

Seed germination and seedling growth of Scots pine in technogenically polluted soils as container media

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Abstract Reforestation of technologically polluted areas has become an increasingly important issue. In this study, seed germination capacity and survival rate and morphometric characteristics of Scots pine (*Pinus sylvestris* L.) seedlings grown in a magnesite-polluted soil medium were investigated in a pot experiment. Significant differences in seed field germination, sprout survival, seedling length at various growth stages, and root collar diameter of the seedling were discovered between the trial variants for the pot trial using growing media from the polluted areas and the control site. In addition, it was observed that the differences between the trial variants depended on seed origin and the level of soil pollution. The data indicate that seed germination and seedling growth were significantly reduced as the levels of pollution increased. These negative effects of the pollution tend to increase as the seedling gets older.

Keywords *Pinus sylvestris* · Technogenic · Emission · Seedlings · Morphometric

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Introduction

Mining activities adversely affect seed germination and seedling growths in their vicinities by polluting the air and soil (Menshikov et al. 1987; Agrawal and Agrawal 1989; Chibrik et al. 2012; Minkina et al. 2013; Vodyanitsky et al. 2016). Technogenically polluted soils are widespread in Russia due to lack of emission control produced by cement and magnesite production, coal incineration, power plants, etc. (Menshikov 1985; Kizilshtein and Levchenko 2003; Gluschenko and Olkhovskaya 2014). A significant increase in the pH of the soil, from a neutral and weakly acid environment within 20.0–25.0 km of the plant to a strongly alkaline environment within 1.0–3.0 km, was observed in snow and soil samples taken within the Satka region. In addition, an increase in the concentration of magnesium compounds, up to 3277–5359 mg/m², which is 28–45 times higher than that in the control area, has been reported for the territory closest to the plant (Menshikov et al. 2012). More than 50,000 ha of forestland has been subjected to the long-term negative impacts of magnesite emissions from the nearby production plant. Both Scots pine (*Pinus sylvestris* L.) and broadleaf forests have been completely killed in the area that surrounds the plant, whereas in the remainder of the territory, trees are in various stages of digression (Menshikov et al. 1987). However, effects of this technogenic pollution on soil and forest ecosystems are not fully investigated. These degraded lands can be converted to a sustainable forest ecosystem by establishing the vegetation cover. Thus, exploring if species

do well in these harsh environments is one of the main challenges of the foresters.

Researchers in this area have presented a variety of opinions on the importance of (i) planting media for pot trials and (ii) providing conditions under which coniferous plants produce and develop seedlings. Some authors consider the medium conditions to be a determining factor in seedling emergence, sprouting, and development (Mityakova 2012; Yerkoeva et al. 2012), whereas others believe that the conditions under which the seeds form are of greater importance (Bender et al. 2012).

Previous studies have been conducted on magnesite pollution (Zavyalov and Menshikov 2009), elevated phytomass (Zavyalov and Menshikov 2010), the morphological and chemical composition of leaves of pilot cultures (*Betula pendula* Roth) (Zavyalov 2013), reproduction (Mohnachev et al. 2013; Mohnachev 2014), and the quality of seeds and seed posterities of pilot cultures (*Pinus sylvestris* L.) (Makhniova et al. 2013; Makhniova and Mohnachev 2014). The aim of the current research is to evaluate the performance of Scots pine in terms of seed formation and germination and seedling growth in a pot trial using soil loaded with different levels of magnesite.

Material and methods

Research area

The research area is located in the western part of the Trans-Urals plain near the town of Satka, Chelyabinsk Region, Russia (55° 04' N, 59° 03' E). The area is located in the central sub-band of the southern boreal forests of the South Urals, and main tree species consist of birch (*Betula*) and Scots pine (*Pinus sylvestris* L.) (Kolesnikov 1969). The soils in the Chelyabinsk area are highly polluted due to magnesite mining (Zavyalov and Menshikov 2009). The climate of the research area is moderately continental with cold, long-lasting winters and short, warm summers. In winter, the area is influenced by arctic cyclones, carrying cold air masses, and a Siberian anticyclone, causing steady warming (Anonymous 1962). In summer, the area is typically under a depression. Snow cover typically averages 165 days per year (Anonymous 1962). The average January temperature is -17°C , whereas that of July is $+19^{\circ}\text{C}$. The average yearly temperature is $0.1\text{--}1.5^{\circ}\text{C}$, and the mean annual rainfall is $300\text{--}450$ mm

(Kolesnikov et al. 1973). The soils of the research area are predominantly gray forest soils with varying degrees of podsolization and humus content, together with soddy-podzolic soils (Anonymous 1964).

