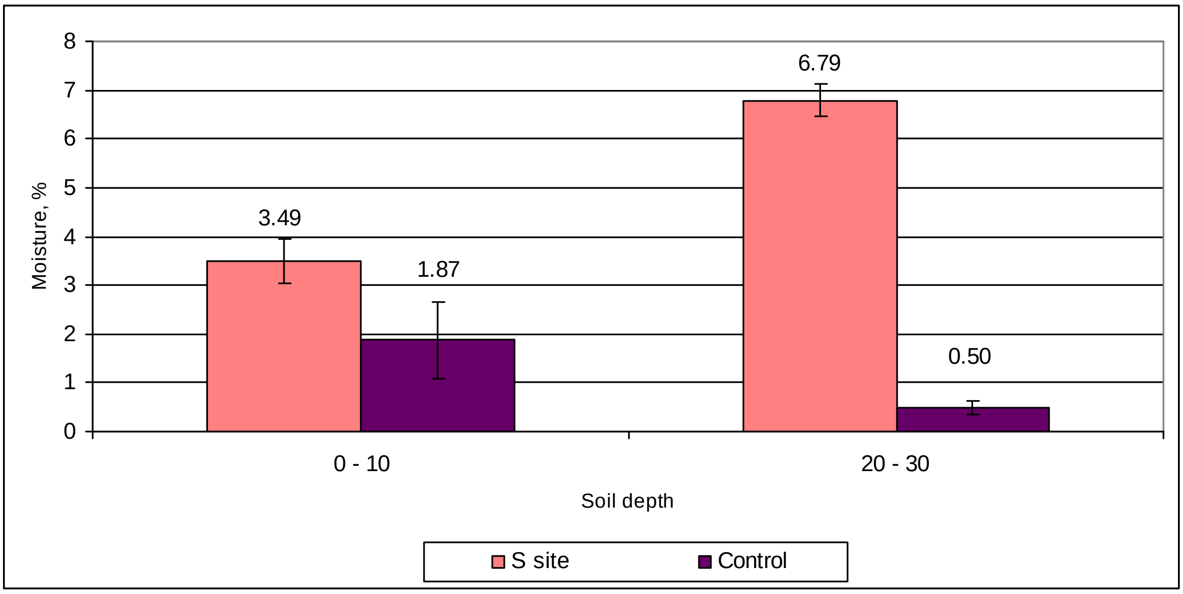


Fig 3.2 Soil moisture content in site amended with sewage sludge (S site) and in control site. Bars represent average values of 6 samples, ± SD

Total carbon variation was similar in both investigation site and was higher in surface soil layer (Fig 3.2). In the site amended with sewage sludge the total carbon content varied significantly from 5.03 ± 0.90 $mg \cdot kg^{-1}$ to 3.45 ± 0.56 $mg \cdot kg^{-1}$, $p<0.05$. In the control site this variation was significant and varied from 5.35 ± 0.97 $mg \cdot kg^{-1}$ to 1.33 ± 0.27 $mg \cdot kg^{-1}$, $p<0.05$. However, between to investigation site the differences significant was only in deeper layer, $p<0.05$.

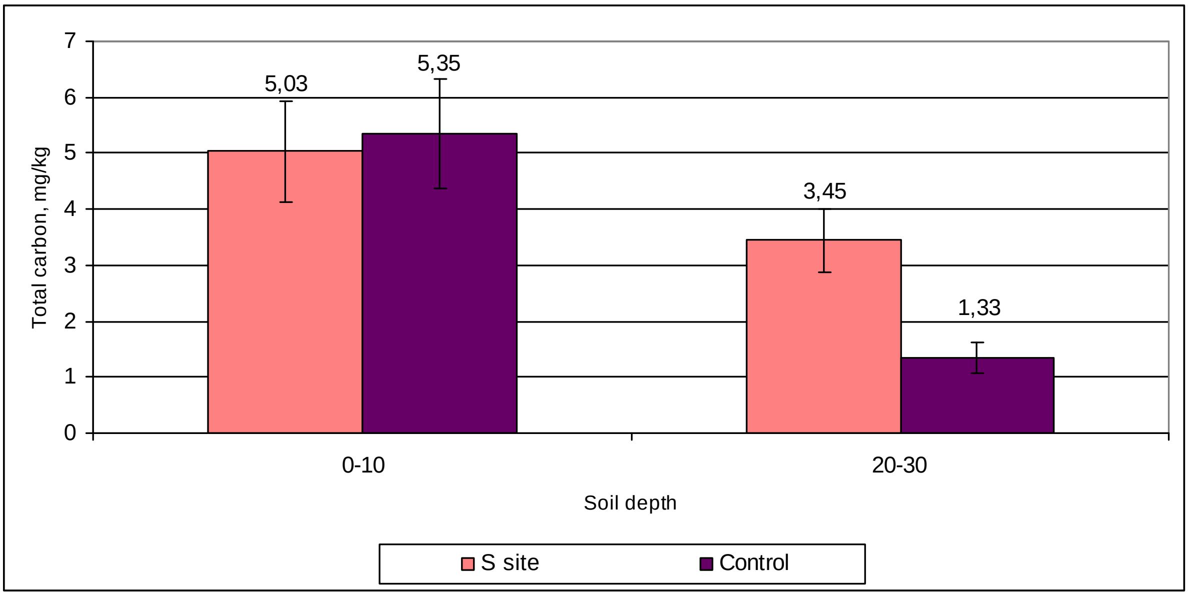


Fig 3.3 Soil Total carbon content in site amended with sewage sludge (S site) and in control site. Bars represent average values of 6 samples, ± SD

The exchangeable acidity in site amended with sewage sludge was significantly smaller that in control site. In the soil layer of 0 – 10 cm of site amended with sewage sludge it was 61.33 ± 22.62 cmol·kg^{-1} and in deeper layer 73.33 ± 39.59 cmol·kg^{-1}, difference was not significant, $p>0.05$. In the control site this variation was significant and varied from 1449.33 ± 84.85 cmol·kg^{-1} to 1199.33 ± 76.36 cmol·kg^{-1}, $p<0.05$. Between site this difference was significant in both layers, $p<0.01$.

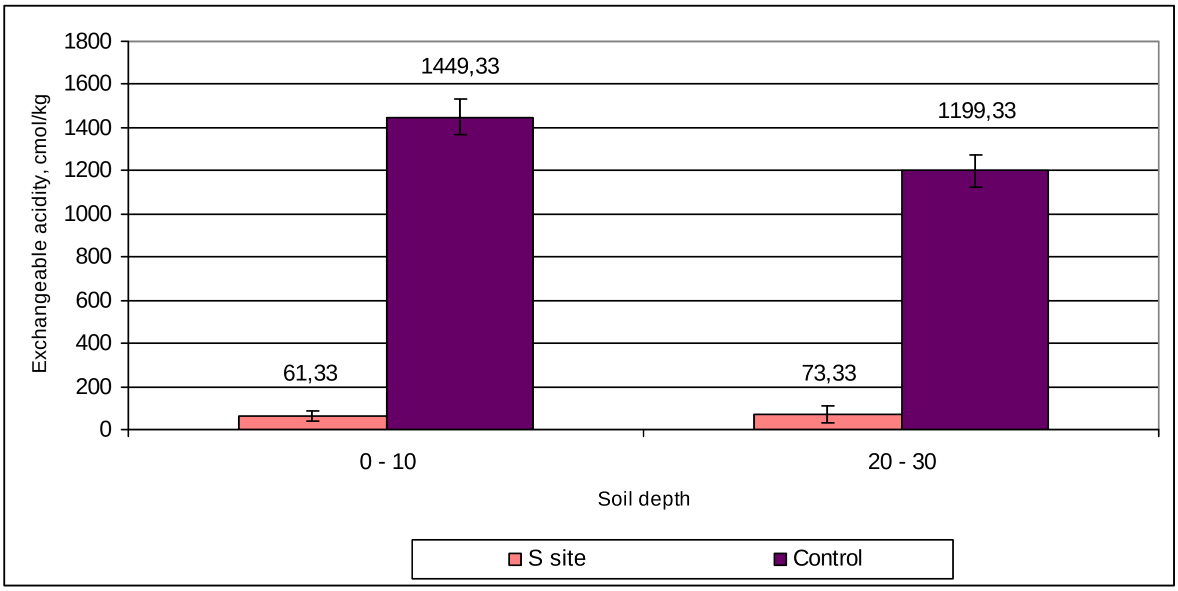


Fig 3.4 Exchangeable acidity in the site amended with sewage sludge (S site) and in control site. Bars represent average values of 6 samples, ± SD

The pH values in the soil depth did not vary, but significant difference was determined between two investigation sites (Fig 3.5), $p<0.05$.

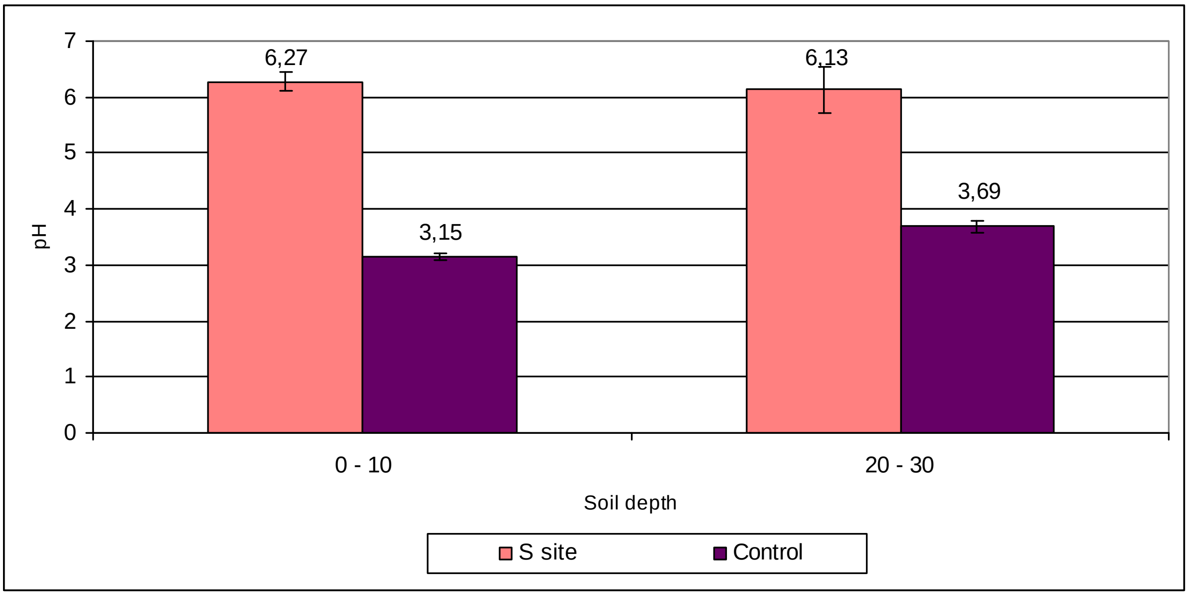


Fig 3.5 pH values in the site amended with sewage sludge (S site) and in control site. Bars represent average values of 6 samples, ± SD

In the site amended with the sewage sludge pH values varied from 6.27 ± 0.17 to 6.13 ± 0.41 in the deeper layer. In the control site the value was 3.15 ± 0.07 in the surface layer and 3.69 ± 0.11 in the deeper layer.

3.3 Trees measurements

Trees morphological parameters

The specific root length (SRL) of birch tree was bigger in site amended with sewage sludge and was equal to 0.33 $m \cdot g^{-1}$ and in control site – about 0.1 $m \cdot g^{-1}$ (Table 3.3). In the case of pine tree the SRL was also bigger in site amended with sewage sludge than in control site, were equal to 0.25 and 0.13 $cm \cdot g^{-1}$ respectively.

Table 3.3 Specific root length (SRL) ($m \cdot g^{-1}$) of birch (*Betula pendula*) and pine (*Pinus sylvestris*) trees in site amended with sewage sludge (S) and in control site (C).

Site	Birch	Pine
S	0.33	0.25
C	0.1	0.13

The average root and shoot length of birch tree was significantly longer in site amended with sewage sludge than in control (Fig 3.6). The average root length was 141 ± 8.48 cm in control site and 390.7 ± 99 cm in site amended with sewage sludge. The shoot length was 471.6 ± 38.6 cm and 862 ± 13 respectively.

The average length of pine tree root and shoots was not significantly smaller in site amended with sewage sludge. The root length varied 404.5 ± 89 cm in site amended with sewage sludge to 446.7 ± 106 in control site. The shoots length in the site amended with sewage sludge was 616.8 ± 57 cm and in control – 702.5 ± 76 cm.

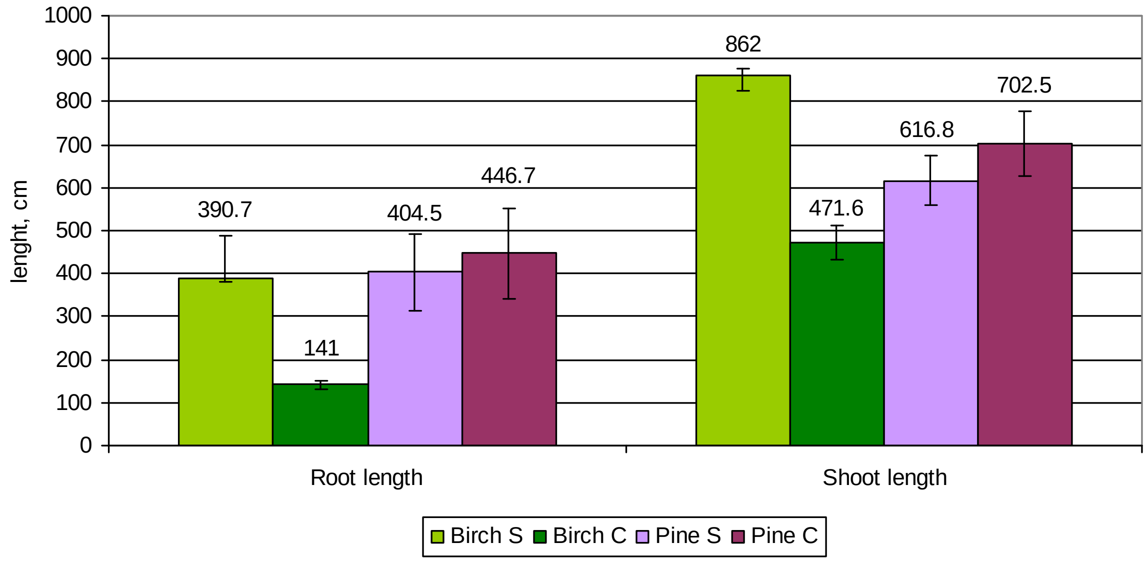


Fig 3.6 Root and shoot lengths of both birch and pine trees in site amended with sewage sludge (Birch S and Pine S) and in control site (Birch C, Pine C). Bars represent the mean values of 3 tree samples, ±SD

The next factor which indicates the environment nutrient content is the number of roots and shoots, or branching (Fig 3.7). The branching of birch roots was significant smaller in control site – 11 ± 1 than in site amended with sewage sludge 21.5 ± 3.5. The shoots branching varied the same as shoot – 19.5 ± 3.5 in control site and 24.5 ± 0.7 in site amended with sewage sludge.

The branching of shoots and roots was not significantly bigger in site amended with sewage sludge of pine tree. Number of roots was 22.5 ± 9.1 in control site and in site amended with sewage sludge – 23 ± 2.8; the number of shoots was: 26.5 ± 0.7 and 31 ± 5.6, respectively.

