Heavy metals in leaves to identify trees that intrude sewage and storm-water pipes

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14 1. Abstract

Tree roots and infrastructural pipes compete for belowground space and root intrusions to 15 sewer/storm water pipes are costly for the municipalities. Destructive identification of trees with 16 roots in pipes will reduce costs and increase wiliness to plant urban trees. We measured the 17 heavy-metal (silver, gold, cadmium, potassium, lead, palladium, rubidium, antimony and zinc) 18 19 concentration in leaves of 10 urban tree/shrub species. Heavy metal concentration is higher in the sewer/storm water than in soil. The concentrations of elements in trees are reflecting the 20 chemistry of the water taken up by. Hence trees using the content of the pipes will have higher 21 22 concentrations. 19 pairs of trees were chosen in Malmö, Sweden, one had been identified making 23 root-intrusions and the other was growing in soil. About 28% more silver was found in the leaves 24 of the trees that had entered the sewers than the control trees. There was a slight increase in 25 potassium levels in trees with roots in storm water pipes. We conclude that the silver concentration in leaves may be used to identify trees with roots intruding sewers. However, there 26 is a large difference between species and the method needs further development before practical 27 28 use.

29 Keywords

Leaf concentration, metal uptake, broadleaved trees, urban trees, root growth, technical
 infrastructure

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35 2. Introduction

The urban environment needs both a well-functioning wastewater system and vital urban vegetation. The occurrence of sewers is hardly questionable and the importance of trees is an emerging area. Recent research has shown that vegetation have positive effects on the human health (Björk et al. 2008) storm-water retention (Bartens et al. 2009) urban biodiversity (Kuhn et al. 2004), air quality and the local climate (Akbari and Konopacki 2004, Svensson and Eliasson 1997). The local temperature is reduced by the shade of trees and the evapotranspiration and act against the urban heat island effect (Akbari and Konopacki 2004).

Combining sewers and urban vegetation and especially trees, may cause problems and is a large
cost for the municipalities. Roots enter cracks in constructions, lift pavements and enter pipes.
Problems related to root intrusions, pipe cracks and backflow, are currently very costly for the
cities. Earlier studies have shown that in both 2003 and 2007 the cost in Sweden was 6 million
euro (Orvesten et al. 2003, Bäckman 2007), the cost is estimated to be 28,4 million euro year⁻¹ in
Germany (Bennerscheidt et al. 2009).

The reason for tree roots to enter waste water pipes is not established scientifically; we however 49 know that the growth environment for urban trees is harsh. We know that roots grow were the 50 soil strength is low, in-between soil layers and along surfaces (Kozlowski et al. 1999). Resent 51 results have shown that the ability of roots to avoid obstacles is due to self-repression of the roots 52 53 that are hindered (Falik et al. 2005). Unfortunately sewer pipes do not seem to be viewed as an obstacle for the roots, instead perhaps a source for water, air or nutrients. Sewer pipes would 54 have a constant supply of both and storm-water pipes would contain an atmosphere of fresh 55 oxygen. 56

Soil moisture is generally low in urban soils. This is mainly due to extensive drainage, high evaporation, low water holding capacity and more or less impermeable ground cover (Burghardt 1994), and the strong compaction of the soils. Apart from being used by the trees in the photosynthesis and for cooling, water is important for transport and solubilizing of nutrients to the roots. However, the nutrient variation in urban soils is very high (Pavao-Zuckerman 2008, Schleuß et al. 1998) with nutrient rich old soils mixed with nutrient poor young soils and rubble and root have a great advantage from growing root to nutrient rich patches.

Most urban soil is compacted both intentionally and unintentionally (Kozlowski 1999), a high bulk density reduces the capacity of the soil to store water and air (Archer and Smith 1972). It is well known that oxygen is absolutely significant for root growth (Bennett 1904) and hypoxia causes roots to die at a redox potential below 200 mV (Stepniewski et al. 1991). This is one factor that limits root growth in urban environments (Jim 1993). Many of these factors interact and make identification of the most decisive factor very difficult.