Experiment

Three Scots pines standing at ages between 35 and 38 years were designated as sampling areas. The stands were planted by the Botanical Garden of Urals Branch of Russian Academy of Science (UB RAS) between 1980 and 1983 using 2 + 0 bare rooted seedlings (Menshikov 1985; Srodnykh and Menshchikov 1992). The areas where stands were located have been receiving different levels of pollution from nearby magnesite processing plants in the Chelyabinsk region. The first sampling (SA-2) is located 1.0 km away from the pollution source, and it is the most heavily polluted site; the second stand (SA-5) is located 3.0 km away from the pollution source, and it is considered a moderately polluted site; and the third site (SA-4) is about 10.0 km away from the magnesite processing plant, and this site is considered the least polluted site (Fig. 1, Table 1). Another stand at the same age growing in the urban Suleya region 20.0 km away from the other sites was designated as control stand (SA-P).

Only a few trees were left surviving in the SA-2 stand at age of 35 years (Fig. 2a), but the level of pollution damage was low in SA-5 and SA-4 stands (Fig. 2b).

Sampling

Between 12 and 25 trees in similar light and crown condition on each site were randomly chosen to sample cones. The cones from the upper and middle parts of the crown were collected prior to their dehiscence in March. The sampled cones were air-dried in laboratory for several weeks. After loosening the carpel, the seeds were removed, separated from the wings, and 1000 seed weights were measured (g). The seed samples were air dried and kept in cold storage for between 6 and 8 weeks at 4°C until the viability test was performed and then they were sown. Completely randomized design (CRD) was used for the experiment. One thousand eighty seeds coming from each sampling area were planted in $55 \times 25 \times 15$ -cm-sized pots filled with sieved soil (<2 mm) from the A1 horizon of the same stand. The seeds were sown uniformly at a depth of $0.5\text{--}0.7$ cm. Following sowing, the seeds were covered manually.

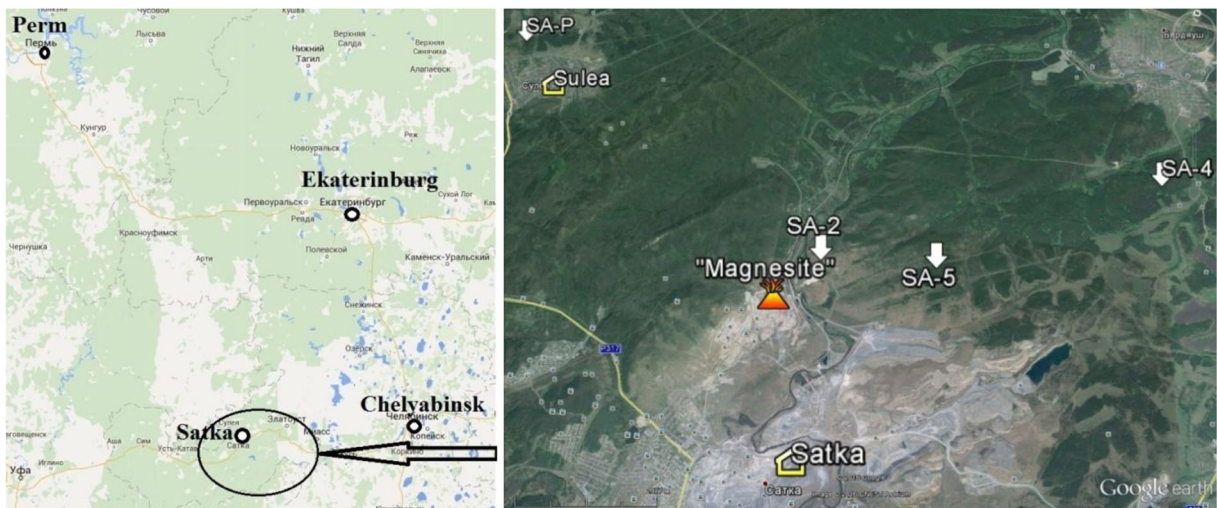


Fig. 1 Research area (left) and locations of sample areas (right)

The emergence rates of seedling (%) on each container were recorded beginning at the 15th day until the 105th day after sowing. The root and hypocotyl lengths of seedlings and the number of sprout cotyledons were measured. The seedling survival rate (%) was calculated as the ratio of surviving seedlings out of emerged ones.