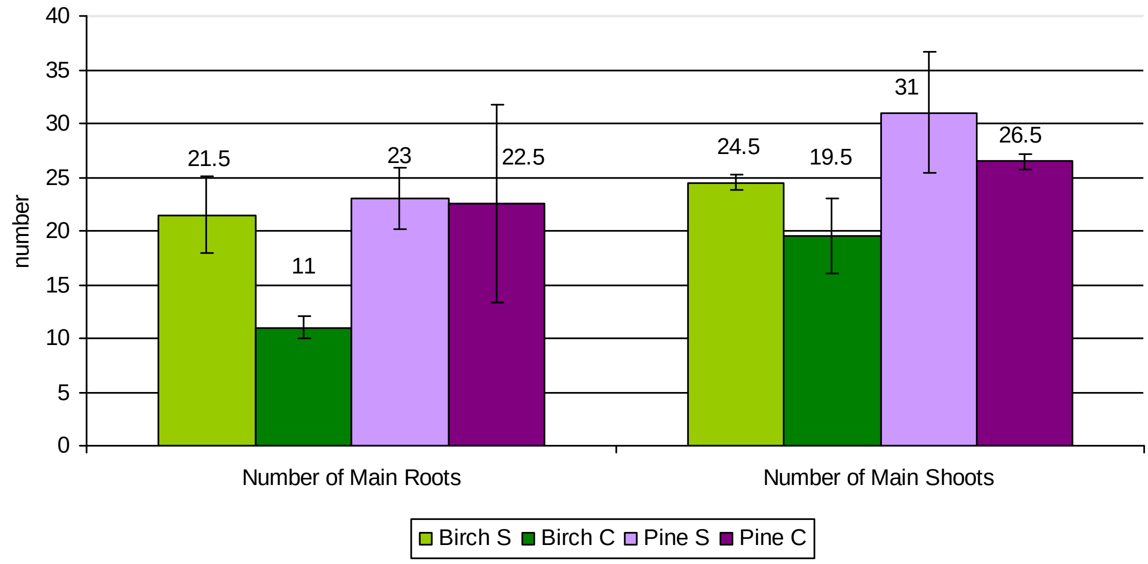


Fig 3.7 Number (branching) of shoots and roots of both tree species in site amended with sewage sludge (Birch S; Pine S) and in control (Birch C; Pine C). Bars represent the mean values of 3 tree samples, ±SD

The other morphological parameter is the length of the tree stem. Of both tree species this parameter was smaller, but not significantly, in the site amended with sewage sludge (Fig 3.8).

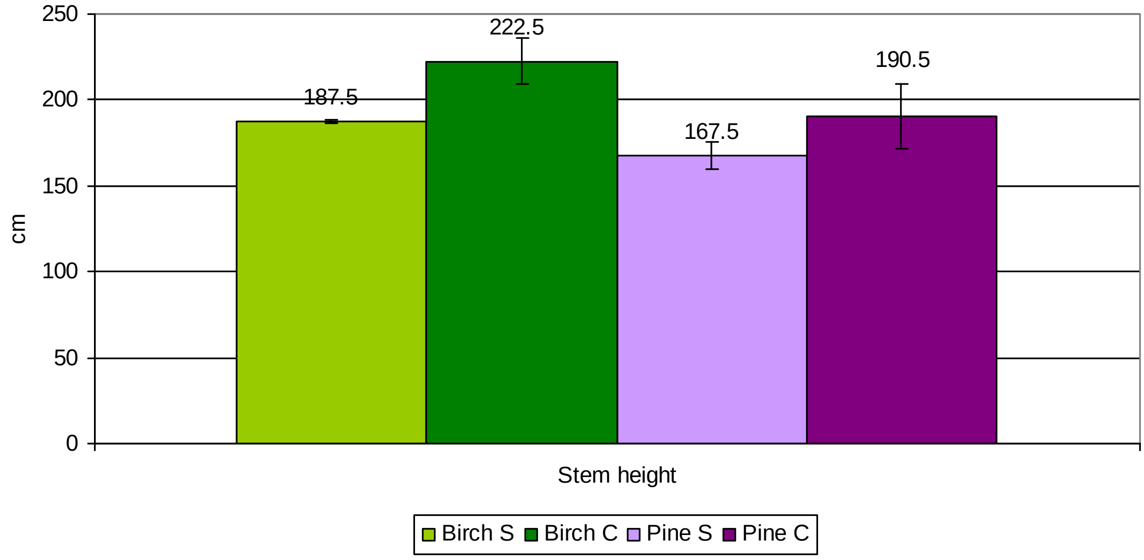


Fig 3. 8 The length of tree stem of both tree species in site amended with sewage sludge (Birch S; Pine S) and in control (Birch C; Pine C). Bars represent the mean values of 3 tree samples, ±SD

The stem length of birch tree was 187.5 ± 0.71 cm in site amended with sewage sludge and 222.5 ± 50.74 cm in the control site. The length of the pine stem was 167.5 ± 7.7 cm and 190.5 ± 19 cm.

The diameter of breast height (DBH, at 30 cm), of birch tree varied form 1.0 cm to 1.4 cm in the site amended with sewage sludge and from 1.0 to 1.8 cm in the control site (Table 3.4).

Table 3.4 The diameter of the breast height (DBH, at 30 cm) of birch (*Betula pendula*) and pine (*Pinus sylvestris*) trees in site amended with sewage sludge (S) and in control site (C).

Site	Birch (DBH, cm)	Pine (DBH, cm)
S	1.0 – 1.4	2.0 – 2.9
C	1.0 – 1.8	2.7 – 3.4

The DBH of pine tree differed more and varied from 2.0 to 2.9 cm in site amended with sewage sludge, from 2.7 to 3.4 cm in the control.

Tree dry mass

The total biomass of birch tree was smaller in the site amended with sewage sludge than in control site, 54 ± 2 and 56 ± 3, however, this difference was not significant ($p > 0.05$) (Fig 3.9).

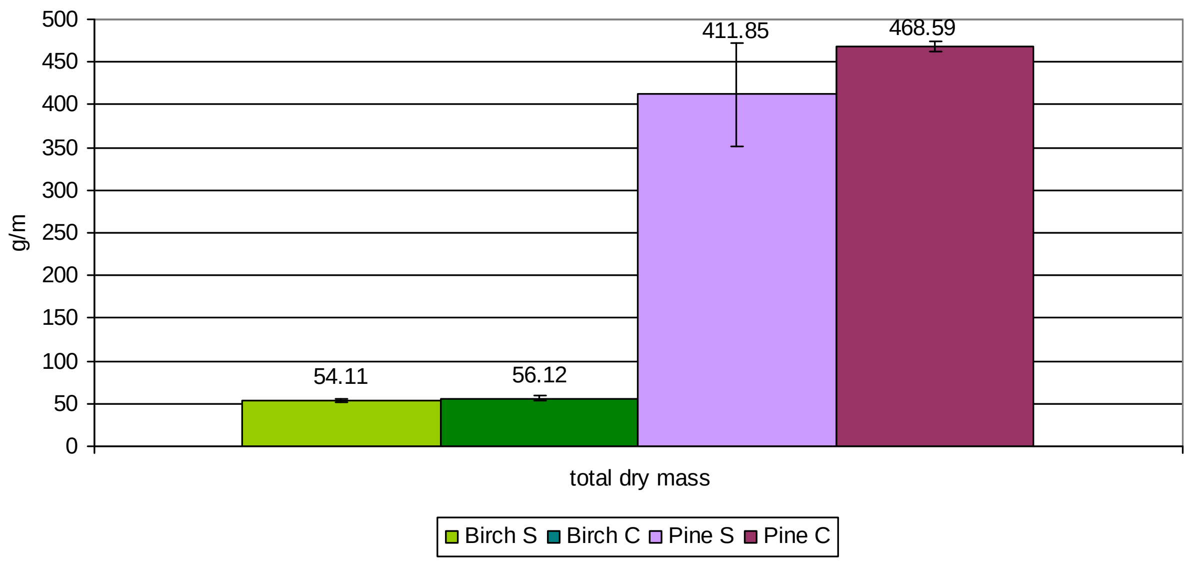


Fig 3.9 Relative total dry mass value of birch and pine tree in the site amended with sewage sludge (Birch S; Pine S) and in control (Birch C; Pine C), $g{\cdot}m^{-1}$. Bars represent mean values of 3 samples and SD.

Statistically not significant difference ($p < 0.05$) was also determined between pine trees in both investigated sites: 412±60 $g{\cdot}m^{-1}$ in site amended with sewage sludge and 468± 5 $g{\cdot}m^{-1}$ in control site.

The mass of different parts of birch tree differ in both investigated sites (Fig 3.10). The biomass of birch leaves in site amended with sewage sludge was 16.76 ± 1 g and in control site 14 ± 0.9 g, difference statistically not significant, $p > 0.05$.

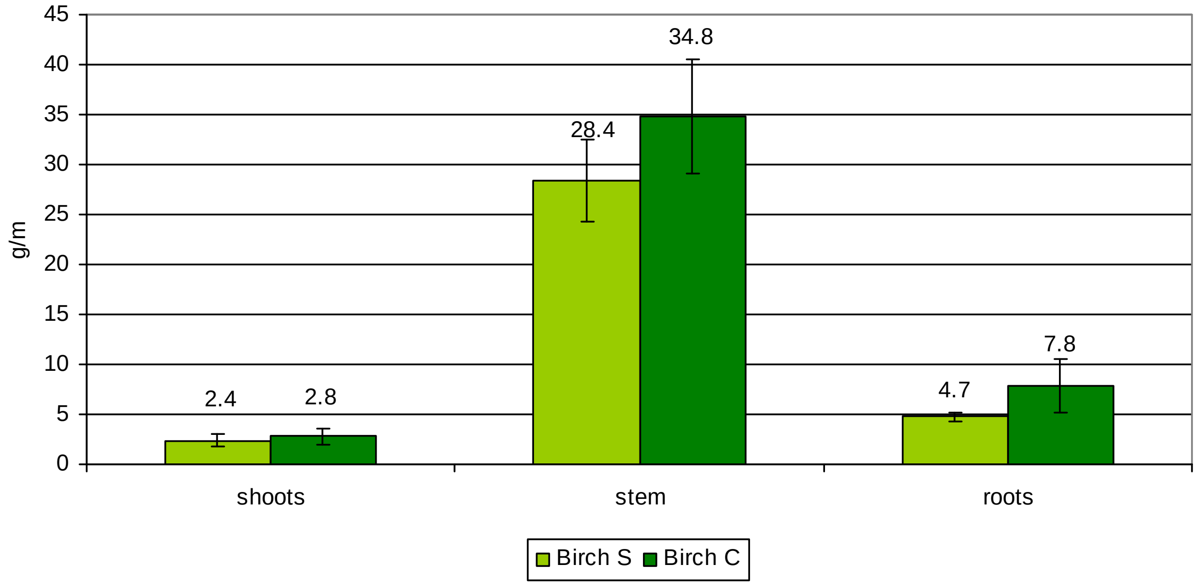


Fig 3.10 Biomass of birch parts in site amended with sewage sludge (Birch S) and in control site (Birch C). Bars represent the mean values of 3 tree samples, ±SD

The other masses of tree parts were recalculated per 1 meter length of shoots, stem and roots. The results revealed that dry mass of all tree part were not significantly smaller in site amended with sewage sludge. The mass of shoots was determined 2.4 ± 0.6 $g{\cdot}m^{-1}$ in site amended with sewage sludge and in the control site 2.8± 0.8 $g{\cdot}m^{-1}$. The mass of birch stem was 28.5 ± 4 $g{\cdot}m^{-1}$ in sewage sludge amended soil and 34.8 ± 5.7 $g{\cdot}m^{-1}$ in control. Root biomass was 4.7 ± 0.48 $g{\cdot}m^{-1}$ and 7.8 ± 2.7 $g{\cdot}m^{-1}$, respectively.

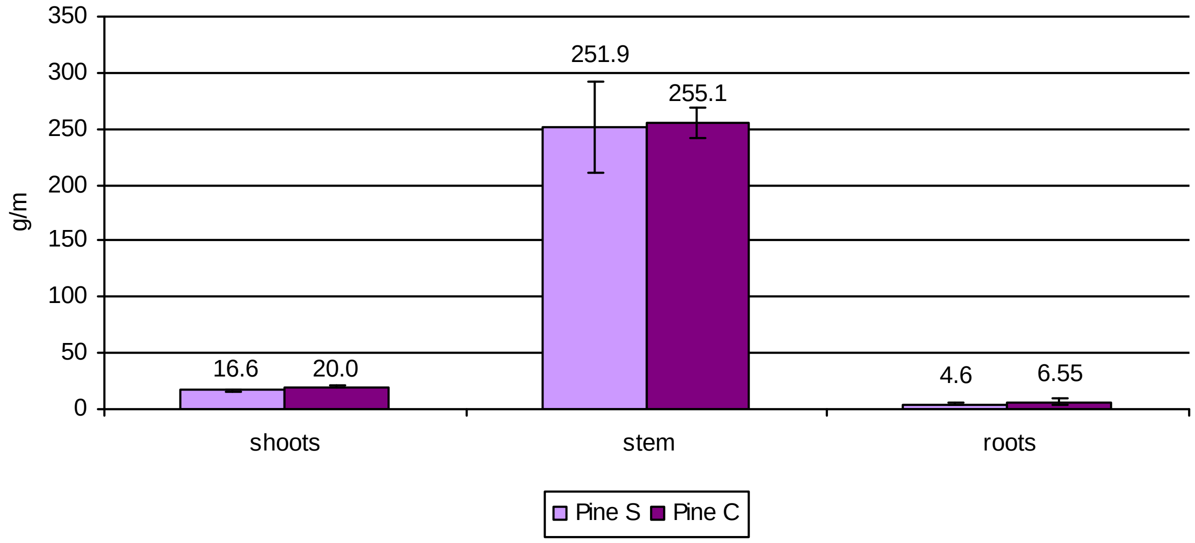


Fig 3.11 Biomass of pine parts in site amended with sewage sludge (Pine S) and in control site (Pine C). Bars represent the mean values of 3 tree samples, ±SD

The mass of different parts of pine tree was also not significantly ($p>0.05$) smaller of trees from site amended with sewage sludge than in control (Fig 3.11). The biomass of needles in control sites was 152.9 ± 33 g, and in site amended with sewage sludge – 175 ± 7.9 g.

The mass of pine shoots was in the site amended with sewage sludge – 16.6 ± 1.2 $g{\cdot}m^{-1}$ and in the control site 20 ± 1.19 $g{\cdot}m^{-1}$. The stem mass of pine tree in site amended with sewage sludge was 252 ± 41 $g{\cdot}m^{-1}$ and in control site – 255 ± 14 $g{\cdot}m^{-1}$ and root mass was 4.6 ± 0.45 $g{\cdot}m^{-1}$ and 6.5 ± 2.75 $g{\cdot}m^{-1}$, respectively.

Table 3.5 Root/shoot ratio of birch (*Betula pendula*) and pine (*Pinus sylvestris*) tree in site amended with sewage sludge (S) and in control site (C).

Site	Birch	Pine
S	0.8	0.13
C	1.78	0.21

The root/shoot ratios of both tree species were bigger in control site (Table 3.5). The birch root biomass was even bigger in control site than the mass of shoots. In the site amended with sewage sludge the ratio was also high, 0.8, comparing with ratios of pine tree, which were 0.13 in site amended with sewage sludge and 0.21 in control site.

In the case of birch tree the smallest difference in two investigation sites was determined between weights of roots, 1.14 (Table 3.6). However, this difference in the case of pine tree was the biggest, 2.59.

Table 3.6 Ratio of different tree parts between two investigation sites (tree part in control/tree part in the soil with sewage sludge).

Tree seedlings	Leaves/needles	Shoots	Stem	Root	Total
Betula pendula, n=3	1.29	1.48	1.52	1.14	1.44
Pinus sylvestris, n=3	1.56	1.59	1.28	2.59	1.42

The highest difference in the case of birch tree was determined between stem weights, 1.52. The opposite situation was in the pine tree case; this difference was the smallest, 1.28. The differences between shoots and leaves/needles weights were higher in the pine tree case.

By comparing total biomass of both trees in the two investigation sites was determined that the ratio was smaller in the case of pine tree, 1.42 and 1.44.

3.3 Heavy metals in soil and trees

The Cd total concentration was significantly smaller in control site than in site amended with sewage sludge (Fig 3.12). In recent site it varied not significant in soil depth – 1.33 ± 0.18 in soil surface soil layer to 1.15±0.24 mg·kg^{-1} in the deeper soil layer, $p>0.05$. In control site this variation was from 0.85±0.11 to 0.75±0.07 mg·kg^{-1}, not significant too, $p>0.05$. Between investigation sites difference was significant, $p<0.05$.