70 The research on root intrusions has so far mainly focused on technical solutions like 71 development and testing of the joints between the different pipe elements (Lu et al. 2000; Stål et 72 al. 2005; Whittle 2003), development of liner material that is used to mend pipes from the inside ⁸ and different ways to remove the roots once they are inside the pipes. The removal of roots in 73 pipes is done both mechanically and chemically. The repair of the pipes may be done both from 74 the inside and by excavating the pipes. We now know that no joints are able to completely resist 75 76 root growth (Ridgers et al. 2006, Stål et al. 2005). On the contrary, roots have been shown to 77 enter pipes through perfectly functioning intact joints (Ridgers et al. 2006; Stål 1998, Stål et al. 2005). According to Swedish standards a sewer pipe joint need to resist a pressure of 0.55 MPa 78 (Ridgers et al. 2006), however, an oak root may exert a radial pressure of 1.23 MPa 79

80 (Strechenbach et al. 2009). The pressure a root exerts on the environment is more than twice as81 high as the pressure sewer pipes are constructed to resist.

Efforts have been made to find a safe distance between trees and pipes. A distance of 7 m has been recommended when combining roots and pipes in urban environments. However, this distance has been shown to be vastly underestimated (Östberg et al. 2010, Ostberg et al. 2012). Tree roots are known to grow much further than this and now we believe that there is no secure distance to avoid root intrusions. Poplars and Salix species have been said to be more prone to make root intrusions than other tree species (Orvesten et al. 2003) but most tree species and some bushes are able to enter pipes (Ostberg et al. 2012).

As discussed previously there are frequent conflicts between the vegetation and the technical
infrastructure. This often leads to discussions about removing the trees, but it has been
impossible to positively identify the tree that is causing the root intrusion.

A method to identify the tree individual that has entered a pipe would make studies of causes for
root intrusions possible. This could lead to treatment of the trees in order to avoid root intrusions
or to construct a root hostile environment in the pipe trench.

The chemistry inside a plant is reflecting the chemistry of the water that is taken up by the plant. Consequently, the tissue from a tree that uses the content of a sewer or storm-water pipe will reflect the chemistry in the content of that pipe. Heavy metals are suitable candidates to analyse in plant tissue in order to monitor the concentrations in the soil or other growth media. The heavy metals in sewage and storm-water originate from the products that we produce and use and the content may vary to a large extent in different cities and in different areas of a city. If a root has entered a pipe the tree will take up the metals that are found in the pipes to a larger extent than

trees that have not entered pipes. The metal distribution in the pipes will therefore be reflected inthe tree.

104 One heavy metal that is present in sewage and is generally very rare in soils is silver. Silver is taken up by plants even though the uptake normally is very small (Ratte 1999, Krizkova et al. 105 2008) and may be toxic to poplar cuttings at 1 mg L^{-1} and to Arabidopsis at 0.05 mg L⁻¹ (Wang et 106 al. 2013). However found in low levels in soils silver is enriched in urban soils as compared to 107 rural soils due to historic use in photographic industry when waste was less purified (Andersson 108 and Ladenberger 2010). The use of silver has increased in recent years due to the use as a 109 disinfectant in for example sports-ware. The silver is then released during washing of the clothes 110 and in showers and transported to the sewage. Thus the levels in the sewers are higher than in the 111 112 soils even in urban areas, and there is a constant supply in the sewers as compared to the static levels in soils. In total this has as a result that the available silver levels are higher in sewers than 113 in urban soil. 114

Storm-water receives rare earth metals from the use of car catalysts. As the name suggests they are very rare and do only exist in soil in concentrations below or at the detection limit. The elements of the platinum group, rubidium, platinum, might be used to identify the individual trees that have entered storm-water pipes (Ek et al. 2004). Rare earth metals are not toxic and very stable. This makes them suitable to use for monitoring uptake from storm-water sources.

120 The aim of this study was to find a method to identify the tree individuals that have roots 121 intruding sewers and storm-water pipes. The hypothesis are; 1) leaves of trees that have their 122 roots in a sewer have a higher concentration of silver than trees that do not have entered sewers

and 2) individuals that have roots in the storm-water pipes have a higher concentration ofplatinum and rubidium.