Statistical analysis

The emergence rate, root and hypocotyl lengths of seedlings, the number of sprout cotyledons, and the seedling survival rate were tested with an analysis of variance (ANOVA) procedure for a completely randomized design. STATISTICA was used for all statistical analyses (TIBCO Software Inc., Palo Alto, CA, USA). Results for ANOVA were considered significant at $p < 0.05$.

Results

Seed weight

Thousand seed weights of samples collected from the SA-2 stand were about 12, 20, and 30% lower than those of SA-5, SA-4, and SA-P sites, respectively ($p < 0.05$) (Fig. 3).

Analysis of the viability test indicates that seeds had between 85 and 93% germination rates (Fig. 4). The decrease in the index was correlated with a decrease in the pollution levels from SA-2 to SA-4; however, the differences among all the tree stands were not significant. It is worth noting that seeds from the highly polluted SAs had a consistently lower mass index and higher germination rate than those from other SAs.

In addition, measurements of seedling linear size (root and hypocotyl lengths) showed little distinction

Table 1 Soil pH in the different magnesite contamination zones in the upper (0–10 cm) soil layer in different years (data are from Menshikov et al. (2016))

Severity of pollution	Distance from the emission source (km)	Soil type	pH by year				
			1983	1990	2005	2009	2010
High (SA-2)	1	Gray soils, light loamy	8,9	8.2	8.9	8.8	8.9
Moderate (SA-5)	3	Gray soils, medium loamy	8.8	8.0	8.5	8.0	8.3
Low (SA-4)	10	Gray soils, light loamy	7.7	7.0	7.6	7.4	7.3