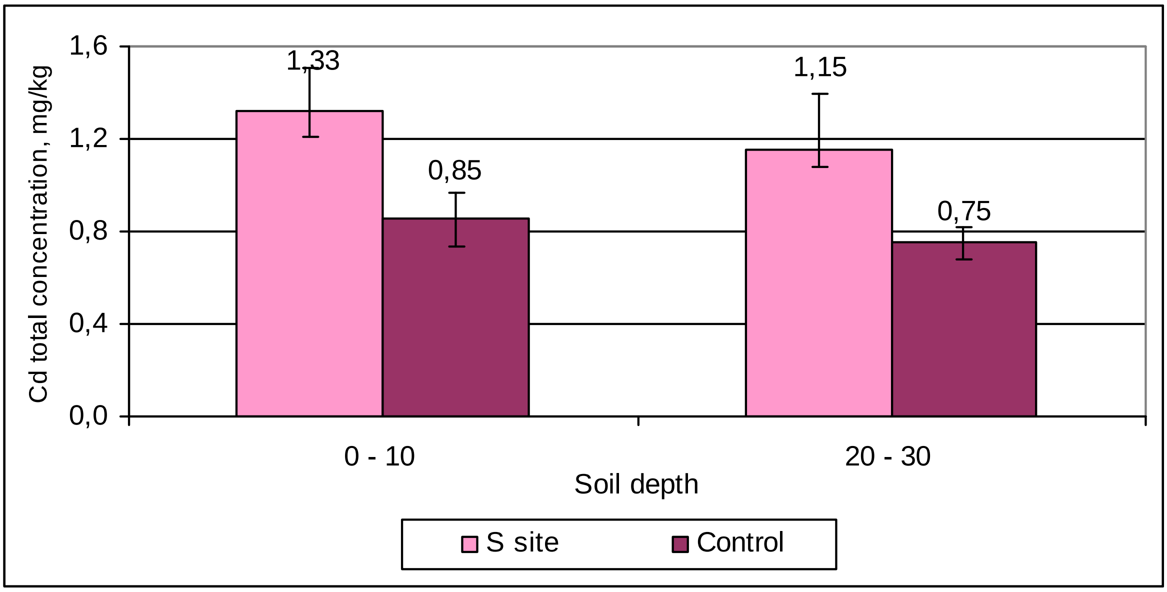


Fig 3.12 Cd total concentration in the soil amended with sewage sludge (S site) and in control soil. Bars represent average values of 12 samples ± SD

The Pb concentration also determined to be higher in the soil amended with sewage sludge than in control site (Fig 3.13). In the site amended with sewage sludge Pb concentration was 38.83±8.72 in surface layer and 42.92±2.42 mg·kg^{-1} in deeper layer, $p>0.05$. In the control site it varied in depth not significant, $p>0.05$, and was 24.78±0.63 and 23.00±1.18 mg·kg^{-1}. However, between site this differences was significant, $p<0.05$.

The total Cu concentration was also higher in site amended with sewage sludge than in the control site. In site amended with sewage sludge it varied not significant from 9.9±0.07 to 9.35±4.41 mg·kg^{-1}, $p>0.05$ (Fig 3.14).

Cu concentration in control site varied also not significantly from 4.00±1.04 to 4.53±1.92 mg·kg^{-1} in the deeper soil layer, $p>0.05$. Between site this difference was significant only in the upper soil layer, $p<0.05$.

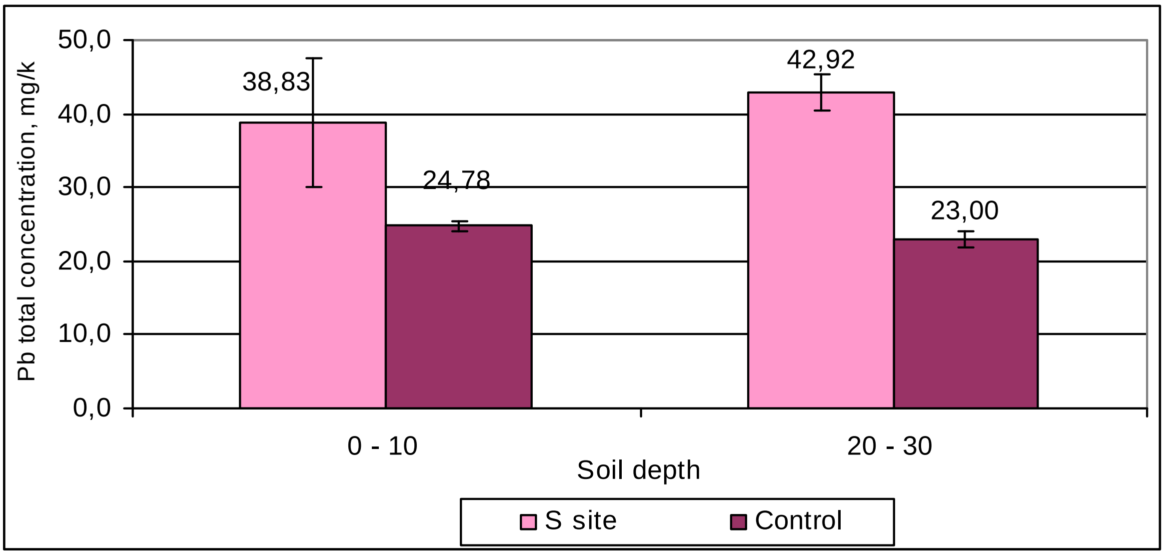


Fig 3.13 Pb total concentration in the soil amended with sewage sludge (S site) and in control soil. Bars represent average values of 12 samples ± SD

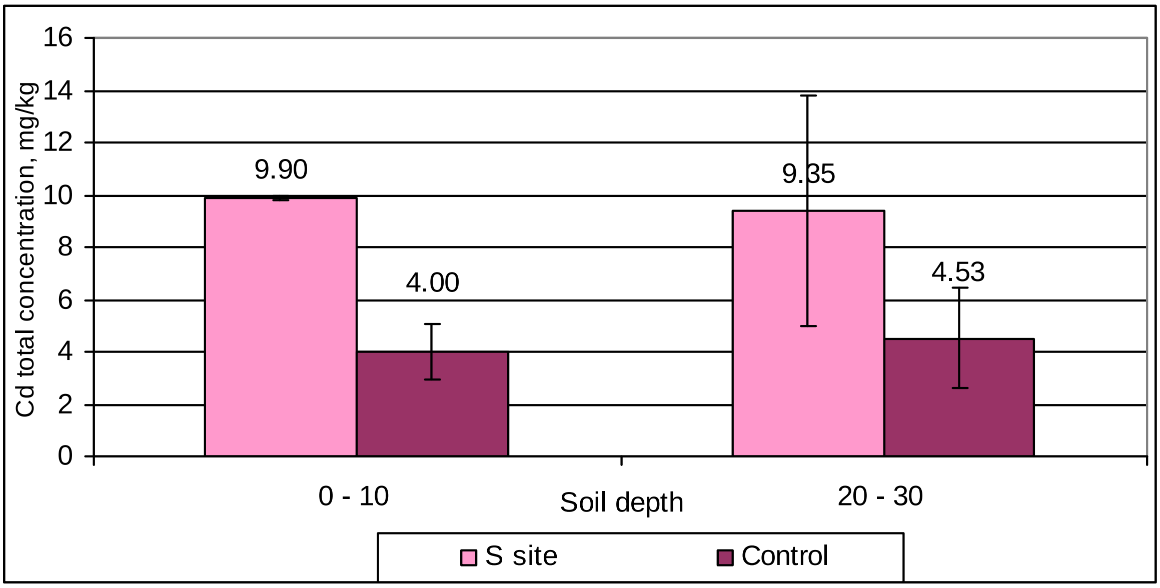


Fig 3.14 Cu total concentration in the soil amended with sewage sludge (S site) and in control soil. Bars represent average values of 12 samples ± SD.

The mobile forms of HMs varied differently then in the case of total forms. The Cd mobile concentration was approximately the same in both soil layers and was equal 0.16 ± 0.07 and 0.06 ± 0.04 mg·kg^{-1} (Fig 3.15).

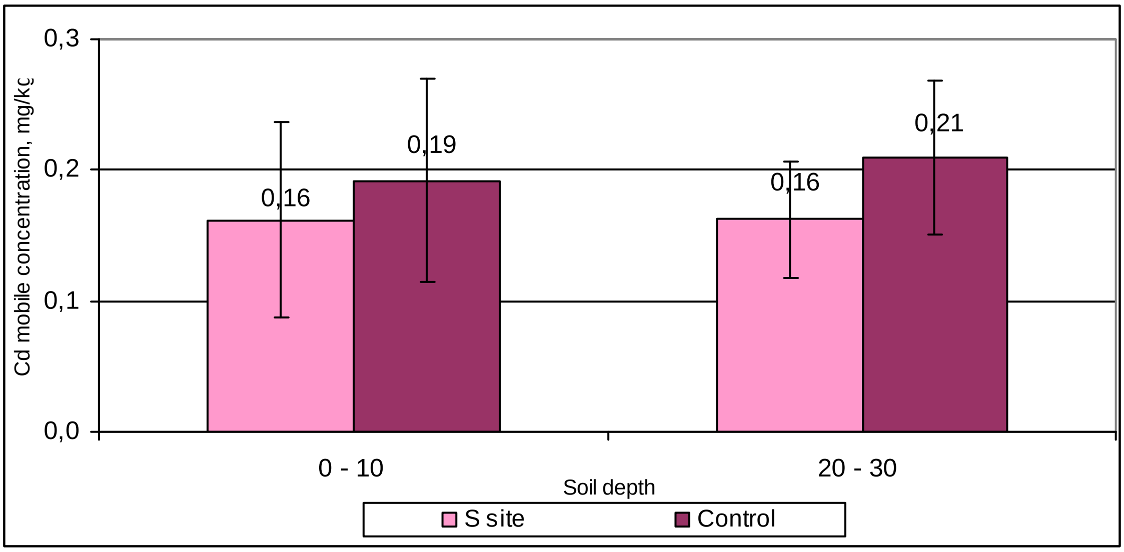


Fig 3.15 Cd mobile concentration in soil solution in the soil amended with sewage sludge (S site) and control site. Bars represent average values of 12 samples ± SD

The concentration in control site was higher that in site amended with sewage sludge and varied from 0.19 ± 0.08 to 0.21 ± 0.06 $mg{\cdot}kg^{-1}$ in the deeper layer. The difference was not significant between sites, $p>0.05$.

The Pb concentration also was smaller in site amended with sewage sludge (Fig 3.16). And varied from 0.28 ± 0.03 $mg{\cdot}kg^{-1}$ in surface soil layer to 0.24 ± 0.05 $mg{\cdot}kg^{-1}$ in the deeper layer, $p>0.05$.

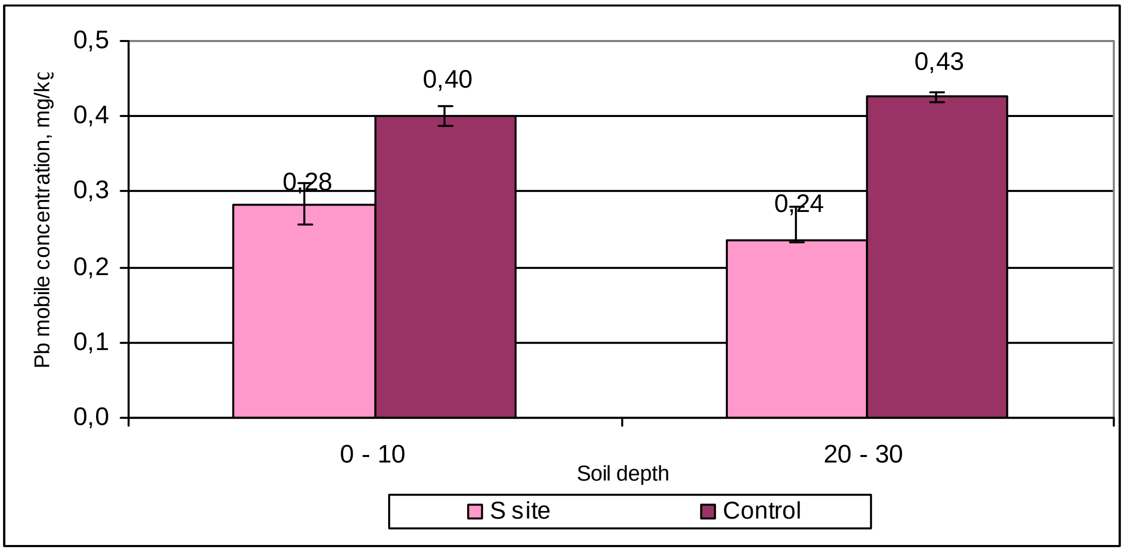


Fig 3.16 Pb mobile concentration in soil solution in site amended with sewage sludge (S site) and in control site. Bars represent average values of 12 samples ± SD

In control site these Pb mobile concentration values were equal to 0.40 ± 0.03 $mg{\cdot}kg^{-1}$ and 0.43 ± 0.00 $mg{\cdot}kg^{-1}$, $p>0.05$. However, between sites it varied significantly, $p<0.05$.

The Cu mobile form concentration opposite from previous HMs was higher in site amended with sewage sludge (Fig 3.17).

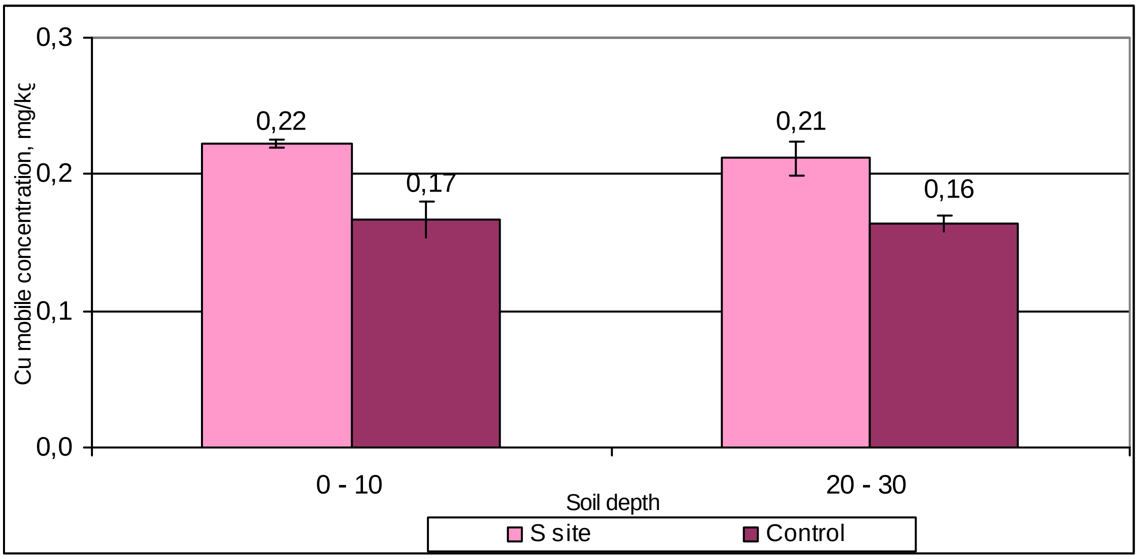


Fig 3.17 Cu mobile concentration in the soil solution in site amended with sewage sludge (S site) and in control site. Bars represent average values of 12 samples ± SD

In site amended with sewage sludge Cu mobile concentration was equal to 0.22 ± 0.00 mg·kg^{-1} and 0.21 ± 0.01 mg·kg^{-1}. In control site these concentrations varied from 0.17 ± 0.01 mg·kg^{-1} to 0.16 ± 0.01 mg·kg^{-1}. Variation between site was significant, $p<0.05$.

The percentage of Cd mobile form was highest of investigated HMs (Table 3.7). In the site amended with sewage sludge it was 12.21 % in surface layer and 14.06 % in deeper layer. In control site the percentage was 22.49 and 27.94, respectively.

