Does sewage sludge amendment to soil enhance the development of Silver birch and Scots pine?

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Abstract

Sewage sludge can be used to improve forestry soil properties, because it is rich in phosphorus, nitrogen and organic material and, thus, can enhance the growth of tree seedlings in poor quality soils. Our study was performed on a site amended with industrial sewage sludge and afforested with birch and pine seedlings. To evaluate the growth of tree seedlings, tree dry biomass, height, diameter, root/shoot ratio, specific root length, shoot and root length were calculated. Higher concentrations of heavy metals and no significant increase in the biomass of trees on sewage sludge amended soil suggest an inhibitory effect of heavy metals on tree biomass growth.

The site treated with sewage sludge had significantly higher soil moisture content, soil pH, total copper and total lead concentrations and significantly lower exchangeable acidity. Tree tissues at the sewage sludge treated site contain significantly higher concentrations of copper and cadmium. Therefore, both positive and negative impacts of treatment are apparent. In terms of management strategies, it is recommended that the chemical quality of sewage sludge is analyzed prior to possible field applications and only sewage sludges with toxic heavy metal concentrations below accepted safety limits are applied.

Keywords: Betula pendula Roth., biomass, Pinus sylvestris L., heavy metals, root/shoot ratio, sewage sludge, specific root length.

Introduction

The potential utilization of sewage sludge as a fertilizer in forestry is much debated (Gradeckas, A. *et al.*, 1998; Bojarczuk, K. *et al.*, 2002; Bramryd, T. 2002; Katinas, V. *et al.*, 2002; Pikka, J. 2005; Hermle, S. *et al.*, 2006). Deforested

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soils usually lack nutrients and are mainly acidic, especially in exploited peat areas (Gradeckas, A. *et al.*, 1998). Sewage sludge contains components that are potentially beneficial for soils (such as organic matter, phosphorus, nitrogen, calcium and magnesium). However, sludges can have high concentrations of heavy metals (HMs), especially cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn), typically originating from industry. At high concentrations, HMs can be phytotoxic and cause reduced tree growth or even death (Kabata-Pendias, A. and Pendias, H. 2001). Toxic metal ions present in the substrate may also adversely affect trees by damaging roots, which leads to inhibition of the transport of water and nutrients to upper parts of the plant (Kupčinskienė, E. 2006).

The distribution and mobility of HMs mostly depends on soil properties, which controls their mobility within soil systems and their availability to trees. Specific soil properties (pH, exchangeable acidity (H^+ and Al^{3+}), soil moisture, soil texture and organic matter (SOM)) are the key factors that describe soil quality and tree growing conditions (ICP Forest Manual, 2005).

The level of adsorption of HMs, and associated phytotoxicity, mainly depends on tree species. For example, *Betula* and *Salix* tree species are considered as metal tolerant and accumulators (Eltrop, L. *et al.,* 1991; Kahle, H. 1993). Experiments on two *Salix* clones failed to show inhibition effects on growth for any HM treatment (max. 41.4 Cd, 655 mg·kg^{-1} Pb) (Vandecasteele, B. 2004). However, another study revealed that in acidic subsoil *Salix viminalis* displayed a significant growth reduction following the increased Zn and Cd accumulation (Hermle, S. *et al.,* 2006). In contrast, analysis of birch and pine trees in a site treated with industrial sewage sludge (The Taruškos Forest site, Lithuania) did not reveal any negative effects for either tree species (Katinas, V. *et al.,* 2002; Baltrėnaitė, E. and Butkus, D. 2007).

Other investigations have shown that birch grown in metal polluted soil decreased above-ground biomass (Bojarczuk, K. *et al.,* 2002). Silver birch *(Betula pendula Roth.)* grown in polluted substrate was characterized by high biomass allocation to roots (60% versus 30–40% in the control substrate). However, fertilization with sewage sludge, which mainly consists of nutritious organic material, can accelerate tree growth, and increase biomass allocation to foliage (Bojarczuk, K. *et al.,* 2002).

The heavy metals Cu, Cd and Pb have phytotoxic and synergistic effects (Breckle, S.W. and Kahle, H. 1991; Arduini, I. *et al.,* 1994; Kabata-Pendias, A. and Pendias, H. 2001). For example, Cu is an essential metal for normal plant growth and development. Cu participates in numerous physiological processes and is an essential cofactor for many metalloproteins. However, excess Cu inhibits plant growth and disturbs important cellular processes (*i.e.* photosynthetic electron transport) (Bojarczuk, K. 2004; Yruela, I. 2005). The determined phytotoxic concentrations of Cu in the soil was 60–125 mg·kg^{-1} (Kabata-Pendias, A. and Pendias, H. 2001).