125 3. Materials and methods

126 3.1 Site and tree selection

The study was performed in a paired design where the trees were chosen using a tree database of 127 the trees in Malmö, Sweden (55° 36' N, 13° 0' E). The Malmö tree database contains the location 128 of the trees and the tree species and is coupled to a map of the location of confirmed root 129 intrusions identified using internal filming of the pipes. The recording was done as a continuous 130 inspection of the pipe condition and is part of the municipalities' management regime. 19 131 detected root intrusions were chosen as the base of this study. Trees were considered to have 132 133 done root intrusions if they were located in close proximity of a confirmed root intrusion and were no other tree was located closer than 15 m and used in the study. A second tree or bush of 134 the same species and age growing under the same conditions were also selected to represent the 135 136 control in the pair (table 1). The control tree was always located having the sampled tree between itself and the point of the root intrusion. 137

Most individuals are of the species White willow (*Salix alba*) that is one of the most common tree/bush in Malmö. The size of the trees and bushes varies but they are all well-established growing on the site for more than 5 years. Both trees that have intruded sewers and storm water pipes are include in the study, 9 pairs coupled to sewers and 10 pairs coupled to storm water. Ten different species were included in the study; Swedish white beam (*Sorbus intermedia*), Horsechestnut (*Aesculus hippocastanum*), Ash (*Fraxinus excelsior*), Black poplar (*Populus* x *canadensis*), Bush rose (*Rosa* sp), Planes (*Platanus* × *hispanica*), Lilac (*Syringa vulgaris*), Sweet
cherry (*Prunus avium*), Rowan (*Sorbus aucuparia*), and White willow (*Salix alba*).

146 3.2 Site and tree information

147 All storm water pipes were made from concrete, but the sewer pipes were both concrete and148 PVC, commonly used in Sweden. One very old sewer pipe was made from clay.

The location of the sites and the ground cover was noted on site. The ground cover was either hard cover or grass. Most sites were situated in housing areas close to a school or a kinder garden, many were situated at a small biking path, and some were situated next to a road. The pipes were located at different depths from the surface, and were of different size.

153 3.3 Leaf collection and analysis

154 3.3.1 Pre sampling and leaf analysis

Initially an extended analysis of silver (Ag), gold (Au), cadmium (Cd), potassium (K), phosphorus (P), lead (Pb), palladium (Pd), platinum (Pt), rubidium (Rb), antimony (Sb), and zinc (Zn) concentration in the leaves was done in a few leaf samples from trees that had done root intrusions to select the elements to study further. All elements apart from platinum were possible to analyse in the leaves. Phosphorus, palladium, and antimony were either below the detection limit not considered interesting from the results of the initial analysis and were therefore removed from the study.

162 3.3.2 Main leaf sampling and analysis

Leaves were collected at the 23nd of September 2010, from the south facing sun-exposed part of the crown. In a few cases collection of leaves had to be from a slightly other direction but sunexposed leaves were always collected. The leaves were washed with distilled water on site to remove any deposits that might interfere with the analysis. The leaves were dried at 70°C for a minimum of three days until constant weight and stored awaiting analysis at 40°C. The leaf samples were dissolved in concentrated nitric acid in a microwave oven and then analysed for the elements chosen as a result of the initial analysis (ICP-MS, Perkin Elmer).

The concentration of the heavy metals silver, gold, cadmium, lead, rubidium and zinc together with the potassium concentration in the surface soil at 11 sites close to the trees that had been included in the study was provided from an earlier study (Andersson and Landenberger 2010). In this study the samples were collected at approximately 2-15 cm depth. The difference depending on the occurrence of a root zone in some of the sites and the soil was sampled below that zone. The samples were then extracted using nitric acid (7M) and analysed for total content of the elements using ICP-MS at the lab of SGU (Perkin Elmer).

177 3.3.3 Data treatment and statistics

The differences in elemental concentration between trees that had intruded both sewer and storm-water pipes and control trees were tested using t-test. The differences in elemental concentration between trees that had entered sewer pipes and storm water pipes were also tested using paired t-test. The relationship between heavy metal levels in the soil and in the leaves was tested using linear regression. Difference in heavy metal content of the leaves from trees growing in sewers and storm-water pipes and with grass or hardcover was analysed using t-test. All statistical analysis was done using SPSS 18.0, (IBM statistics).

- 185 4. Results
- 186 4.1 Intrusions in sewer pipes

The leaves from the trees that have entered the sewer-pipes in Malmö have a higher silver (Ag) concentration $(0.0091\pm0,0041 \ \mu g \ g^{-1} \ dry$ weight) than the leaves that originates from trees that most likely do not have their roots in the sewers $(0,0071\pm0.0030 \ \mu g \ g^{-1} \ dry$ weight, table 2). There is also more silver in the leaves from trees that are root intruding sewers than storm-water pipes $(0.0049\pm0.0013 \ \mu g \ g^{-1} \ dry$ weight, p= 0,036, t-test).