Table 3.7 The percentage of mobile form of HMs in site amenended with sewage sludge (S) and in Control site.

Site	**Soil depth (cm)**	
S	**0 - 10**	**20 - 30**
Cd	12.21	14.06
Pb	0.73	0.95
Cu	2.25	2.26
C		
Cd	22.49	27.94
Pb	1.61	1.85
Cu	4.17	3.62

The Cu mobility second after Cd and varied from 2.25 % in surface soil layer to 2.26 % in deeper layer in the site amended with sewage sludge. In control site this variation was 4.17 and 3.62 %. The Pb percentage of mobile form in the site amended with sewage sludge was 0.73 in the surface and 0.95 % in the deeper layer. In control site this percentage was equal to 1.61 and 1.85 respectively. The bigger percentage of mobile form HMs in deeper soil layers could be related with less organic matter in this layer.

Cu, Cd and Pb in trees

Cu concentrations in roots and shoots of both tree species were higher in site amended with sewage sludge (Fig 3.18).

Cu concentration in birch tree from site amended with sewage sludge was highest in shoots – 5.25±0.24 mg·kg^{-1}, in leaves was 3.89±0.29 mg·kg^{-1}, in stem – 3.26±0.47 and in roots – 2.52±0.16 mg·kg^{-1}. In control site the concentrations of Cu in birch tree parts distributed in following sequence: 3.97±0.55 mg·kg^{-1} in the stem, in the leaves – 3.94±0.05 mg·kg^{-1}, 3.28±0.02 mg·kg^{-1} in shoots and in roots – 2.36±1.25 mg·kg^{-1}.

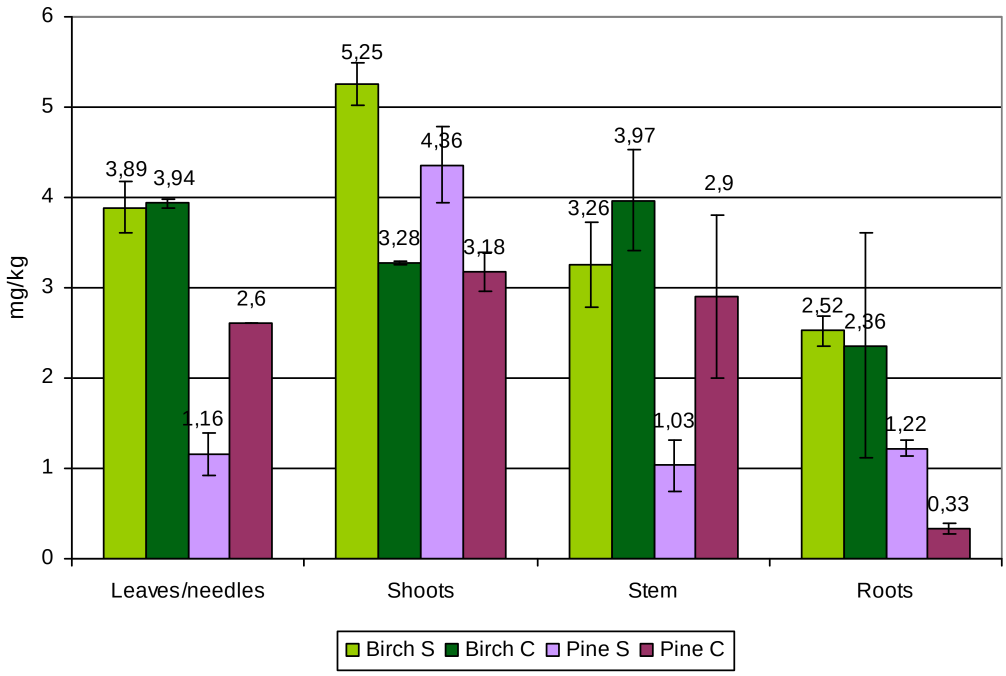


Fig 3.18 Cu concentrations in both tree species different parts. Bars represent average values of 3 samples ± SD

In pine tree Cu concentrations in site amended with sewage sludge were: 4.36±0.42 mg·kg^{-1} in shoots, 1.22±0.09 mg·kg^{-1} in roots, 1.16±0.24 mg·kg^{-1} in needles, and in the stem – 1.03±0.29 mg·kg^{-1}. In the control site the highest Cu concentration also was in shoots – 3.18±0.22 mg·kg^{-1}, lower was determined in stem – 2.9±0.9 mg·kg^{-1}, in needles – 2.6±0 mg·kg^{-1} and in the roots – 0.33 ±0.06 mg·kg^{-1}.

In all the birch tree parts Cd concentrations were lower in control site, except leaves (Fig 3.19). In the parts of birch from site amended with sewage sludge Cd concentration was: in shoots - 1.69±0.19 mg·kg^{-1}, in leaves was 1.46±0.036 mg·kg^{-1}, in roots – 1.31±0.06 and in the stem – 0.79±0.12 mg·kg^{-1}. In control site the concentrations of Cd in birch tree parts distributed in following sequence: 2.07±0.5 mg·kg^{-1} in the leaves, in the shoots – 1.64±0.05 mg·kg^{-1}, 0.78±0.01 mg·kg^{-1} in stem and in roots – 0.61±0.5 mg·kg^{-1}.

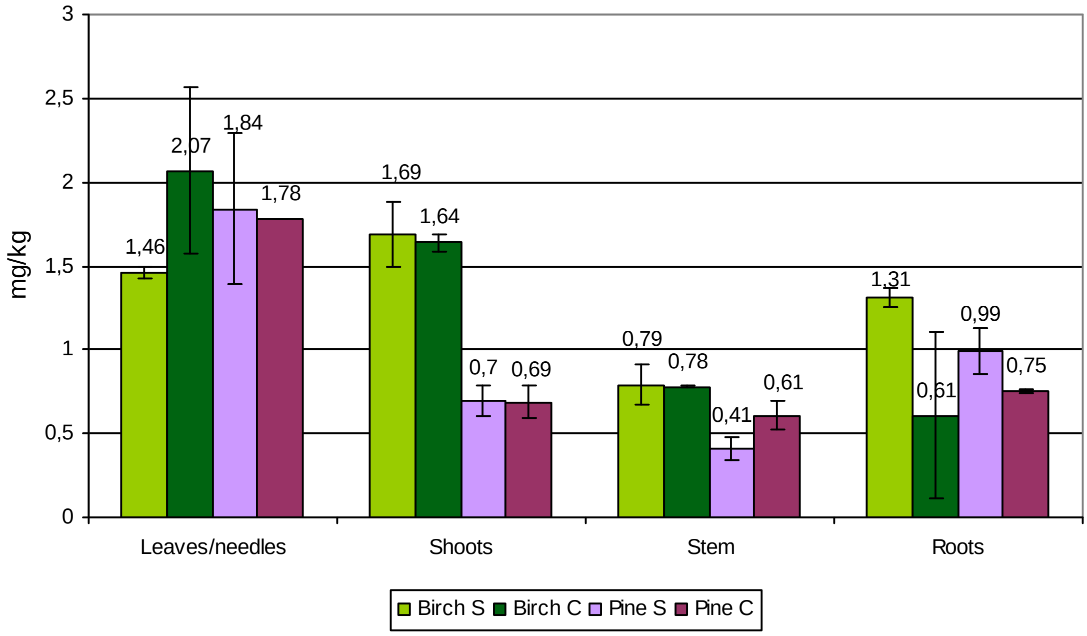


Fig 3.19 Cd concentrations in both tree species different parts. Bars represent average values of 3 samples ± SD

In pine tree Cd concentrations in site amended with sewage sludge were higher, except stem, than in the control site and were: 1.84±0.45 mg·kg^{-1} in needles, 0.99±0.14 mg·kg^{-1} in roots, 0.7±0.09 mg·kg^{-1} in shoots, and in the stem – 0.41±0.07 mg·kg^{-1}. In the control site the highest Cd concentration also was in needles – 1.78±0 mg·kg^{-1}, lower was determined in roots – 0.75±0.01 mg·kg^{-1}, in shoots – 0.69±0.1 mg·kg^{-1} and in the stem – 0.61±0.09 mg·kg^{-1}.

The Pb concentrations in birch tree parts were smaller in site amended with sewage and in the stem it did not differ a lot (Fig 3.20). The highest concentration was in shoots – 0.54±0.03 mg·kg^{-1}, in roots – 0.4±0.06 mg·kg^{-1}, in stem was 0.36±0.01 mg·kg^{-1} and in leaves – 0.25±0.07 mg·kg^{-1}. In control site the concentrations varied in the same sequence as in site amended with sewage sludge: 1.62±0.05 mg·kg^{-1} in shoots, in roots – 0.63±0.2 mg·kg^{-1}, in stem – 0.3±0.11 mg·kg^{-1} and in leaves – 0.27±0.04 mg·kg^{-1}.

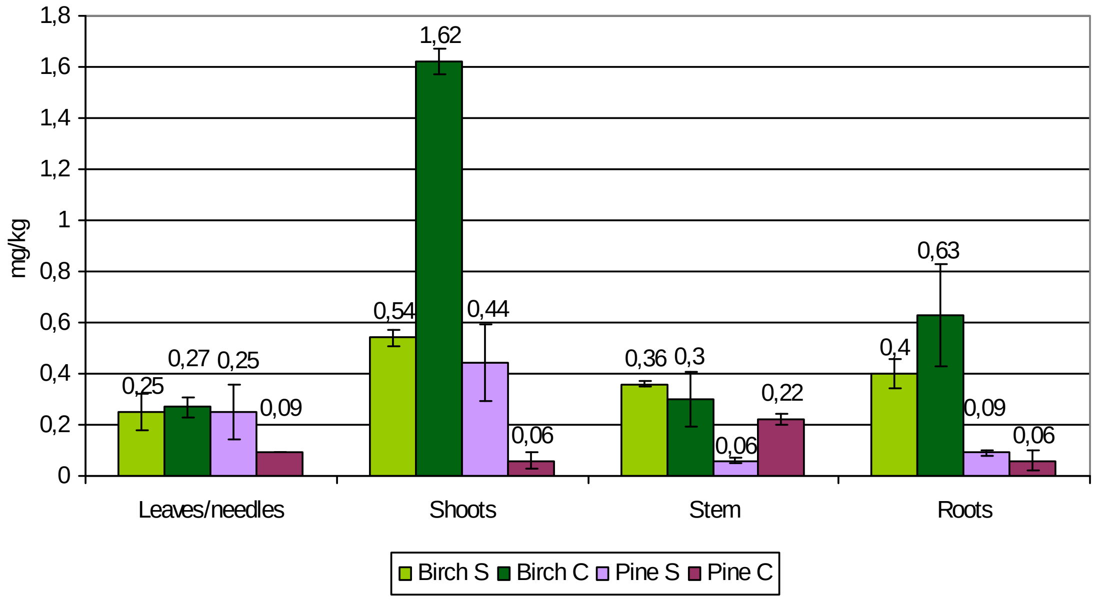


Fig 3.20 Pb concentrations in both tree species different parts. Bars represent average values of 3 samples ± SD

In pine tree the situation is opposite then in birch tree. Pb concentrations were higher in all parts except in stem in site amended with sewage sludge. In the site amended with sewage sludge the highest concentration was determined in the shoots – 0.44±0.15 mg·kg^{-1}, in the needles it was 0.25±0.11 mg·kg^{-1}, 0.09±0.008 mg·kg^{-1} in roots, and in the stem – 0.06±0.009 mg·kg^{-1}. In the control site the highest Pb concentration was in stem – 0.22±0.02 mg·kg^{-1}, in needles – 0.09±0 mg·kg^{-1}, in shoots – 0.06±0.03 mg·kg^{-1} and in the roots – 0.06±0.04 mg·kg^{-1}.

Review of chapter 4

Soil properties

Soil has an inherent potential to resist (stability) and recover from (resilience) environmental stresses (Griffiths *et al.* 2005). Soil is important to plants as source of nutrients and water and also as growth fundament. However, the plant growing conditions depends from many soil properties such as pH, soil texture, moisture, aeration ect. In this study we have focused on such soil properties as soil moisture, total carbon (organic carbon), exchangeable acidity and pH. The results showed the statistically significant difference between two investigation sites. This showed that in sewage sludge amended soil the growing conditions considered to be better than in control site.

For example, soil moisture content was bigger of the soil amended with sewage sludge. However, in upper layer this difference was not as significant as in deeper layer. These results reveal the better moisture capacity in site amended with sewage sludge. As it is known the soil moisture has influence on the transportation of the soil solution through the roots. Lower moister content can also indicate suppression of the diffusion and the mass flow in soil and to plant.

The average carbon content in the soil was also greater in sewage sludge amended site than in control. The upper layer carbon content of both sites was approximately equal as it is high in organic compounds in both sites. However, in deeper layer the carbon content was significantly bigger in site amended with sewage sludge. This shows the higher content in organic matter soils than in control site. The changes in soil carbon are known to influence soil physical properties (Denef *et al.* 2001) and due to higher content of soil carbon soil are more physically stable than the unamended soil (Sort and Alcaniz 2001).

The comparison of pH two methodologies shows that rich in more organics soil is more stable in acidic fluctuation during the year (Eckert, Sims 1995). As difference value between pH determination with water and $CaCl_2$ was smallest it shows that minerals in soil are stored better and this indicates also HMs stability in it.

As soil in control site is more acidic it could have the influence on the growth of the tree seedlings. Firstly, more acidic soil conditions can increase availability of Al^{+3}, which are known to disturb normal development of tree roots and minimize the uptake of important nutrients to the plant, like Ca^{2+} or Mg^{2+} (Kupčinskienė 2006). It is also well documented that low pH increases the mobility of toxic HMs which are easily uptaken by plants (Kabata – Pendias; Pendias 2001).

Finally, exchangeable acidity indicates soil disturbances due to high Al^{+3} concentrations which, as discussed previously, are toxic to plants and soil organisms (Sparks 1995). Toxic effect of aluminium (acidic, pH <5.5) cause the thickness and shortness of root fibres, leading to decrease assimilation of nutrients from the soil, and resulting slow development of plant (Göransson, Eldhuset 1995; Bojarczuk *et al.* 2002).

To summarize our results of the soil properties, the growth of plants in control site can be suppressed by acidic environment and higher concentrations of Al ions. However, in the site amended with sewage sludge the higher concentrations of HMs could have negative impact on plant growth. Despite that fact, soil in this site has higher pH value, and more organic matter, better moisture content.

Tree dry mass

According to more favourable soil conditions in the tree biomass was expected to be smaller in control site (Pikka 2005). It is particularly related with roots system, because the poor soil conditions such as low pH, high exchangeable acidity value are known to have negative influence on root system development (Kupčinskienė 2006). However, the biomass of roots was smaller in the site amended with sewage sludge. The same tendency was supported by a root/shoot ratio. According to this ratio, the growth of roots was better developed than shoots in control site. This could be related with the higher concentration of Al^{3+} in the soil that was found to cause the shoot growth reduction because nutrients and water transportation from roots is diminished (Påhlsson 1991).

The smaller root and shoot ratio in site amended with sewage sludge shows the increased transpiration of the plant, which leads to the loss of plant water content, reduced photosynthesis rate and plant growth decline (Kozlowski 1997). In another words, plant nutrients and water of the plant is lost faster than received. In addition, the lower values of root/shoot ratio could be the result of nitrogen application which in case of sewage sludge is highly feasible (Bojarczuk *et al* 2002). The sewage sludge is also enriched with phosphorus and this might have positive impact on root development (Gradeckas *et al.* 1998; Pikka 2005).

To conclude, the tree dry masses of different compartments of both tree species in site amended with sewage sludge were smaller or similar than in control site. According to root and shoot growth proportions it was determined that **in control** site plants tend to accumulate **more biomass in roots** than in the site amended with sewage sludge, because of **poor nutritious environment**.

Morphology

The height of the both tree seedlings and stem diameter were slightly bigger in control site. More precisely, birch tree differ more in stem length and pine in stem diameter. The reduced stem diameter may be cause of elevated Cd concentrations in the tree seedlings from site amended with sewage sludge because it is determined to be responsible for the decreased diameter of xylem vessels in plants (Barcelo and Poschenrider 1990; Hagemeyer *et al.* 1994).