Lead is a general protoplasmic toxic metal, which is cumulative and slow-acting (Sharma, P. and Dubey, R.S. 2005). It has a wide range of negative effects on: hormonal status, membrane structure, water potential, electron transport and enzyme activation (Sharma, P. and Dubey, R.S. 2005). However, Pb becomes harmful to plants when concentrations in the soil reaches 100–200 $mg \cdot kg^{-1}$ (Bergman, W. 1986; Kabata-Pendias, A. and Pendias, H. 2001). When Pb is combined with other metals, it displays synergistic effects. For example, root elongation rates of beech (*Fagus sylvatica*) seedlings were significantly reduced by ~30% by 44 $mg \cdot kg^{-1}$ plant-available Pb, but the same effect was observed with only 24 $mg \cdot kg^{-1}$ Pb when combined with 2 $mg \cdot kg^{-1}$ Cd (Breckle, S.W. and Kahle, H. 1991). Decrease in birch biomass was observed when Pb concentrations in soil reached 18 and Cd was 3.6 $mg \cdot kg^{-1}$.

Investigations on different tree species showed that the concentration of metals in soil decreased the growth of shoots and roots by ~50% when Pb concentrations were in the range of 519 to >1280 (285–445) mg Pb kg^{-1} dry soil and Cu were 48–232 (<40–110) mg Cu kg^{-1} dry soil, respectively (An, Y.J. 2006). Typically, Cu is more toxic than Pb, and root growth is more sensitive to the toxicity endpoint than shoot growth in Cu or Pb amended soils.

Cadmium disturbs the uptake, transport and use of Ca, Mg, P and K and water uptake by plants. Cadmium decreases nitrate absorption and its transport from roots to shoots, by inhibiting nitrate reductase activity in shoots (Balestrasse, K.B. *et al.,* 2003). Arduini, I. *et al.,* (1994) found tap-root elongation of stone pine (*Pinus pinea*) and maritime pine (*Pinus pinaster*) was drastically reduced by 5 $\mu m \cdot kg^{-1}$ Cd^{2+} and $Cd^{2+} + Cu^{2+}$ treatments. Burton, K.W. *et al.,* (1986) showed a Cd concentration of 2.5 $mg \cdot kg^{-1}$ significantly decreased the biomass of shoots and roots of *Picea sitchensis*.

The objectives of the investigations were to determine the influence of sewage sludge with high concentrations of heavy metals after 10 years from application on: i) the forest soil properties; ii) growth of Silver birch and Scots pine (*Pinus sylvestris L.)* trees (biomass, stem diameter, and height), iii) growth traits (root/shoot ratio, specific root length, and root/shoot maximum lengths), and iv) heavy metal concentrations in tree components (root, shoots, leaves and needles etc.).

Materials and methods

Site description

The experimental site is located in Gitėnai Forest, near Panevėžys town (Lithuania) *(Figure 1).* Panevėžys is a Mid-lowland Climatic region and is part of the Mūšos-Nevėžis subregion (Climatic regionalism 2010). The average precipitation in this subregion is 500–600 mm annually, and the prevailing winds are south-westerly.

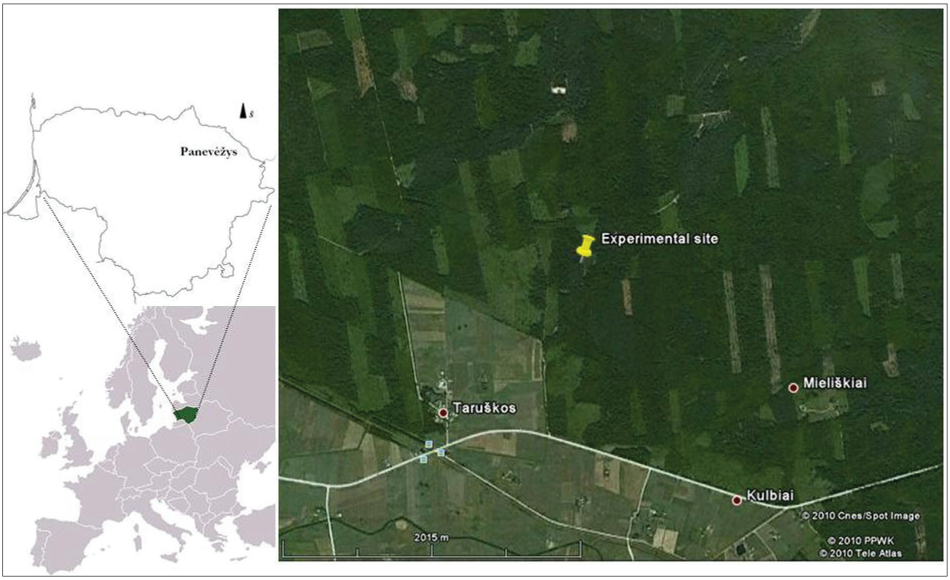

Fig. 1. Experimental site of industrial sewage sludge utilization in the Gitėnai Forest within the Taruškos Forest, located in Panevėžys region (Lithuania), (55°44′ N; 24°33′ E)

In 1998, ~300 t/ha of industrial sewage sludge was spread on the 2-ha experimental site and after one year birch (*Betula pendula*) and pine (*Pinus sylvestris*) seedlings were planted. The experiment was started in order to define the industrial sludge impact on soil chemical composition after 9–10 years of application. A more detailed experimental description is available (Katinas, V. *et al*., 2002).