192 4.2 Intrusions in storm-water pipes

The elements that were analysed did not differ between the trees that had entered storm-water 193 pipes and their control (table 3). Neither has the ground cover any impact on the amount of 194 195 potassium that was taken up by the trees. (ANOVA, p=0.48, Kruskal-Wallis non-parametric test, p=0.32). However, there was more K, Cd and Zn in the trees that had entered the storm water 196 pipes than those entering the sewers (K; p=0.046, Cd; p=0.021, Zn; 0.032). This result was, 197 198 however, also found in the control trees, the levels of Cd was higher in the control trees close to the storm-water pipes than the trees close to the sewers, and there was an indication of 199 differences in the K and Zn levels (K; p=0.067, Cd; p=0.030, Zn; 0.056). The potassium level in 200 the control trees close to the sewers varied between 4.9 and 13.9 mg g^{-1} dry weight, in the sewer 201 intruding trees between 5.4 and 13.5 mg g⁻¹ dry weight, in the trees close to the storm-water 202 pipes 8.3 and 17.7 mg g⁻¹ dry weight, and in the storm-water intruding trees 8.3 and 20.8 mg g⁻¹ 203 dry weight. 204

4.3 Differences among tree species

The different tree species took up silver to different extent (table 3).The number of replicas is very low and should be taken into consideration. There is a relation between the soil content of silver and the silver concentration in the leaves if one tree is removed for the analysis (fig 1). In this case 53% of the variation in leaf silver concentration can be attributed to differences in the soil silver concentration. When the outlier is not removed only 14% of the variation in leaf silver concentration may be explained by the soil concentration.

5. Discussion

The higher silver concentration in the city trees indicates that the tree has reached the sewers. Thus this method may be used to identify trees that have done root-intrusions. Compared to other methods this is not destructive and may be done without damaging the trees and the pipes.

However, there is a difference between tree species in how much silver they take up. To make this method useful we would need to make a database on silver levels in different tree species growing in urban soils to compare the concentration in the potential root intruder with the levels in trees only growing in soil. Alternatively take additional samples from a reference tree in the same environment. These may be difficult to find as the absence of roots in the pipes is difficult to establish.

There is approximately 28% more silver in the leaves of the trees that had entered the sewers than the control trees. The difference is significant, however this should be compared to that there is 50% more silver in willow and chestnut than in planes. Few replicas of each species was analysed why these number should be considered with caution.

226 The silver levels in the leaves of the trees responsible for root intrusions is comparable to levels found in plants growing on soils that have been fertilized with sewer sludge. The silver 227 concentration in Salix growing in sludge is 0.012-0.014 µg g⁻¹dry mass (Hasselgren 2008) as 228 229 compared to 0.007 and 0.009 μ g/g dry weights in the urban trees of this study. In the sludge fertilised soils were the willow grew the soil silver concentration was 10 times higher than in the 230 leaves 0.10 µg g⁻¹dry weight soil in the top 30 cm and 0.06 down to 60 cm depth. In beech 231 growing in a forest soil not far from where the current study was conducted the concentration of 232 silver in the leaves varied across the season from 0.019 μ g g⁻¹ early and late in the season to 233 0.008 µg g⁻¹ in late August calculated from Tyler and Olsson (2006). In *Picea abies* and *Abies* 234 sibirica growing in unfertilized forests the levels are similar or even slightly higher than in this 235 study, the needles contain 0.01 μ g g⁻¹ dry weight (Silkina and Vinokurova 2008) and in *Abies* 236 *fabri* from acid soils in a altitudinal gradient the leaf concentration was 0.015 μ g g⁻¹ (Sun et al 237 2011) indicating that soil pH is an important factor for the Ag uptake. Hyper accumulating native 238 Turkish trees, Euphorbia macroclada, Verbascum cheiranthifolium Boiss and Astragalus 239 gummife, has a higher twig and root concentration, up to 0.5 μ g g⁻¹ biomass (Sagiroglu et al 240 2006). 241