However, the length and branching of roots and shoots of birch tree were bigger in site amended with sewage sludge than in control. In addition, roots in control site had tendency to elongate more than branch, for example, the maximum length of birch root varied from 20 to 80 cm and in the site amended with sludge from 13 – 54 cm. Moreover, it is known that infertile soils produce root systems with long, poorly branched surface roots, whereas fertile ones produce well-branched roots that may penetrate deeper into the soil (Crow 2005). In the case of pine tree the branching of roots and shoots was slightly bigger in site amended with sewage sludge but their average length was smaller comparing with control site.

These differences among species can be explained by root physiological differences, for example, the highest concentration of birch coarse roots accumulates in deeper layers (13 – 16 cm) than pine tree's (5 – 15 cm) (Laitakari 1934). And as it mentioned before in fertile soil roots penetrates easier into deeper layers. This suggests that pine roots have been exposed by sewage sludge for a longer time. Due to the fact that highly contaminated with HMs sewage sludge was spread on the surface of the soil. The leaching of HMs in to deeper layers was suppressed by organic matter from sewage sludge, the peat layer of the soil and Fe – Mn geochemical barrier (Katinas *et al.* 2002).

One of commonly used parameters, specific root length (SRL), is an important indicator to determine the carbon allocation in to the root system and indicates soil nutritious environment. In our case the roots in control site have bigger mass density as their SRL value is lower (Eissenstat 1991). The lower values of SRL indicate the decreased ability of plant to uptake nutrients (Hartikainen *et al.* 2001) and higher SRL values indicate soils richer in nutrients (Ryser 1998) and exhibits high hydraulic conductivity of roots (Eissenstat 1997). These factors also explain differences of root/shoot ratio between sites. In control site to adapt to less favourable conditions trees had to develop root system than above ground mass. And in the site amended with sewage sludge the roots which have bigger absorptive surface could more easily transport nutrients and water to surface parts and expanding above mass. However, the roots are thin and long, and accumulation of carbon is lower, this could lead to weaker roots system and increased risk of mechanical disturbance (strong wind, floods ect.).

To finalize the above results, site amended with sewage sludge had more favourable conditions for tree growth, however, plant biomass and morphology (except root branching) did not support this tendency. This means that sewage sludge might have additional constituents (e.g. heavy metals) that inhibited tree development.

Cu, Cd and Pb concentrations

It is known, that HMs in sewage sludge seem to be an inhibiting factor for tree biomass development, for example, according to Arduini *et al.* (1994), only 0.005 mg·kg^{-1} Cd reduced the spruce tree root elongation and the other research determined that 0.005 mg·kg^{-1} of Cu in solution can reduce biomass of pine trees (Arduini *et al.* 1998). In our study plant available Cd is in 0.16 mg·kg^{-1} concentration in soil amended with sewage sludge may have negative influence to pine root elongation and 0.22 mg·kg^{-1} of Cu to pine biomass. However, possible synergistic effect also should be considered (Arduini *et al.* 1994).

The Pb influence is hard to predict because it is very stable in the soil and usually in very acidic environment it is met in plant available form as in control site. In our study the plant available Pb form in site amended with sewage sludge did not reach 0.3 mg·kg^{-1}. In other studies was determined that tree growth reduction appears with 18 mg·kg^{-1} of plant available Pb in the soil with addition of Cd (Breckle, Kahle 1991).

The higher concentrations of all three HMs were determined in the birch tree seedlings than in pine tree. This reveals the statement that birch tends to extract more HMs from soil than pine. However, the concentrations did not differ a lot in trees from sewage sludge applied and control site.

It is determined that normal contents in plants of Cd and Cu are 0.1 – 1.0 mg·kg^{-1} and 1 – 10 mg·kg^{-1}. The Cu toxicity in plants may occur when the tissue level exceeds 20 – 30 Cu mg·kg^{-1}, the generally growth reduction in plants occurs at a tissue level of 3 Cd mg·kg^{-1} (Pais and Jones 1997). In our study the concentration in birch tree tissues of Cd exceeds 5 mg·kg^{-1} and Cu – 14.9 mg·kg^{-1} in the site amended with sewage sludge. However, these concentration in pine tree was about 3.9 and 7 mg·kg^{-1}.

In addition, no significant relationship between tree growth and HMs accumulation in tree tissues was noticed. However, some tendencies were highlighted. For example, higher Cd and Cu concentrations was determined in shoots and roots in trees from site amended with sewage sludge. It is especially seen differences of Cd in birch roots and Cu in its shoots. In the case of pine tree both concentration of these HMs were higher in shoots and roots, more significantly in roots. And as it discussed previously the root system of pine tree was more reduced than birch tree. It is known the Cu and Cd are inhibitors of tree biomass development especially root system (Arduini *et al.* 1994).

4. Soil – tree system modelling

4.1. PHYTO – DSS vs 7.0 model

The Phyto-DSS is a type model that considers the whole system related with plant use for phytoextraction. It calculates plant growth, water flux, component (e.g. contaminant or nutrients) flux, and costs – income of exploitation large vegetated areas. These simulations reveal the feasibility, risk, and potential outcomes of phytomanagement. The system requires daily climate data, as well as data on the substrate and the plants. The Phyto-DSS makes an economic assessment by comparing the costs of phytoremediation with the cost of inaction, and the best alternative technology (contaminated soil treatment).

The Phyto-DSS was created in 2000 at the, *Instituto de Recursos Naturales y Agrobiología de Sevilla* in Spain (during OECD fellowship). Subsequently, the Phyto-DSS was developed at HortResearch, Palmerston North, New Zealand (2001 – 2004), and the Swiss Federal Institute of Technology, Zurich, Switzerland (2005 – 2007).

The model is mostly based on plant water use and the soluble of HMs in the soil solution. Further is described the main principles and equations of the model.

The authors consider the amount of metal removed by the plant is therefore proportional to the transpiration rate over a given period of time (Robinson *et al.* 2003).

$$M \propto \int_0^t T dt, \qquad (4.1)$$

where: M – the metal concentration in the above-ground dry biomass (mg·kg^{-1}); T – transpiration rate; t – time period.

It is also considered M to be proportional to the metal concentration in soil solution, because total amount of metal that accumulates in the plant is related to the metal concentration in the soil solution.

$$M \propto [C], \qquad (4.2)$$

where: $[C]$ is the soluble metal concentration (mg·L^{-1}) in the soil solution.

The total amount of metal that accumulates in the plant does not usually equal the accumulated product of the soil solution concentration times the volume of water transpired by the plant. For a metal to be translocated to the aerial parts of a plant, it has to enter the root, either via the symplastic or apoplastic pathways where some active or passive filtering may occur.

Here, it is defined the root absorption factor (ϕ) as a dimensionless parameter that represents the root xylem/soil solution metal concentration ratio.

$$\phi = \frac{[C]_r}{[C]}, \tag{4.3}$$

where $[C]_r$ is the soluble metal concentration (mg L^{-1}) in the root xylem.

The parameter ϕ is a simple parameter which covers the hardly understood biogeochemical factors that influence the flow of metals from the soil into the roots. These factors are: rhizobiological activity, root exudates, temperature, moisture, pH and the concentration of competing ions. The issue is further complicated by the fact that ϕ could change depending on the metal concentration in the soil solution.

This change in ϕ over a concentration range can be modelled by adding a decay constant *K*.

$$\phi(C) = \frac{\phi_1 C_1}{C_1 + K(C - C_1)}, \tag{4.4}$$

where ϕ *(C)* – root absorption factor at soil solution concentration *C* (mg L^{-1}); ϕ_1 – the measured root absorption factor at concentration C_1 (mg L^{-1}); *K* ($0 \le K < 1$) is the decay constant, as RAF decreases because of the soluble component in the soil increases. *K* describes the rate of this decrease.

The plant specific ϕ can be approximated using the plant's total water use, above-ground dry biomass, and the metal concentration in soil solution. The assuming relationship holds:

$$\phi \cong \frac{MB}{TC}, \tag{4.5}$$

where *B* – above-ground dry biomass (kg); *T* – the total water use (L).

The model also assumes that potential metal uptake depends on root density. Plant metal-uptake causes a change in soil metal concentration (mg kg^{-1}) at depth *d*, and this change is calculated as:

$$\Delta[M]_z = \frac{1}{\rho_z}\int_0^{\tau} R_z TC\phi dt, \tag{4.6}$$

where: $[M]z$ – change in contaminant metal concentration (mg·kg^{-1}) at depth *z*, ρ_z – bulk density of the soil (g·cm^{-2}) at depth *z*; *t* – time (days); R_z – root density fraction (root mass at depth *z*)/(total root mass);

Economic evaluation

To determine the economic viability of phytoextraction it must be compared to the best alternative technology and the economic cost of inaction. Phytoextraction will be implemented only if it is the most financially attractive long-term option. The cost of phytoextraction *V* (US$) can be determined by:

$$V = A + \int_0^t (c_1 + c_2 - P_1 \times V_1 - P_2 \times V_2)dt + \sum_{x=1,2,3..t} \int_0^x (c_1 + c_2 - P_1 \times V_1 - P_2 \times V_2)dt \times \frac{1}{100}, \qquad (4.7)$$

where A – total area (ha); c_1 – cost of planting (Lt/ha); c_2 – cost of production (Lt/ha); P_1 – the production of saleable biomass (t/ha); V_1 – the value of the biomass (Lt/t); P_2 – the production of bio-ore (t/ha); V_2 – the value of the bio-ore (Lt/t) and I – interest rate (%).

The cost of phytoextraction can be compared with the cost of the best alternative technology over the same period of time. If the alternative technology results in the land being remediated in a shorter period of time, then the cost of this technology *(V_a)* will be offset by the now-enhanced earnings off the land.

$$V_a = A \times (c(1 + \frac{1}{100})^{t1} - L(1 + \frac{1}{100})^{(t2-t1)}), \qquad (4.8)$$

where A – total area (ha); $t1$ – time needed for conventional technology to remediate land (yr); $t2$ – time taken for phytoextraction to remediate land (yr); c – cost of alternative technology (Lt/ha); L – earnings from the land (Lt).

The cost of inaction *(Vi)* compared to phytoextraction is determined by:

$$V_i = (LG + RP + LE + FC)(1 + \frac{1}{100})^{-1}, \qquad (4.9)$$

where LG – cost of legislation (Lt); RP – the cost of loss of reputation/goodwill (Lt); LE – loss of earnings off the land (Lt/yr); FC – future costs (Lt),

Limitations of model

PHYTO DSS model has following limitations:

– Environmental conditions, such as drought, are not considered.
– Considered only HMs accumulation in the above plant biomass.
– For simplicity and utility model considers a uniform moisture distribution in the soil. In this case the remediation time can be considered shorter than actual. During longer period the concentration of HMs in plants can exceed few times real amounts.
– There is no possibility to simulate mixed plantations.

4.2. Soil – tree modelling methodology

Simulation period was chosen from 1999 first decade to 2008 first decade. The simulated area was 1 ha.

Climate issues were settled with Climate simulator (Fig 4.1). The specific to Panevėžys region monthly rainfall amount was entered. The average rainfall was estimated – 672 mm and annual evapotranspiration was 367 mm.

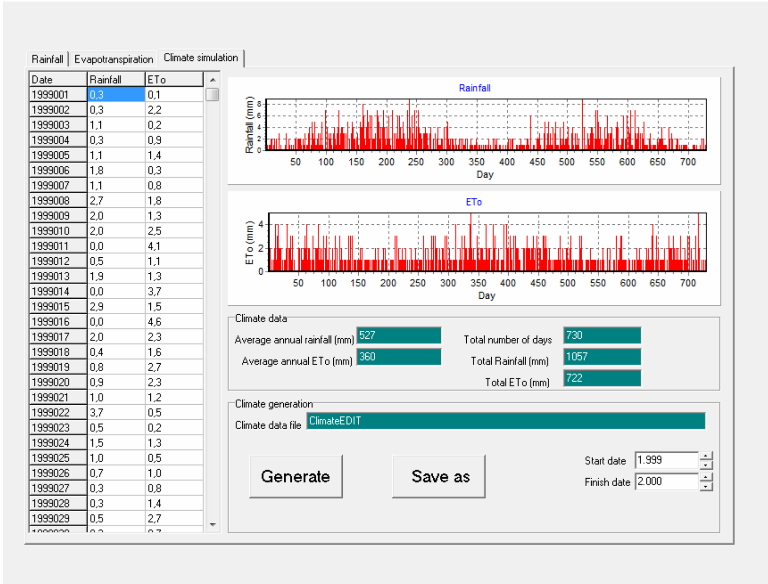


Fig 4.1 Window of Climate Simulator

The soil type was chosen as peaty soil. We simulated 50 cm soil layer as model considers mostly surface soil layer dynamics. The soil density was chosen specific to peat – 1.6 $kg{\cdot}L^{-1}$ (Buivydaitė, Motuzas 2000). The initial moisture content was 5 %, as it was determined during research. It is also considered that rainfall infiltrates – 100% in to the soil. The maximum soil depth used in simulation is 40 cm, as it should be less than chosen soil layer depth.

The component (HMs) concentration in the soil was chosen to be highest in the surface soil layer, specifically in depth of 10 – 20 cm. The main distribution of HMs was in 40 cm depth (Torri and Lavado 2007).

For the birch tree as it is deciduous there was settled the transpiration period during 288 and 120 days of the year (except winter). The root mainly distribution was in 15 – 20 cm soil depth (Laitakari 1934).

In the case of pine tree specific issue is that it is evergreen tree species this influences continuing transpiration and biomass growth over the year. The lateral root distribution was considered mostly in 10 – 15 cm depth soil layer (Laitakari 1934).

The total initial concentrations of HMs in the most contaminated part of the soil profile were chosen: Pb – 45 $mg{\cdot}kg^{-1}$, Cd – 7.3 $mg{\cdot}kg^{-1}$, and Cu – 9 $mg{\cdot}kg^{-1}$. The background concentration of these metals were determined – Pb is 12 $mg{\cdot}kg^{-1}$, Cd is 2.2 $mg{\cdot}kg^{-1}$, and Cu – 3.1 $mg{\cdot}kg^{-1}$. (Katinas *et al* 2002)

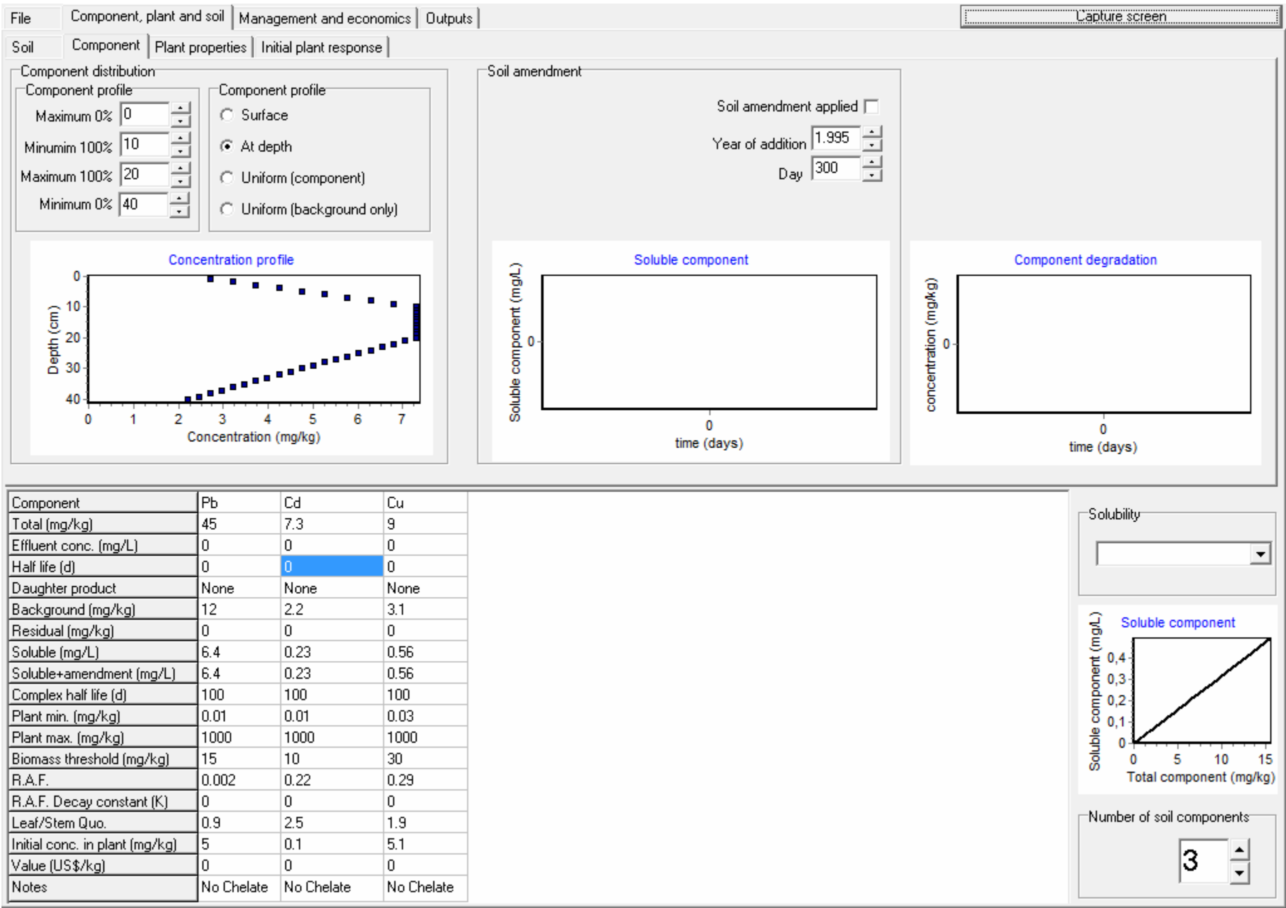


Fig 4.2. The Phyto DSS model active window

The most important in this model is the concentration of soluble, or mobile, forms of HMs. We have chosen mobile forms considered the most contaminated HMs total concentration. For Pb it was 6.4 mg·L^{-1}, Cd – 0.23 mg·L^{-1}, and for Cu was – 0.56 mg·L^{-1} (Katinas *et al*. 2002).