Table 1 shows the HM concentrations of the industrial sludge (from Panevėžys town) and HM background concentrations of the site soil before experiment started. According to the Lithuanian regulation 'LAND 20-2005', the sewage sludge is Category II and can be used in forestry or agriculture only once every three years. High concentrations of Cu and Pb in sewage sludge were due to industrial activities in Panevėžys, typically electroplating and refrigerator manufacturing.

Table 1. Mean concentrations of HMs and phosphorus in Panevėžys industrial sewage sludge and background concentrations in experimental site before trees were planted in 1998 (published in Katinas, V. et al. 2002)

HMs	Background concentration of experimental site ($mg \cdot kg^{-1}$)	Concentration in industrial sewage sludge ($mg \cdot kg^{-1}$)
Cu	3.1–9.9	291
Pb	11.0–15.0	1,456
Cd	2.2–5.0	6.2
P	490.0–273.0	21,764

Soil sampling

Sampling was carried out in May 2007. Sites measuring 50x50 m were chosen at the site where the sewage sludge was applied (Site S) and in the adjacent forest area (~200 m from the contaminated plot), which was chosen as the background control (Site C). Before soil sampling the litter layer was removed. From both the C and S sites, six composite soil samples (mix of five subsamples) were taken (at 0–10 and 20–30 cm depths). Samples were transported at 4°C to the laboratory and then air-dried. For further analysis, soil samples were oven-dried at 105°C and fractionated through a 2.0 mm sieve *(Retsch, As 2006*).

Tree sampling

Ten year-old *Betula pendula* and *Pinus sylvestris* trees were sampled from both the C and S sites (three birch and pine trees per plot). Tree height and the diameter at 30 cm height (±1 mm) above the ground were measured. Leaves or needles, shoots, stem and roots (coarse >2.0 mm and fine <2.0 mm diameter) were shredded. Each component was weighed (±0.05 g) and measured (±1 mm).

Soil properties

Soil moisture content was determined in 15 g of each sample that was dried at 105°C to constant mass. Soil pH was measured by agitating air-dried soil in a mechanical shaker (*Gerhardt, Rotoshake RS 12*) in 0.01 M $CaCl_2$ solution for 1 hour, and waiting for another hour prior to pH measurement using a calibrated digital pH meter (pH 538 WTV). For total carbon determination, air-dried soil was fractionated through a 2.0 mm sieve *(Retsch, As 2006),* milled, homogenized and 100 mg soil samples were taken. Total C content was analyzed by dry combustion using a Total Organic Carbon Analyzer (TOC-V by SHIMADZU) at 900°C. Exchangeable acidity was determined in 0.1 $mol \cdot l^{-1}$ $BaCl_2$ soil solution. After two hours of shaking the soil extract was titrated with a 0.05 $mol \cdot l^{-1}$ NaOH solution at pH ≤7.8 (ICP Forest Manual, 2006).

Total and mobile Cd, Cu and Pb

Mobile Cd, Cu and Pb were measured in extraction of neutral salt 0.01 M $CaCl_2$, at 1:10 ratio. The solution was mixed and shaken for 16 h, at 20°C. Wet digestion was employed. Each soil sample (weighing 0.5 g, within 10 ml of HNO_3 and 2 ml of HCl solution) was digested for 31 minute in *Mileston*

ETHOS digester (Soon, Y.K. and Abboud, S. 1993). Total metal concentrations in solutions were analyzed using a *Buck Scientific 210 VGP* Atomic Absorption Spectrophotometer (FAAS and GFAAS).

Cd, Cu and Pb concentrations in tree seedlings

Each tree component (roots, stem, shoots and needles/leaves) was shredded and then incinerated at 400°C to ash. Before HM analysis, tree component ashes were powdered and pressed. Metal analysis was performed using the X-Ray Fluorescence Spectrometer in the University of Wolverhampton (UK), using pulverized samples embedded in a wax base.

Statistical analysis

Each sample was measured in duplicate. T-test analysis was performed to determine significant differences between the two investigation sites ($p<0.05$). Data analysis was carried out using *Statistica* (version 7.0) software.

Results

Soil properties

Soil moisture content was higher at Site S than at Site C *(Table 2).* At Site C soil moisture content varied significantly ($p<0.05$) and was greater in the upper soil layer than in deeper soil and the difference of moisture content values was significant between sites ($p<0.05$).

Site C was significantly ($p<0.05$) more acidic (topsoil and subsoil) than Site S *(Table 2).* Exchangeable acidity at Site S was significantly less than at Site

Table 2. Selected soil properties (n=6, mean±1SD) in the soil from the control site (C) and the soil amended with sewage sludge (S)

Site, soil depth, (cm)	Moisture % ±1SD[a]	pH ±1SD[a]	Exchang. acidity, cmol/kg ±1SD[b]	TC, $mg\cdot kg^{-1}$ ±1SD
0–10 (C)	1.87±0.79	3.15±0.07	1449±85	5.35±0.97
20–30 (C)	0.50±0.14	3.69±0.11	1199±76	1.33±0.27
0–10 (S)	3.49±0.44	6.27±0.17	61.3±22.6	5.03±0.90
20–30 (S)	6.79±0.32	6.13±0.41	73.3±39.6	3.45±0.56

significance between sites [a]$p<0.05$, [b]$p<0.01$.