The soil in urban Malmö contains a higher variation in silver than the rural surrounding (Andersson and Landerberger 2010). The variation of silver in the surface soil in Malmö is between 0.04 to 0.17 mg kg⁻¹ soil with extreme values 0f 0.33 and 0.56 mg kg⁻¹ soil. This variation interferes with the analysis and must be considered. This large variation is probably due to a patchy pollution pattern and perhaps also to a high variation in pH. Analysis of the soil in combination with the leaf sampling would be very important to compensate for the silver content in the soil. However this interference from the soil may be compensated for by also sampling one tree that have no contact with a pipe but still grows in a similar soil as the tree that is investigated and study the difference between the two. The silver concentrations in the soil may vary significantly within a city and the uptake is related to this concentration. An alternative is to take a soil sample at the same time as the leaves and compensate for the uptake from the soil. To conclude this method needs to be developed further before it can be used in practice. The longevity of the success is also uncertain as the use of silver might be restricted in the future.

Trees intruding storm-water pipes do not seem to be possible to identify by their platinum and rubidium leaf concentrations. The levels taken up seem to be very low and variable. Rubidium is taken up in the same way as potassium and therefor used as a marker for studying potassium uptake.

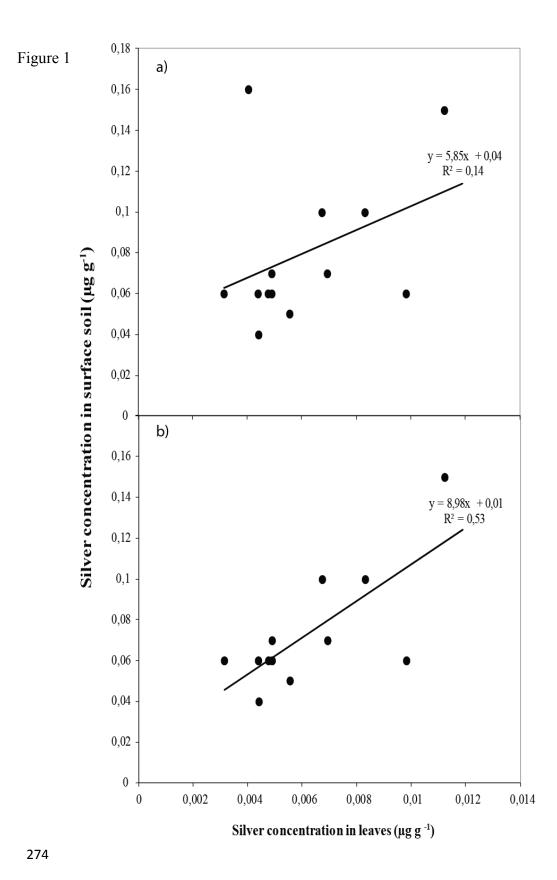
The potassium level in the trees was higher when the trees with access to storm-water. One could expect that the potassium that is a very mobile element in soil and that is taken up passively by trees would be more available if the tree had a continuous supply of storm-water. This would indicate that rubidium would be possible to use as a marker element to study root intrusion into pipes. The potassium levels in the leaves found in some trees are below optimum (Bergman 1988) indicating potassium deficiency. This lower potassium level could be a result of a stronger growth in the trees doing root intrusions leading to a dilution of potassium in the tissue.

To conclude the method to analyse silver levels in leafs to identify trees that have entered sewer pipes may be used, however with care. There are differences among tree species in how they take up silver and the levels in the soil may interfere with the results. Thus analysing the leaves in a

- 270 pairwise way and complementing with analysis of silver in the soil would be a way to overcome
- these problems.