The threshold tree biomass concentrations were chosen according Pais and Jones (1997) and Kabata – Pendias and Pendias (2001) were for pine tree: Pb – 30 mg·kg^{-1}, for Cd – 5 mg·kg^{-1} and for Cu – 20 mg·kg^{-1}; and for birch tree: 30, 10, 30 mg·kg^{-1}, as birch considered more tolerant to HMs (Eltrop *et al*. 1991; Kahle 1993).

The Root absorption factor (R.A.F) was estimated according to 4.5 equation. The data, such as HMs concentration in above biomass and dry biomass, was chosen from our research work. The birch tree RAF for Pb was estimated to be 0.002, Cd – 0.22 and Cu – 0.29. The pine tree RAF differed and for Pb was 0.0015, Cd – 0.16 and Cu – 0.14. The R.A.F. Constant (K) was not considered, K = 0. This means that absorption of roots during the years considered being constant.

As many components have a higher concentration in the leaves than in the stems. It is important to consider HMs translocation to leaves/needles because they can decompose and return HMs in the soil. For birch tree we estimated leaf and stem ratio according our research data and for

Pb it was 0.9, Cd – 2.5 and Cu – 1.9. For pine tree as it is evergreen this factor is not so efficient, however, we estimated that for Pb ratio is 5, for Cd – 5.6, and for Cu – 1.4.

The initial concentration in plants of HMs was chosen: Pb – 5 $mg \cdot kg^{-1}$, Cd – 0.05 $mg \cdot kg^{-1}$, and Cu – 5.1 $mg \cdot kg^{-1}$ for pine tree and for birch – Pb – 5 $mg \cdot kg^{-1}$, Cd – 0.1 $mg \cdot kg^{-1}$, and Cu – 5.1 $mg \cdot kg^{-1}$ (Kabata – Pendias, Pendias 2001).

For further comparison of estimated and measured data we considered that in 1 m^2 was growing 1 birch tree and 2 pine trees. As our biomass was weighted after drying it (as dry weight) it was added 1 kg to birch tree biomass and 2.5 kg to pine tree (thicker stem and heavier needles) biomass.

Economic evaluation scenario consists of phytoremediation and biomass use for biofuel. There is a possibility after year period to gain profit from biomass, however in our case after 10 year the amount of biomass is not so efficient for calculation of profit. We considered that cost of site assessment could be 8000 US dollars (19800.80 Lt); the cost of planting – about 4600 – 5300 US dollars (11385.46 – 13118.03 Lt) for 1 ha, and value of biomass could be from 2000 to 3000 US dollars (4950.20 – 7425.30 Lt). The capping of soil initial cost was chosen to be 160 000 US dollars (396 016 Lt) per 1 ha for 1 year period (Terry, Banuelos 1999). The cost of inaction consist of loss of productivity which evaluated from 4600 – 5300 US dollars/ha/yr (11385.46 – 13118.03 Lt/ha/yr); reputation – 1 million dollars (2.4 mln. Lt), and litigation cost over 200 000 US dollars (495020 Lt).

4.3. Soil – tree system modelling results

The estimated and measured concentration in the soil did not reveal any big difference, except Cd concentration which was almost five times bigger than measured (Fig 4.3, Fig 4.4.). In two plantations was estimated about 6.8 mg·kg^{-1} and measured value was 1.24 mg·kg^{-1}.

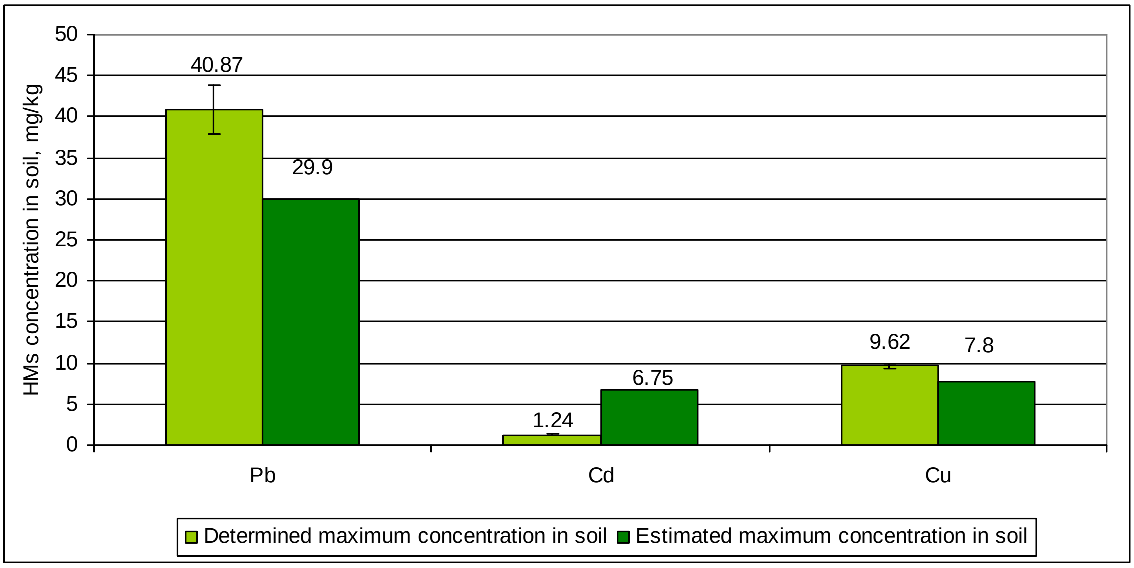


Fig 4.3. The measured and estimated HMs maximum concentrations in the soil of the birch tree plantation

In the case of birch tree and pine tree the estimated Pb concentrations were 29.90 mg·kg^{-1} in birch tree case, 34.50 mg·kg^{-1} in case of pine tree and measured value was 40.87 mg·kg^{-1}. The estimated Cu concentration was similar in the soil of both plantations – 7.80 mg·kg^{-1}. The measured value was 9.65 mg·kg^{-1}.

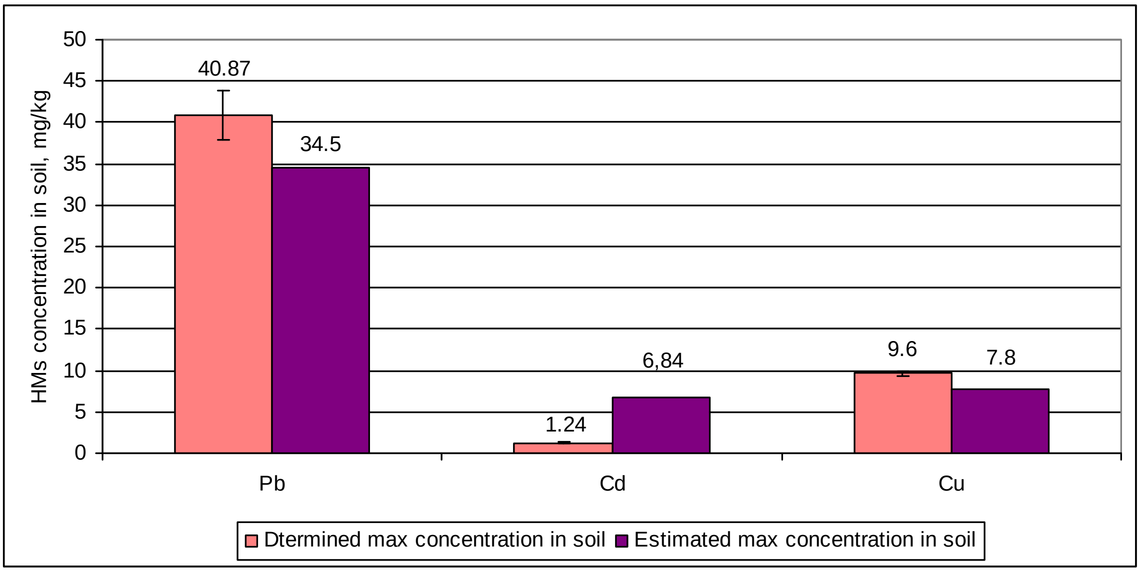


Fig 4.4. The measured and estimated HMs maximum concentrations in the soil of the birch tree plantation

The extracted masses of HMs in trees in birch tree case were smaller than measured values (Fig 4.5). The Pb value measured was 0.02 kg·ha^{-1} and estimated 0.01 kg·ha^{-1}. The Cd values were similar and were equal to 0.06 kg·ha^{-1}. The bigger difference was among Cu values – 0.18 kg·ha^{-1} was measured and 0.16 kg·ha^{-1} predicted.

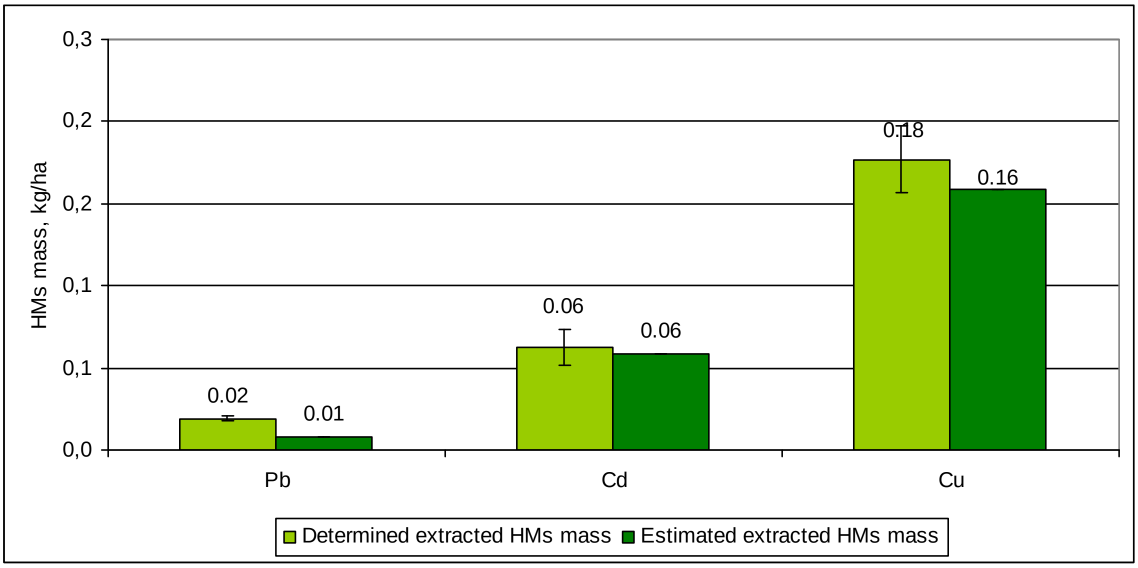


Fig 4.5. HMs accumulated in the birch tree seedlings comparison of estimated and measured data

In the case of pine tree the Pb and Cu estimated and measured values did not differ a lot (Fig 4.6). Pb measured value was equal to 0.05 kg·ha^{-1} and estimated to 0.03 kg·ha^{-1}. Cu was equal to 0.48 and 0.5 kg·ha^{-1} respectively. The biggest difference between values was in case of Cd, measured value was 0.21 and estimated even – 0.32 kg·ha^{-1}.

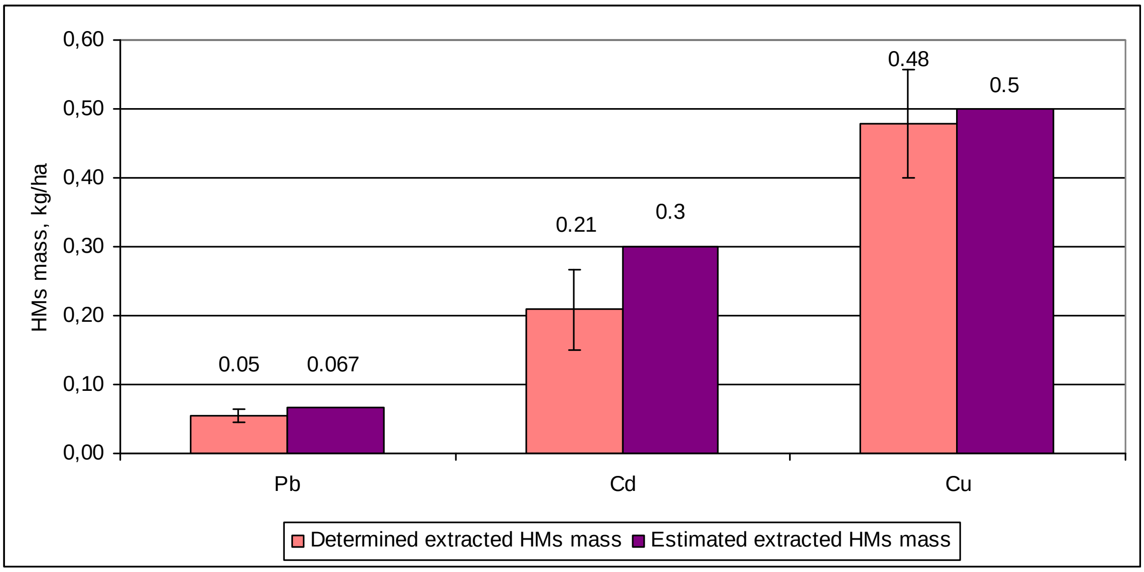


Fig 4.6. HMs accumulated in the pine tree seedlings comparison of estimated and measured data

The measured biomass in the case of birch tree was equal to 1.12 kg·m^{-2} and estimated was 1.20 kg·m^{-2}.