C *(Table 2)*. However, at Site S the difference was not significant with depth, ($p>0.05$), but it was remarkable at Site C ($p<0.05$). Between the two sites this difference was significant ($p<0.01$) in both soil layers. Total carbon (TC) variation was similar in both sites and was higher in the surface soil layer *(Table 2)*. However, between the two sites a considerable difference ($p<0.05$) was only found in the 20–30 cm soil layer.

Metal contamination of soil

Total Cu concentrations were higher at Site S than at Site C *(Table 3)*. Copper concentrations within sites C and S did not vary significantly between soil layers ($p>0.05$). However, between both sites, Cu concentration differences between upper soil layers were significant ($p<0.05$).

Table 3. Total concentrations and mobile fraction (mean value ±1SD, n=6) of Cu, Cd and Pb in two soil layers (0–10 cm and 20–30 cm) at the site amended with sewage sludge (S) and at the control site (C)

Site, soil depth, (cm)	Cu		Cd		Pb	
	Total [a], $mg \cdot kg^{-1}$	Mobile, %	Total [a], $mg \cdot kg^{-1}$	Mobile, %	Total [a], $mg \cdot kg^{-1}$	Mobile, %
0–10 (C)	4.00±1.04	4.4±0.8	0.85±0.11	36.1±13.1	24.78±0.63	1.6±0.1
20–30 (C)	4.53±1.92	2.5±0.37	0.75±0.07	47.5±16.5	23.00±1.18	1.6±0.5
0–10 (S)	9.9±0.07	2.1±0.3	1.33±0.18	13.8±0.8	38.83±8.72	0.7±0.2
20–30 (S)	9.35±4.41	1.5±0.2	1.15±0.24	18.7±0.8	42.92±2.42	0.6±0.2

Significance between sites [a]$p<0.05$.

Total Cd concentration was significantly less at Site C than at Site S ($p<0.05$) *(Table 3)*. However, Cd concentration varied with depth insignificantly ($p>0.05$) at both sites. Pb concentrations were significantly ($p<0.05$) higher at Site S than at Site C. As was the general case with HMs, Pb did not vary significantly between soil layers ($p>0.05$).

The mobile fraction of HMs was distributed in the sequence: Cd>Cu>Pb *(Table 3)*.

Contamination of tree tissue

Copper concentrations in roots and shoots of both tree species were significantly higher ($p<0.05$) at Site S than at Site C *(Figure 2)*. Copper concentrations in the birch tree from Site S was 5.3±0.2 in shoots and 2.5±0.2 $mg \cdot kg^{-1}$ in roots. At Site C the concentrations of Cu in the birch tree components was 3.3±0.02 in shoots and 2.4±1.3 $mg \cdot kg^{-1}$ in roots.

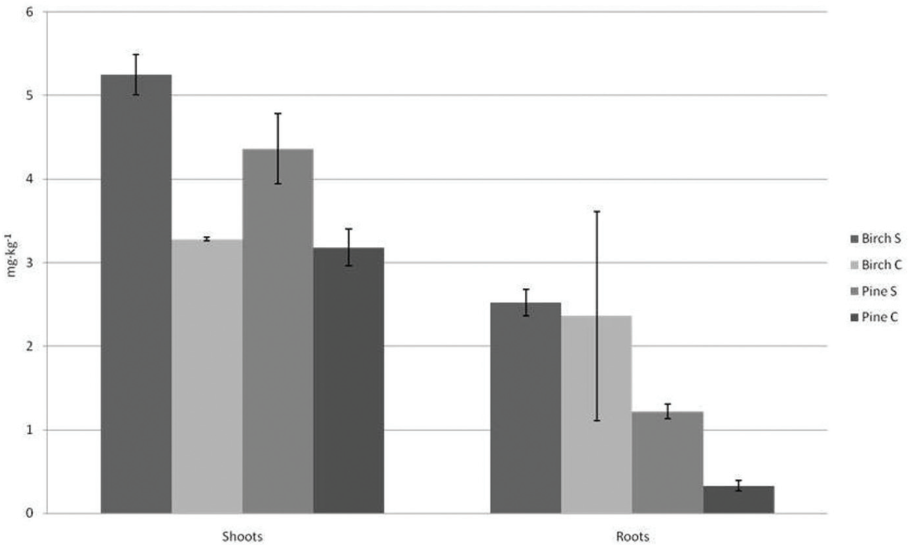

Fig. 2. Copper concentrations in roots and shoots of both tree species in the site amended with sewage sludge (Birch S; Pine S) and in the control site (Birch C; Pine C). Bars represent mean values of three samples ±1SD

In pine tree components Cu concentrations from Site S were 4.4±0.4 in shoots and 1.2±0.1 mg·kg^{-1} in roots. At Site C, higher Cu concentrations were measured in shoots (3.2±0.2 mg·kg^{-1}) than in roots (0.3 ±0.1 mg·kg^{-1}). Differences between Cu values in shoots and between Cu values in roots from both sites were significant ($p<0.05$).