272

273 FIGURE





Tables 275

276 Table1.

			Stem circumference		Dina	
			circumterence	Surface	Pipe	Pipe
Site	Species	Location	(cm)	cover	type	material
					Storm	
Brodda park	White willow	Park	30	Grass	water Storm	Cnc
Madrialgången	Rowan	Biking path	10	Grass	water	Cnc
			50	<u> </u>	Storm	
Nottettens förskola	White willow	Pre-school	50	Grass	water Storm	Cnc
Ärtholm/pildam	Poplar	Next to road	40	Grass	water	Cnc
	· ·				Storm	
Kyrkrosvägen	Horse chestnut	Biking path	17	Grass	water	Cnc
	\ A /l=:+=; =*	Park, Biking	20	Create	Storm	Care
Bellevue allé	White willow *	path	20	Grass	water Storm	Cnc
Bellevue skola	London plane	Pre-school	40	Grass	water	Cnc
					Storm	
Agnesfridsvägen	White willow	Next to road	25	Hard cover	water	Cnc
/			25		Storm	Care
Kantyxegatan	White willow	Next to road	35	Hard cover	water Storm	Cnc
Högaholmsförskola	White willow	Pre-school	Bush	Grass	water	Cnc
Bergaskolan	London plane	School yard	50	Hard cover	Sewer	PVC
Bergaskolan	Lilac	Bike parking	Bush	Grass	Sewer	Cnc
		Biking path /				
Husie Kyrkoväg	White willow *	School yard	40	Grass	Sewer	Cnc
Annelundsskolan	White willow	Pre-school	30	Grass	Sewer	Cnc
Rosengårdsskolan	Roses	School yard	Bush	Hard cover	Sewer	Cnc
-inneskolan	Sweet cherry Swedish white	School yard	20	Hard cover	Sewer	Clay
Malmö Högskola	beam	Next to road	15-25	Hard cover	Sewer	Cnc
olkparken	Horse chestnut	Park	35	Grass	Sewer	PVC
Slottsstaden	Ash	School yard	25	Hard cover	Sewer	Cnc

					Sewer vs. Storm water	
Sewer	Control	Intruding	Paired difference	p-value	Control	p-value
Silver	0.0071±0.003	0.0091±0.004	0.0020	<0.014	-0.008	0.62 ns
Gold	0.033±0.035	0.024±0.017	-0.009	0.21 ns	0.013	0.26 ns
Cadmium	0.26±0.47	0.20±0.38	-0.06	0.26 ns	-0.95	0.03
Lead	0.019±0.018	0.015±0.012	-0.004	0.39 ns	-0.95	0.49 ns
Rubidium	6.1±5.2	5.9±3.9	-0.20	0.84 ns	-0.99	0.78 ns
Zinc	47.39±56.8	33.5±25.6	-13.85	0.38 ns	-79.8	0.056
Potassium (mg g⁻¹)	9.8±3.4	10.1±3.5	0.3	0.55 ns	-3.3	0.067
Storm water					Intruding	
Silver	0.0085±0.0087	0.0049±0.001	-0.003	0.66 ns	0.004	0.04
Gold	0.020±0.015	0.016±0.010	-0.002	0.49 ns	0.007	0.24 ns
Cadmium	1.21±0.95	1.10±0.88	-0.094	0.24 ns	-0.91	0.02
Lead	0.06±0.17	0.11±0.17	0.042	0.09 ns	-0.09	0.13 ns
Rubidium	7.09±3.2	7.74±3.80	0.567	0.52 ns	-1.83	0.47 ns
		159.4±137.9	28.3	0.29 ns	-125	0.03
Zinc	127.2±84.7	133.4±137.5				

Table 3. Mean silver concentration in different species of trees growing in urban areas in the City ofMalmö, Sweden.

Species	Number of	Silver concentration	
	individuals	(µg g ⁻¹)	
Swedish white-beam	2	0.014	
Horse-chestnut	4	0.010	
Ash	2	0.009	
Willow	16	0.006	
Sweet cherry	2	0.006	
Lilac	2	0.006	
Rowen	2	0.005	
Poplar	2	0.005	
Rosa	2	0.004	
Plane	4	0.004	

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377 Figure captions and table legends

Table 1. Description of the trees and their surrounding environment that are include in the study. The site, species, location, size at sampling, (circumference at 1 m), surface cover, pipe type, and pipe material (Cnc - concrete, PVC- polyvinylchloride, Clay) are listed.

381

Table 2. Mean metal concentration ($\mu g g^{-1}$) in leaves form urban trees that had made root intrusions in sewers or storm water pipes and control trees of the same species and age. The mean difference between intruding and control trees, between trees intruding sewers and storm water pipes and the p-values (paired t-test).

386

Table 3. Mean silver concentration in leaves of different tree species growing in urban areas in
the City of Malmö, Sweden.

389

Figure 1. Relationship between the silver concentration in leaves for woody species that have grown in urban areas in Malmö and the silver concentration in the surface soil in the close vicinity of the sites were the trees are growing (a). The same relationship when one outlier was removed from the analysis (b).

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