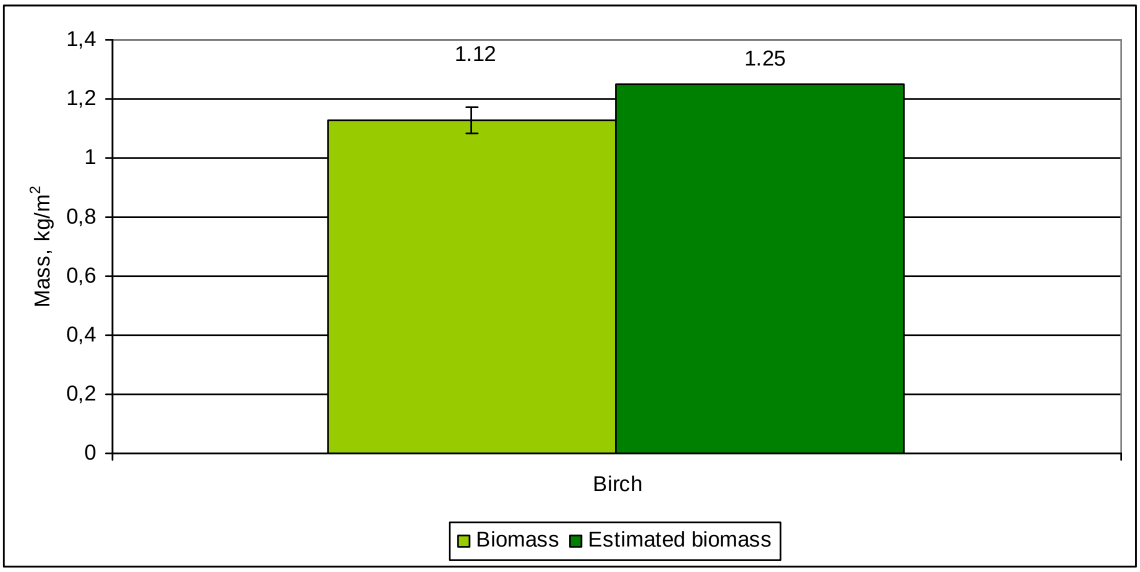


Fig 4.7. The measured and estimated birch tree biomass, kg·m^{-2}

The estimated biomass of pine was smaller than measured. And was equal to 4.2 kg·m^{-2}, the measured was 6.22 kg·m^{-2}.

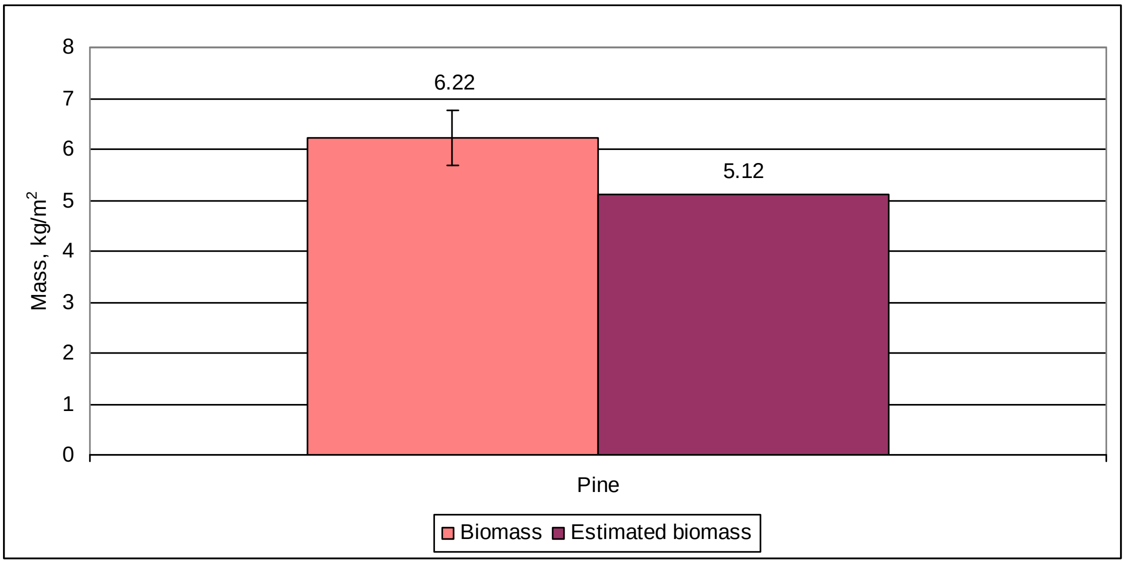
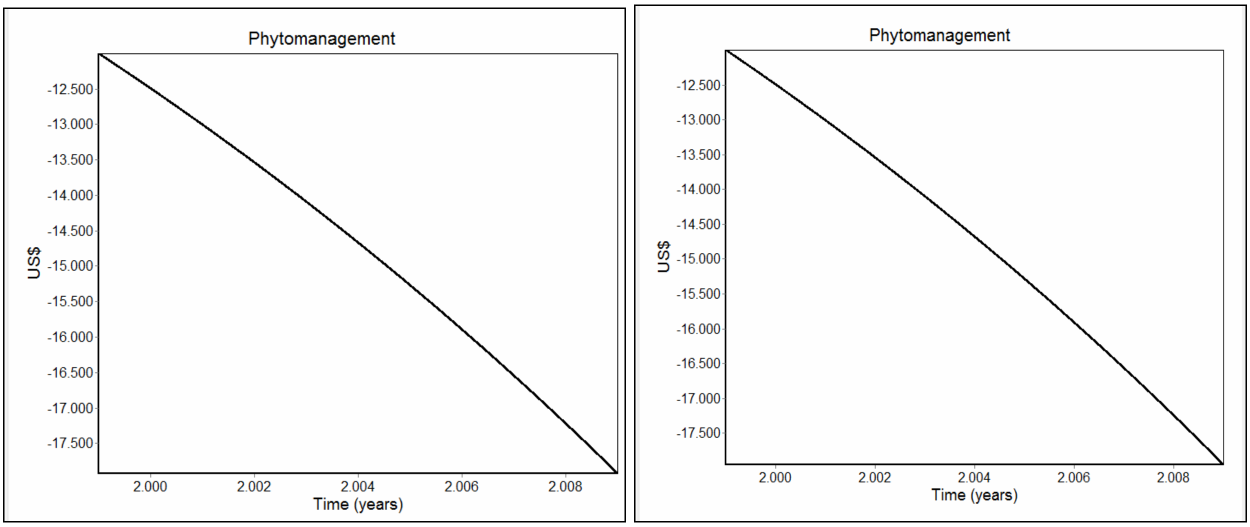


Fig 4.8. The measured and estimated pine tree biomass, kg·m^{-2}

The cost of phytomanagement was determined to be the best solution despite the long time period. After 9 year period the cost of phytomanagement activity (trees planting, growing) was about 18 000 US dollars (45 653.4 Lt) of both tree species plantations.



a) b)

Fig 4.8. The cost of planting Birch trees (a) and pine tree (b) in area for phytoremediation

However, the cost of alternative soil treatment as for example, capping, was approximately 180 438 US dollars (457644.9 Lt).

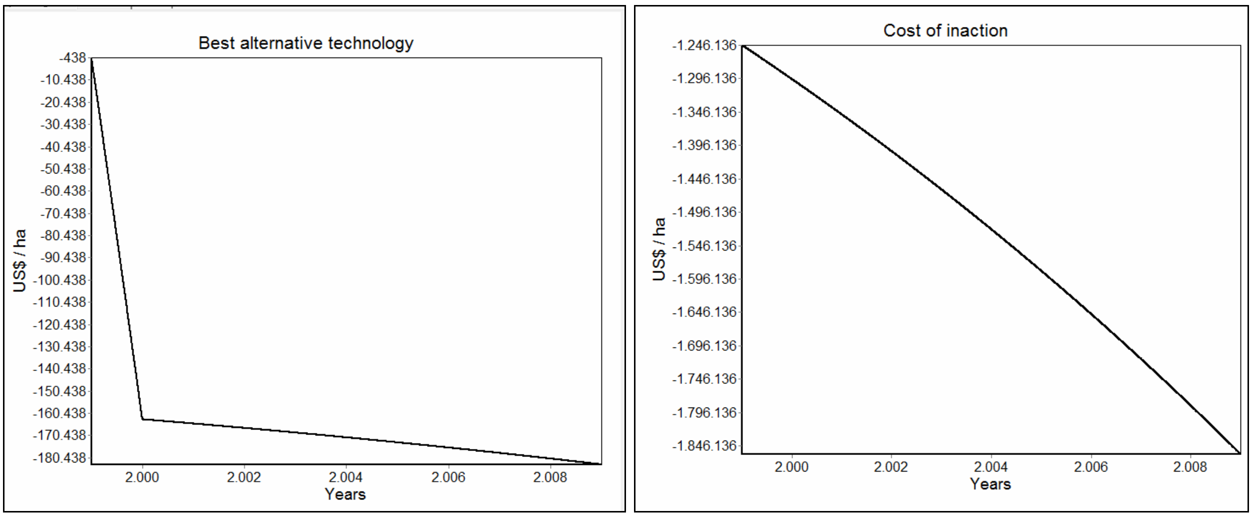


Fig 4.9. The cost of capping action to clean the soil and the cots of inaction

The inaction according to our entered data was the most economically irrelevant. The cost of inaction after 9 years period could reach over 1.8 million US dollars (4565340.0 Lt).

Review of chapter 4

The Phyto DSS model revealed similar values to measured data of biomass production. However, the Pb and Cu concentrations in the soil were estimated differed from measure 26% and 19%. The biggest difference was determined between Cd values in the soil, 81%. The Pb decrease in the soil is caused by water flow as its big amount was leached; it is also related with high entered initial soluble concentration. The Cu was highly accumulated in plants this is because it is one of essentials metals for the plants. In the case of Cd considered high concentration in soil could be related with small soluble concentration in the soil solution and also slow accumulation in the trees.

The estimated amounts of HMs in the trees were similar to measured values. In the pine trees the Cd and Cu measured differ 25% and 4% from estimated ones. **Pb** measured value from estimated differed **30%**. In the birch case the Cu and Cd the values did not differ a lot either, about 11% and 1%. However, as in pine case the most difference was between **Pb** values **50%.** In both cases Pb measured amount was bigger than estimated. This is highly related with estimated root absorption parameter for Pb (the lowest). The root absorption factor was estimated according to our present research data. It would better before simulation to run laboratory researches and precisely determine initial data.

The economic results revealed that phytomanagement could be the most economically efficient. However, different values of trees did not show the differences between plantations. This could be explained by different cost of initial production (tree seedlings; 2 for pine and 1 for birch) which was higher for pine tree. However, the biomass production was bigger than birch tree and this could give greater income in the future.

The Phyto DSS model is very simplified and quit easy to use for prediction the phytoextraction possible features in environmental and economical fields. However, the precise initial data is required to forecast future biomass growth and HMs accumulation, such as root development, metal uptake rate, water uptake rate. The climate conditions also should be considered very precisely, because model is sensitive to changes in water regime (rainfall, evapotranspiration).

Conclusions

1. Soil properties were determined to be better in the site amended with sewage sludge, however the development of investigated trees was slower than in control site (e.g. birch roots, shoot, stem and leaves biomass was 39, 18, 15, 22% smaller; in pine case: 30, 1.2, 17, 36%, respectively; the stem height of birch was 16% and pine – 12% smaller) which suggest the possible inhibitory effect of heavy metals.
2. Higher concentrations of Cu and Cd were determined in soil amended with sewage sludge than in control site (60 and 36%, respectively) and in contaminated trees Cu and Cd concentrations were higher (Cu – 37% in birch and 27% in pine shoots; 6% in birch and 73% in pine roots; Cd was 3% in birch and 1.4% in pine shoots; 53% in birch and 24% in pine roots) than in control trees.
3. During 10 year period pine trees produced 87% higher biomass and accumulated about 60% more heavy metals (Cu, Cd and Pb) than birch trees.
4. The differences between simulated with Phyto DSS model and measured accumulated heavy metal values did not exceed 30%, except Pb which differed about 30 – 50 %.

Recommendations

1. According to our investigation data we recommend to plant pine tree plantation if the sites are amended with sewage sludge. Due to the fact, that pine tree accumulate higher amount of heavy metals and gain more biomass than birch trees. This option is more favourable economically (bigger income from biomass production) and environmentally (higher soil cleaning rate).
2. For evaluation of trees development we could recommend to use functional traits for indication better soil nutritious environment, such as: specific root length (SRL), root/shoot ratio, root branching; and for possible toxicity effect of heavy metals or other harmful compounds: tree height and stem diameter (if trees are of the same age), tree biomass (dry mass).
3. The model Phyto DSS can be recommended to use for prediction of heavy metals accumulation and biomass growth rates of pine and birch trees, respectively.

References

An, Y.J. 2006 Assessment of comparative toxicities of lead and copper using plant assay. Chemosphere. 62: 1359–1365.

Antoniadis, V.; Robinson, J.S.; Alloway, B.J. 2008 Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. *Chemosphere* 71: 759 – 764.

Aplinkos apsaugos agentūra. 2006. Visuomenės informavimas apie nuotekų ir dumblo tvarkymą pagal 1991 m. gegužės 21 d. Tarybos direktyvos Nr. 91/271/EEB dėl miesto nuotekų valymo 16 straipsnį. Ataskaita.

Athar, R.; Ahmad, M. 2002 Heavy metal toxicity: Effect on plant growth and metal uptake by wheat, and on free living azotobacter. *Water, air and soil pollution.* 138: 165-180.

Arcak, S.; Karaca, A.; Haktanır, K. 2006. Investigations on sewage sludge: chemical composition and effects on some chemical properties of soil. Available on internet: <http://www.toprak.org.tr/isd/isd_62.htm>

Arduini, I.; Godbold, D. L. and Onnis, A. 1994. Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus pinaster* seedlings. *Physiologia Plantarum.* 92(4): 675 – 680.

Arduini, L.; Godbold, D.L.; Onnis, A.; Stefani, A. 1998. Heavy metals influence mineral nutrition of tree seedlings. Chemosphere 36: 739 – 744.

Adalsteinsson, S.; Gussarsson, M.; Asp, H.; Jensen, P. 1997. Monitoring copper-induced changes in fine root geometry of birch (*Betula pendula*) using nutrient film technique (NFT). Physiological Plant. 99: 379 – 384.

Bayes, C.D., Taylor, C.M.A. and Moffat, A.J. 1991. Sewage sludge utilisation in forestry: the UK research programme. In: *Alternative uses for sewage sludge*, ed. J.E. Hall. Pergamon Press, Oxford, 115–138.

Balestrasse, K.B., Benavides, M.P. AND Gakkego S.M. 2003. Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. *Functional Plant Biology*. 30: 57 – 64.

Baltrėnaitė, E.; Butkus, D. 2007. Accumulation of heavy metals in tree seedlings from soil amended with sewage sludge. *Ekologija*. 53 (4): 68 – 76.

Barceló, J.; Poschenrieder, C. 1990. Plant water relations as affected by heavy metal stress: a review. *Journal of Plant Nutrition*. 13: 1 – 37.

Bozkurt, M. A.; Yarilgaç, T. 2003. The Effects of Sewage Sludge Applications on the Yield, Growth, Nutrition and Heavy Metal Accumulation in Apple Trees Growing in Dry Conditions. *Turkish Journal of Agric For*. 27: 285 – 292.

Bojarczuk, K. 2004. Effect of toxic metals on the development of Poplar (*Popus tremula*×*P. alba*) cultured *in vitro*. 13 (2): 115 – 120.

Bojarczuk, K.; Karolewski, P.; Oleksyn, J.; Kieliszewska – Rokicka, B.; Zytkowiak, R.; Tjoelker, M. G. 2002. Effect of polluted soil and growth and physiology of silver birch (*Betula pendula*) seedlings. *Polish Journal of Environmental studies* 11(5): 3 – 492.

Breckle, S. W.; Kahle, H. 1991. Effects of toxic heavy metals (Cd, Pb) on growth and mineral nutrition of beech (*Fagus sylavtica* L.). *Plant Ecology*. 101(1): 43 – 53.

Buivydaitė V., Motuzas A. 2000. Pagrindinės dirvoțemio fizikinės savybės. *Geologijos pagrindų ir dirvotyros laboratoriniai darbai*. p. 44 – 48.

Burton, K. W.; Morgan, E. A.; Roig A. 1986. Interactive effects of cadmium, copper and nickel on the growth of sitka spurce and studies of metal uptake from nutrient solutions. *New Phytologist*. 103(3): 549 – 557.