In birch tree shoots and roots, Cd concentrations were lower at Site C *(Figure 3)*. In the components of birch trees from Site S, Cd concentrations were 1.7±0.2 in shoots and 1.3±0.1 mg·kg^{-1} in roots. At Site C the concentrations of Cd in birch tree components varied from 1.6±0.05 in shoots to 0.6±0.5 mg·kg^{-1} in roots. However, Cd concentration differences between trees from both investigation sites are insignificant ($p>0.05$).

In pine tree components, Cd concentrations at Site S were higher than Site C: 1±0.1 in roots and 0.7±0.01 mg·kg^{-1} in shoots. At Site C, higher Cd concentrations were also found in roots: 0.8±0.01 and in shoots 0.7±0.1 mg·kg^{-1}. Cadmium concentrations in roots between investigation sites were significantly different ($p<0.05$).

Lead concentrations in birch tree components were lower at Site S than at Site C *(Figure 4)*. At Site S the concentration was 0.6±0.03 in shoots and 0.4±0.06 mg·kg^{-1} in roots. At Site C the concentrations were 1.6±0.1 in shoots and 0.6±0.2 mg·kg^{-1} in roots. The lead concentration in shoots between investigation sites are significantly different ($p<0.05$).

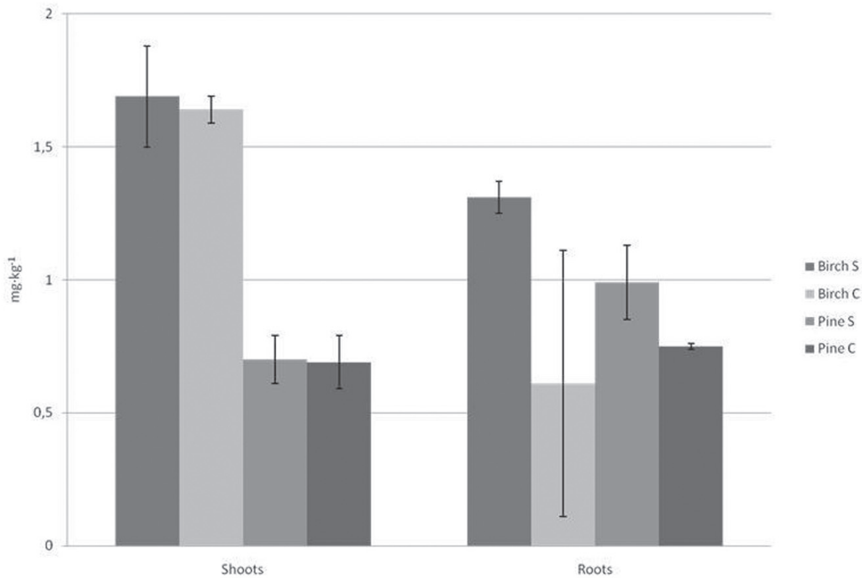

Fig. 3. Cadmium concentrations in roots and shoots of both tree species in the site amended with sewage sludge (Birch S; Pine S) and in the control site (Birch C; Pine C). Bars represent mean values of three samples ±1SD

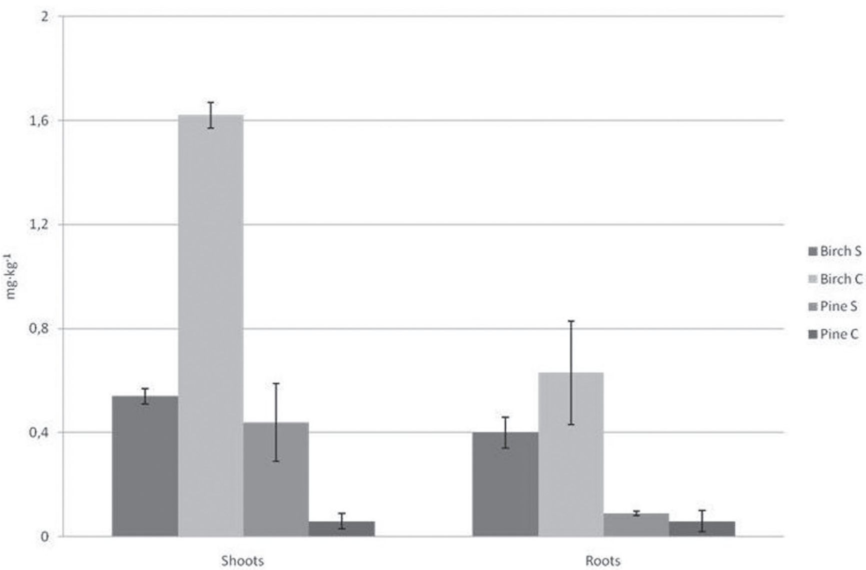

Fig. 4. Lead concentrations in roots and shoots of both tree species in the site amended with sewage sludge (Birch S; Pine S) and in the control site (Birch C; Pine C). Bars represent mean values of three samples ±1SD

In pine tree components, Pb concentrations were lower at Site C than at Site S. The concentration was 0.4±0.2 in shoots and 0.1±0.01 $mg \cdot kg^{-1}$ in roots at Site S, Pb concentrations were similar in shoots (0.06±0.03) and roots (0.06±0.04 $mg \cdot kg^{-1}$) at Site C. Lead concentrations in shoots are significantly different between investigation sites ($p<0.05$).

Tree biomass and growth

Total and different tree components dry mass

The total biomass of birch and pine trees was less at Site S than at Site C, but these differences were not significant ($p>0.05$) *(Table 4)*. Dry mass of different trees components were greater in the control site, but these differences are insignificant ($p>0.05$). However, pine root mass was significantly ($p<0.05$) less at Site S (16±2 g) than at Site C (30±1 g).

Table 5. The diameter (cm) at 30 cm height and stem height (cm) of birch and pine trees at the site amended with sewage sludge (S) and at the control site (C), mean, ±1SD, n = 3

Tree species	Diameter	Stem height
Birch C	1.2±0.2	188±0.7
Birch S	1.4±0.4	223±13
Pine C	2.6±0.5	168± 8
Pine S	3.0±0.3	190±19

Stem diameter and height

The diameter (at 30 cm height) of birch trees varied from 1.0–1.8 cm at Site C and from 1.0–1.4 cm at Site S *(Table 5)*. The diameter of pine tree varied from 2.7–3.4 cm at Site C and from 2.0–2.9 cm at Site S and no significant differences were detected between stem diameter mean values at both sites ($p>0.05$). The stem height of both tree species was insignificant ($p>0.05$) between both sites.

Table 4. Total dry mass and mass of different components of birch and pine tree in the site amended with sewage sludge (Birch S; Pine S) and in the control (Birch C; Pine C), g/tree; mean value ±1SD, n=3

Tree species	Leaves/ needles	Shoots	Stem	Roots	Total biomass
Pine S	111±26	122±18	458±41	16±2.0*	749±156
Pine C	175± 8	144± 7	528±84	30±1.0*	821±21
Birch S	14± 4	19± 7	53± 7	12±2.5	101±12
Birch C	19± 8	28± 3	70±18	24±2.0	112± 5

*difference significant, p <0.05.

Tree development traits

The root/shoot ratio of birch tree was significantly ($p<0.05$) larger at Site C *(Table 6).* The birch root biomass was even greater (1.78±0.07) at Site C than the mass of shoots. At Site S the ratio was also high (0.80±0.12), compared with the ratios of pine trees. These were 0.13±0.01 at Site S and 0.21±0.02 at Site C, but the difference was insignificant ($p>0.05$). The specific root length (SRL) of birch tree was significantly shorter at Site S than at Site C ($p<0.05$). In the case of pine trees, SRL was also significantly shorter at Site S than at Site C ($p<0.05$).

Table 6. Root/shoot ratio, Specific root length (SRL) ($m \cdot g^{-1}$), Shoots and roots max. length (cm) of birch (Betula pendula) and pine (Pinus sylvestris) tree at the site amended with sewage sludge (S) and at the control site (C), n=3 ±1SD

Tree species	Tree development traits			
	Root/shoot ratio	SRL	Shoot max	Root max
Birch C	1.78±0.07	0.06±0.01	47.1–77.1	20.4–81.0
Birch S	0.80±0.12	0.30±0.10	47.5–70.0	13.4–53.0
Pine C	0.21±0.02	0.19±0.04	47.5–73.5	22.0–47.5
Pine S	0.13±0.01	0.26±0.08	42.0–67.5	17.5–35.1

The maximum root and shoot lengths of birch and pine trees were longer at Site C than at Site S. The maximum lengths of pine tree seedlings roots and shoots were also less at Site S. The number (branching) of roots and shoots is an important factor that indicates environmental nutrient status *(Figure 5).* The branching of birch roots was significantly ($p<0.05$) less at Site C (11±1) than Site S (22±4). Shoot branching varied from 20±4 at Site C to 25±1 at Site S.

The branching of pine shoots and roots was not significantly greater for the pine trees at Site S. The number of roots was 23±9 at Site C and 23±3 at Site S ($p<0.05$). The number of shoots was: 27±1 and 31±6, respectively ($p<0.05$).

Discussion

More favourable growth conditions in soil amended with sewage sludge

Soil is important to plants as a source of nutrients and water and has an inherent potential to resist (stability) and recover (resilience) from environmental stresses (Griffiths, B.S. *et al.,* 2005). Plant growing conditions depend on many soil properties, including pH, texture, moisture and aeration. It is known that soil moisture, carbon content, exchangeable acidity and pH are good indicators of conditions for vegetation growth (ICP Forest Manual, 2006).