Cheung, Y.H.; Wong, M.H.; Tam, N.F.Y. 2004. Root and shoot elongation as an assessment of heavy metal toxicity and 'Zn Equivalent Value' of edible crops. *Hydrobiologia*. 188 – 189(1): 377 – 389.

Crow, P. 2005 The influence of soil and species on tree root depth. Forestry Commission. Information note. Available in internet: <www.forestresearch.gov.uk>.

D'Aoust, AL.; Delisle, C.; Girouard, R.; Gonzalez, A; Bernier-Cardou, M. 1994. Containerized spruce seedlings: relative importance of measured morphological and physiological variables in characterizing seedlings for reforestation. Inf. Rep. LAU-X-110E. Sainte-Foy, QC: Natural Resources Canada, Canadian Forest Service– Quebec Region. 28 p.

Dahlin, S.; Witter, E.; Mårtensson, A.; Turner A.; Bååth E. 1997. Where's the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. *Soil biology and biochemistry*. 29(9 – 10): 1405 – 1415.

Denef, K.; Six, J.; Paustian, K. & Merckx, R. 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles. *Soil Biology and Biochemistry* 33: 2145 – 2153.

Eckert, D.; Sims, J.T. 1995. Recomendation for soil testing procedures. For North Eastern United States. *Second edition.* Recommended Soil pH and Lime Requirement Tests. Chapter 3.

Eissenstat, D.M. 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytologist*. 118(1): 63 – 68.

Eissenstat, DM. 1997. Trade-offs in root form and function. In: Jackson LE, ed. *Ecology in agriculture*. San Diego, CA, USA:Academic Press. 173 – 199.

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

Eutech Instruments Pte Ltd. [online]. 1997 Information on Measurement of pH in Soil. Available in internet: <http://www.eutechinst.com/techtips/tech-tips6.htm>.

European Commission (2001b) *Disposal and recycling routes fro sewage sludge Part 3 – Scientific and Technical Report* European Commission DG Environment

Gasiūnas, V. 1997. Impact of fertilization with sewage sludge on agrochemical qualities and amounts of heavy metals in soil. p.249-258 In: Vilniaus miesto nuotekų dumblo panaudojimas sunaikintoms žemėms rekultivuoti ir tręšti. Collection of publications. p. 315.

Göransson, A.; Eldhuset, T.D. 1995. Effects of aluminium ions on uptake of calcium, magnesium and nitrogen in *Betula pendula* seedlings growing at high and nutrient apply rates. *Water, air and soil Pollution.* 83: 351 – 361.

Gradeckas, A., Kubertavičiene, L. and Gradeckas, A. 1998. Utilization of wastewater sludge as a fertilizer in short rotation forests on cut away peatlands. *Baltic Forestry*, 4(2): 7 – 13.

Griffiths, B.S.; Hallett, P.D.; Kuan H.L.; Pitkin, Y.; Aitken, M.N. 2005. Biological and physical resilience of soil amended with heavy metal-contaminated sewage sludge. European Journal of Soil Science 56: 197 – 205.

Gussarsson, M.; Asp, H.; Adalsteinsson, S.; Jensén, P. 1996. Enhancement of cadmium effects on growth and nutrient composition of birch (Betula pendula) by buthionine sulphoximine (BSO). *Journal of Experimental Botany*. 47: 211-215.

Hagemeyer, J.; Heppel, T.; Breckle S. W. 1994. Effects of Cd and Zn on the development of annual xylem rings of young Norway spruce (*Picea abies*) plants. *Trees – Structure and Function*. 8 (5): 223 – 227.

Han, F. X.; Banin, A.; Triplett, G. B. 2001. Redistribution of Heavy Metals in Arid-Zone Soils Under A Wetting-Drying Cycle Soil Moisture Regime. *Soil Science.* 166(1): 18 – 28.

Hart, S. 2008. Nutrient availability in goats. Available on internet: <http://www2.luresext.edu/goats/training/nutrition.html> Žr. 2009 05 26.

Hartikainen, H; Pietola, L.; Simojoki, A.; Xue, T. 2001. *Quantification of fine root responses to selenium toxicity.* Agricultural and food science in Finland. 10:53 – 58.

Harri, R.W. 1992. *Root-Shoot ratio.* Arboriculture 18(1):39 – 42.

Hue, N.V., Ranjith, S.A. 1994. Sewage sludges in Hawaii: chemical composition and reactions with soils and plants. *Water Air Soil Pollution*. 72: 265–283.

Hutchings, T. 2002. The opportunities for woodland on contaminated land. Forest commission. Information note. www.forestry.gov.uk

Human health and environmental impacts of using sewage sludge on forestry and for restoration of derelict land. 2007. Project funders /partners: SNIFFER, SEPA, EHS, Scottish Executive. Final report.

ICP forest manual. 2006. Part III. Sampling and analysis of soil. Available in internet: <http://www.icp-forests.org/Manual.htm>

Iqbal, M.Z.; Shazia, Y. 2004. Differential tolerance of *Albizia lebbeck* and *Leucaena leucocephala* at toxic levels of lead and cadmium. *Polish Journal of Environmental Studies*. 13(4):439 – 442.

Yruela, *I.* 2005. Copper in plants. *Brazilian Journal of Plant Physiology*. 17: 145 – 156.

Joseph, L.U., Andrea, L.C. and Mal, T.K. 2002. Effects of lead contamination on the growth of *Lythrum salicaria*. *Environmental Pollution*. 120(2): 319 – 323.

Kabata – Pendias, A.; Pendias H. 2001. Trace elements in soils and plants. Third edition. ISBN 0849315751. CRC Press. p. 413.

Kadūnas, V.; Budavičius, V.; Gregorauskienė, V.; Katinas, V.; Kliaugiene, A; Radzevicius, A.; Taraskevicius, R. 1999. Geochemical atlas of Lithuania. Geological institute. Vilnius. p. 89.

Kahle, H. 1993. Response of roots trees to heavy metals. *Environmental and Experimental Botany* 33 (1)|: 99-119.

Katinas, V.; Kadūnas, V.; Radzevičius, A.; Zinkutė, R. 2002. Processes of chemical element dispersion and redistribution in the environment with wastewater sludge used for recultivation of woodcutting areas. *Geologija* 38:3-11.

Kissel, D.E. and Vendrell, P.F. 2006. Salt pH and salt concentration. A New Method for Measuring Soil pH. University of Georgia. Available in internet: <http://www.clemson.edu/>

Kozlowski, T. T. 1997. Responses of woody plants to flooding and salinity. Tree physiology monograph. 1:29.

Kupčinskienė, E. 2006. *Latentiniai paprastosios pušies pakitimai lokalios taršos aplinkoje* [Latent injuries of scots pine (*Pinus sylvestris* L.) under influence of local pollution] Kaunas: Lututė. p. 256.

Landjeva, S.; Merakchijska – Nikolova, M.; Ganeva, G. 2003. Copper toxicity tolerance in *Aegilops* and *Haynaldia* seedlings. *Biologia Plantarum*. 46(3):479 – 480.

Laitakari, E. 1934. Koivun juuristo (Summary: The root system of birch, Betula verrucosa and odorata). Acta Forestalia Fennica 40: 853–901.

Lazdiņa1, D.; Lazdiņš, A., Kariņš, Z.; Kāposts, V. 2007. Effect of sewage sludge fertilization in short-rotation willow plantations. Journal of Environmental engineering and landscape management. 15 (2): 105 – 111.

Levinsky, B. 2002. Humates and Humic Acids. How do they work? TeraVita Limited, Lancaster, PA .Public lecture. Read in institute SunFire, Philadelphia.

Mahmood, T.; Islam, K.R.; Muhhamad, S. 2007. Toxic effects of heavy metals on early growth and tolerance of cereal crops. *Pak. J. Bot*. 39(2):451 – 462.

Manceau, A., Boisset, M.C., Sarret, G., Hazemann, J.L., Mench, M., Cambier, P. and Prost, R. 1996. Direct determination of lead speciation in contaminated soil by EXAFS spectroscopy. *Environmental Science and Technology* 30:1540 –1552.

Máthé-Gáspár, G; Máthé, P.; Szabó, L.; Orgoványi, B.; Uzinger, N.; Anton, A. (2005) After-effect of heavy metal pollution in a brown forest soil. *Acta Biologica Szegediensis.* 49 (1 – 2): 71 – 72.

Mažvila J. 2001. Sunkieji metalai Lietuvos dirvožemiuose ir augaluose. Monografija. Lietuvos žemdirbystės institutas. Agrocheminių tyrimų centras. p. 343.

Menon, M.; Hermle, S.; Günthardt – Goerg, M.S. and Sculin, R. 2007. Effects of heavy metal soil pollution and acid rain on growth and water use efficiency of a young model forest ecosystem. *Plant and soil*. 297(1 – 2): 171 – 183.

Metcalf, E. 2003. Wastewater engineering: treatment, disposal, and reuse, fourth ed. McGraw-Hill Publishing Company Ltd., New York.

Miller, A.; Hills, A. 2000. The Importance of soil pH. *Published by* Agriculture Western Australia. 78:2.

Moffat 2006. *Information Note: Use of Sewage Sludges and Composts in Forestry*. Forestry Commission.

Montse, T.; Joan, R. 2006. Leaching of heavy metals (Cu, Ni and Zn) and organic matter after sewage sludge application to Mediterranean forest soils. *Sci. total environment*. 363(1 – 3):11 – 22.

Nóbrega, R. S. A.; Boas, R. C. V.; Nóbrega, J. C. A.; de Paula, A. M.; Moreira, F. M. S. 2007. Utilization of sewage sludge in the seedling growth of aroeira (*Schinus terebynthifolius* Raddi) seedlings. *Rev. Árvore* [online]. 31(2): 239 – 246.

Ostonen, I.; Püttsepp, Ü.; Biel, C˙; Alberton, O.; Bakker, M. R.; Löhmus, K.; Majdi, H.; Metcalfe, D.; Olsthoorn, A. F. M.; Pronk, A.; Vanguelova, E.; Weih, M.; Brunner I. 2007 Specific root length as an indicator of environmental change. *Plant biosystems*. 141(3): 426-442.

Österås, A. H. 2004. Interactions between calcium and heavy metals in Norway spruce: Accumulation and binding of metals in wood and bark. Doctoral thesis. Comprehensive summury. Stockholm University, Faculty of Science, Department of Botany. p. 52.

Påhlsson, A. B. 1991. Influence of aluminium on biomass, nutrients, soluble carbohydrates and phenols in beech (*Fagus sylvatica*). *Physiologia Plantarum* 78 (1): 79 – 84.

Pais, I.; Jones, J.B. Jr. 1997. The handbook of trace elements. St. Lucie Press, Boca Raton, Florida.

.Parat, C.; Denaix, L.; Leveque, J.; Chaussod, R.; Andreux, F. 2007. The organic carbon derived from sewage sludge as a key parameter determining the fate of trace metals. *Chemosphere* 69: 636 – 643.

Parent L.E. and Tremblay C. 2003. Soil Acidity Determination Methods for Organic Soils and Peat Materials. Chapter 5. p. 93 – 105. In: Parent L.E., Ilnicki P. Organic soils and peat material for sustainable agriculture. CRC Press. ISBN 0849314585. 205 p.

Pikka, J. 2005. Use of wastewater sludge for soil improvement in afforesting cutover peatlands. Metsanduslikud uurimused / Forestry Studies. 42:95 – 105.

Terry, N.;. Banuelos, G. S. 1999. Phytoremediation of contaminated soil and water. CRC Press. ISBN 1566704502, 9781566704502. p. 389.

Ryser, P. 1996 The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Functional Ecology*. 10: 717 – 723.

Ryser, P; Emerson, R. 2007 Growth, root and leaf structure, and biomass allocation in *Leucanthemum vulgare* Lam. (Asteraceae) as influenced by heavy-metal-containing slag. *Plant and soil*. 301(1 – 2):315 – 324.

Robinson, B.H.; Fernández, J.E.; Madejón, P.; Marañón, T.; Murillo, J.M.; Green, S.R.; Clothier, BE. 2003 Phytoextraction: an assessment of biogeochemical and economic viability. Plant and Soil. 249(1): 117 – 125.

Sanchez Monedero, M.A., Mondini, C., De Nobili, M., Leita, L., Roig, A., 2004. Land applications of biosolids. Soil response to different stabilization degree or treated organic matter. *Waste Manage* 24 (4): 325–332.

Selivanovskaja, S. Yu.; Latypova V.Z. 2006. Effects of composted sewage sludge on microbial biomass, activity and pine seedlings in nursery forest. *Waste Management* 26: 1253 – 1258.

Sewage sludge for land restoration. 2004. Briefing notes. Available in internet: <http://www.water.org.uk/static/files_archive/2Sludge_for_Land_restoration_briefing_Notes_v2_march_15.doc>.

Singh, R.P. and Agrawal, M. 2007 Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. *Chemosphere*. 67(11):2290 – 2240.

Sharma, P.; Dubey, R.S. 2005 Lead Toxicity in plants. *Brazilian Journal of Plant Physiology*. 17(1):35-52.

Slattery, W.J. and Ronnfeldt, G.R. 1992. Seasonal variation of pH, aluminium and manganese in acid soils from North – Eastern Victoria. *Australian Journal of Experimental Agriculture* 32 (8): 1105 – 1112.

Sort, X. & Alcaniz, J.M. 1999. Effects of sewage sludge amendment on soil aggregation. *Land Degradation and Development*. 10: 3–12.

Sparks, D. 1995. Environmental Soil Chemistry. San Diego, USA, Academic Press.

Šottníková, A.; Lunáčková, L.; Masarovičová, E.; Lux A. and Streško V. 2003. Changes in the Rooting and Growth of Willows and Poplars Induced by Cadmium. *Biologija Plantarum.* 46(1): 129 – 131.

Torri, S.I. and Lavado, R.S. 2007. Dynamics of Cd, Cu and Pb added to soil through different kinds of sewage sludge. *Waste management* 28: 821 – 832.

Vandecasteele, B.; Meers, E.; Vervaeke, P.; De Vos, B.; Quataert, P.; Tack, F.M.G. 2004. Growth and trace metal accumulation of two *Salix* clones on sediment – derived soils with increasing contamination levels. *Chemosphere.* 58: 995-1002.

Wójcik, M. and A. Tukendorf. 1999. Cd-tolerance of maize, rye and wheat seedlings, *Acta Physiologia. Plantarum.* 21: 99 – 107.

ANNEX

Publications of the author

Published articles:

1. Vaitkutė, D.; Baltrėnaitė, E. 2008. Soil pH – comparison of two analysis methods. 11th Lithuanian Conference of Junior Researches – “Science – Future of Lithuania”, April 3rd in Vilnius. 470 – 479.

Articles given to publication:

2. Vaitkutė, D.; Baltrėnaitė, E. 2009. Tree functional traits to determine the in influence of sewage sludge. 2009. 12th Lithuanian Conference of Junior Researches – “Science – Future of Lithuania”, April 2nd in Vilnius.
3. Vasarevičius, S.; Kadūnas, K.; Baltrėnaitė, E.; Vaitkutė, D. 2009. Aplinkos apsaugos reikalavimai ir modelis užterštoms teritorijoms Lietuvoje vertinti. 12-osios jaunųjų mokslininkų konferencijos “Mokslas – Lietuvos ateitis” įvykusios Vilniuje 2009 balandžio 2 d. medžiaga.
4. Vaitkutė, D.; Baltrėnaitė' E.; Booth, C. A.; Fullen, M. A. Sewage sludge impact on Silver birch (*Betula pendula* L.) and on Scots pine (*Pinus sylvestris* L.) growth. In *Preparation.*

Presentations of the author

1. Soil pH – comparison of two analysis methods. Poster presentation in 11th Lithuanian Conference of Junior Researches – “Science – Future of Lithuania” in Vilnius, April 3rd, 2008.
2. pH variation in different land use – two pH analysis methods. Poster presentation in COST 639 meeting in Zurich, 6-8 March 2008.
3. Tree functional traits to determine the in influence of sewage sludge. Oral presentation in 12th Lithuanian Conference of Junior Researches – “Science – Future of Lithuania” in Vilnius, April 2nd, 2009.