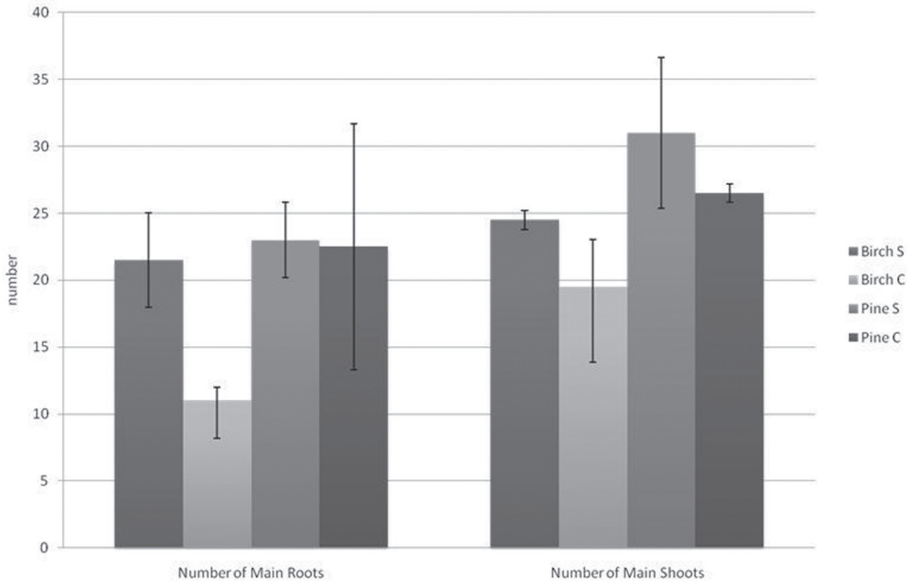

Fig. 5. Number of shoots and roots of both tree species in the site amended with sewage sludge (Birch S; Pine S) and in the control site (Birch C; Pine C). Bars represent mean values of three samples, ±1SD.0

Soil moisture influences the transportation of soil solutions through roots. Lower moisture content can also indicate suppression of the diffusion and the mass flow from soil to plants. Moisture content was higher in soil amended with sewage sludge. These results reveal better moisture capacity in the site amended with sewage sludge.

Changes in soil carbon influence physical properties (Denef, K. *et al.*, 2001) and due to higher soil carbon contents soils are physically more stable than non-amended soils (Sort, X. and Alcaniz, J.M. 1999). Soil carbon content was higher at Site S than at Site C. Carbon content of the upper layer was approximately equal at both sites, as it was the case with organic compounds too. However, in deeper layers carbon content was significantly greater at Site S.

More acidic soil conditions can increase Al^{+3} availability, which in turn can disturb the normal development of tree roots and minimize the uptake of macro-nutrients, such as Ca^{2+} or Mg^{2+} (Kupčinskienė, E. 2006). Low pH also increases the mobility of toxic HMs, which can be taken up more easily by plants (Kabata-Pendias, A. and Pendias, H. 2001). As soil in the control site is more acidic, it could inhibit the growth of tree seedling.

Exchangeable acidity (Al^{+3}, H^{+}) indicates soil disturbances due to high Al^{+3} concentrations which, as discussed previously, are toxic to plants and soil organisms (Sparks, D. 1995). Toxic effects of aluminium (acidic, pH <5.5) increase the thickness and stunt root fibres, leading to decreased assimilation of nutrients from the soil and slowing down plant development (Göransson, A. and Eldhuset, T.D. 1995; Bojarczuk, K. *et al.* 2002).

The results illustrate significant differences between the two investigated sites indicating 1.60–1.99 times higher pH values, 2.59 times more SOM in deeper soil layers and 1.9–13.5 times higher moisture content. Exchangeable acidity (Al^{+3}, H^+) was 23.7 times less in the upper and 16.35 times less in the lower layers of sewage sludge amended soil. This reveals that in the latter the conditions for the trees are better than in that of the control site.

Variation of Cu, Cd and Pb in soil

Our results reveal Pb is the least mobile heavy metal and Cd tends to eluviate to deeper soil. Furthermore, the mobile fraction of HMs is strongly related to soil pH, as it is one of the main factors influencing HM migration (Eckert, D. and Sims, J.T. 1995; Kabata-Pendias, A. and Pendias, H. 2001).

HMs in sewage sludge can inhibit tree biomass development. For example, only 0.005 mg·kg^{-1} Cd reduced spruce tree root elongation (Arduini, I. *et al.*, 1994) and 0.005 mg·kg^{-1} Cu in solution can reduce pine biomass (Arduini, I. *et al.*, 1998). In our study plant available Cd was 0.16 mg·kg^{-1} in soil amended with sewage sludge, which may have had a negative influence on pine root elongation, as 0.22 mg·kg^{-1} Cu have in pine biomass. Furthermore, possible synergistic effects should be considered (Arduini, I. *et al.* 1994).

The influence of Pb is hard to predict, because it is very stable in the soil and is probably only available in the very acidic soil of the control site. In addition, plant available Pb in the site amended with sewage sludge was ≤0.3 mg·kg^{-1}, which is much lower than the 18 mg·kg^{-1} that is known to inhibit tree growth (Breckle, S.W. and Kahle, H. 1991).

Variation of Cu, Cd and Pb in tree

Higher metal concentrations were determined in birch trees than in pine trees, which accords with the hypothesis that birch tends to extract more HMs from soil than pine (Eltrop, L. *et al.*, 1991; Kahle, H. 1993). However, there were minor differences between their concentration in trees from sites both C and S. Normal contents of Cd and Cu in plants are 0.1–1.0 and 1–10 mg·kg^{-1}, respectively (Kabata-Pendias, A. and Pendias, H. 2001; Kupčinskienė, E. 2006. Copper toxicity in plants may occur when the tissue concentration is >20–30 Cu mg·kg^{-1}. Decreased rates of plant growth occur at tissue concentrations of

>3 Cd mg·kg^{-1} (Pais, I., and Jones, J.B. Jr. 1997). In our study, the concentration in birch tree tissues at Site S was >5 Cd and >4.9 mg·kg^{-1} Cu and these concentration in pine tree were ~3.9 and 7.0 mg·kg^{-1}, respectively. In addition, no significant relationship between tree growth and HMs accumulation in tree tissues was observed. However, some tendencies were highlighted. For example, higher Cd and Cu concentrations were determined in shoots and roots in trees from Site S, these differences being particularly evident in the Cd content of birch roots and in the Cu content of birch shoots. In the case of pine trees, concentrations of both of these HMs were higher in shoots and roots, more significantly in roots. Copper and Cd are inhibitors of tree biomass development, especially tree root systems (Arduini, I. *et al.* 1994).

Tree biomass indications

Tree biomass was expected to be less in the control site considering positive influence of sewage sludge on soil properties (Pikka, J. 2005). Tree biomass is strongly related to root systems, because poor soil conditions (low pH and high exchangeable acidity (Al^{+3}, H^{+})) have a negative influence on the development of the root system (Kupčinskienė, E. 2006).

In our study, biomass did not significantly change by the application of sewage sludge with the exception of reduced pine root mass (by 50.0%) without significant change of root/shoot ratio, which is in contrast to a significant reduction of the latter in birch. Better root system development at Site C is associated with the relatively poor nutritional environment and this tendency is remarkable for birch trees (Påhlsson, A.B. 1991; Bojarczuk, K. *et al.* 2002; Gradeckas, A. *et al.* 1998; Katinas, V. *et al.* 2002; Pikka, J. 2005).

Tree growth traits

Specific root length (SRL) is an important indicator to determine carbon allocation into the root system and indicate a nutritious soil environment. In our study, the roots at Site C had greater mass density, as their SRL value was lower (Eissenstat, D.M. 1991), which indicates the decreased ability of plants to uptake nutrients (Hartikainen, H. *et al.* 2001). Higher SRL values indicate soils richer in nutrients (Ryser, P. 1996) and exhibits high hydraulic conductivity of roots (Eissenstat, D.M. 1997). The larger the SRL the more effective is the strategy of allocation of assimilates to the development of short roots (Lõhmus, K. *et al* 1989; Ostonen, I. *et al.* 1999; Wahl, S., and Ryser, P. 2000). However, the length and branching of roots and shoots of birch trees were greater at Site S than at Site C. In addition, roots at Site C tended to elongate more than branches, for example, the maximum length of birch root varied from 20–80 cm and at Site S comparative values were 13–54 cm. Moreover, infertile soils produce root systems with long, poorly branched surface roots; whereas, fertile soils produce well-branched roots that may penetrate deeper into the soil (Crow, P. 2005). In the case of pine trees the branching of roots and shoots was slightly longer at Site S, but their mean length was shorter than at Site C.

These differences among species can be explained by differences in root physiology, for example, the highest concentration of birch coarse roots accumulates in deeper layers (13–16 cm) than pine trees (5–15 cm) (Laitakari, E. 1934). In fertile soil, roots penetrate easier into deeper layers. The spread sewage sludge was contaminated with HMs, but the leaching of HMs into deeper layers may have been suppressed by organic matter from sewage sludge, the surface peat layer and the Fe-Mn geochemical barrier (Katinas, V. *et al.* 2002).

The production of longer, thinner roots may be an important mechanism to compensate for reduced carbon allocation to, and dry matter accumulation by, roots of trees exposed to pollution. These factors may also help explain differences of root/shoot ratio between sites. To adapt to less favourable conditions at Site C, trees had greater propensity to develop root systems at the expense of above-ground biomass. The roots at Site S, which have greater absorptive surfaces, could more easily transport nutrients and water to surface parts and expand above-ground biomass. However, the roots were thin and long, and carbon accumulation was less. This could lead to weaker root systems and increased risk of mechanical disturbance (e.g. by strong winds and floods). The production of longer, thinner roots may be an important mechanism to compensate for reduced carbon allocation to, and dry matter accumulation by, roots of trees exposed to pollution.

Site S had more favourable conditions for tree growth. However, plant biomass and tree growth trends (except root branching) did not support this tendency. This suggests that sewage sludge might have additional constituents (e.g. heavy metals) that inhibited tree development.

Conclusions

The results reveal that soil amended with sewage improves soil quality. However, higher concentrations of metals and no significant increase in the biomass of trees in soil amended with sewage sludge suggest an inhibitory effect of heavy metals on tree biomass growth.

During a 10 year period, pine trees produced 87% more biomass and accumulated ~60% more heavy metals (Cu, Cd and Pb) than birch trees.

For indication of soil nutritious environment it is recommended to use the following tree functional traits: 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) and tree biomass (dry mass).

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