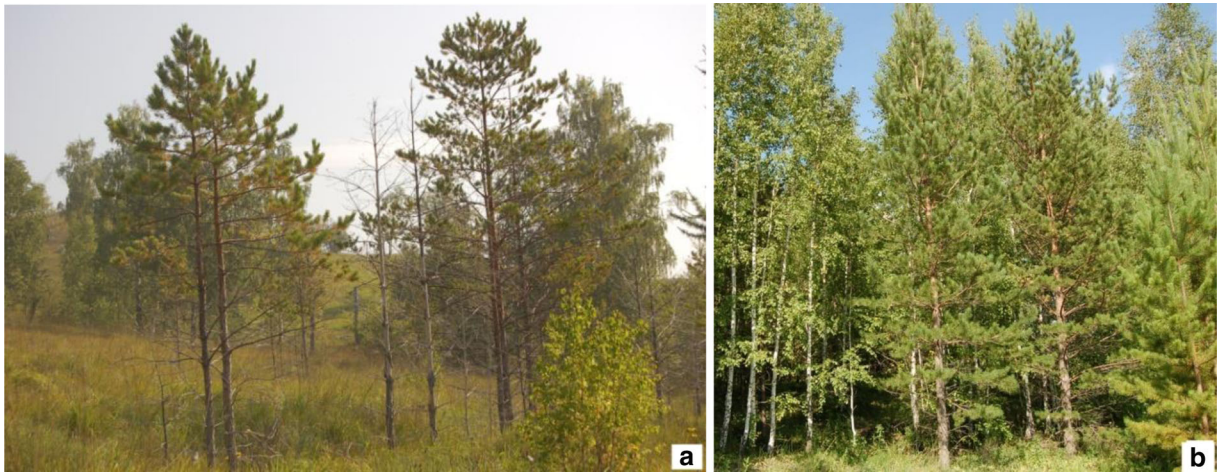


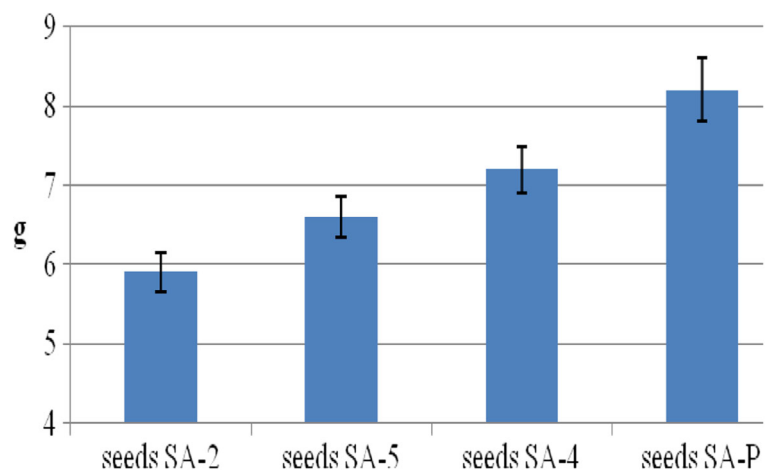
Fig. 2 Sample areas in zones of high (a) and low (b) levels of contamination

between seeds that formed under the different levels of technogenic pollution (Fig. 5).

Number of cotyledons

The number of sprout cotyledons is used as an index for determining genetic differences due to different tree genotypes and ecological conditions. It was found that the number of sprout cotyledons varied from four to nine depending on the SA. The seeds from the background conditions frequently formed sprouts with more than six seed lobes, which is a significant increase over those of the other tree stands ($p < 0.05$) (Fig. 6). The tendency for the number of cotyledons to increase was correlated with the increase in pine seed mass ($r = 0.70$).

Fig. 3 Thousand seed weights of 35-year-old Scots pine plantation trees located in the Chelyabinsk region, Russia

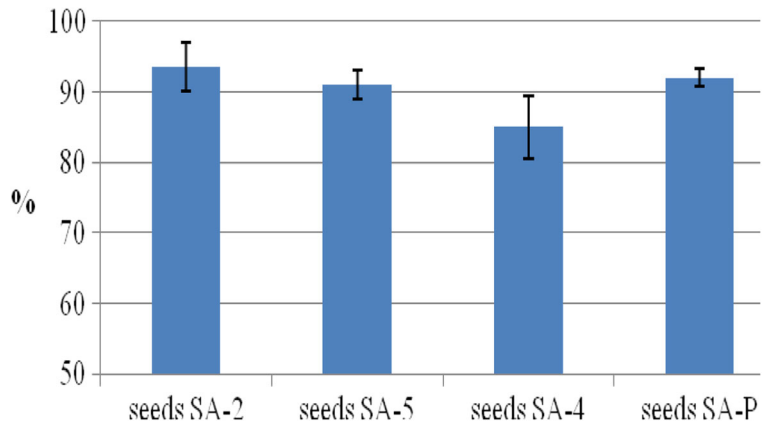


The laboratory study showed that seeds formed under control conditions (SA-P) weighed more and had more cotyledons than those formed under higher levels of technogenic pollution. However, we did not find differences in the sprout germinating capacity and morphometric characteristics between the seed genitures formed under the various levels of technogenic pollution and those formed under control conditions.

Seed germination and sprout/seedling survival rates in the pot trial

In the pot trials, it was found that the most favorable conditions for Scots pine seed germination were provided by the control potting media (Fig. 7). The longest period of seed germination (up to 105.0 days) was

Fig. 4 Seed germination in the laboratory



observed for this planting media, as were the smallest differences in germination index for the seeds from the different sources (63.0–74.0%).

The germination of seeds from sites SA-P and SA-4 was significantly lower in soils from zones of moderate and high levels of contamination than it was in soil from background conditions ($p < 0.05$). The germination of seeds from sites SA-2 and SA-5 in soil with a moderate level of pollution was 1.2–2.3 times higher than that for the same seeds in soil with a high level of pollution (Fig. 7).

Our results show that the potting medium from the area with high technogenic pollution had a negative impact on the germination of seeds from all tree stands (Fig. 7). The differences in the germination index were significant ($p < 0.05$). Excluding the seeds from SA-P, the potting media from both the highly and moderately polluted areas were equally unfavorable for seed germination. It was also found that SA-4 seed germination was inversely correlated with the level of technogenic pollution in the potting medium, i.e., the higher the pollution level, the lower the germination index (Fig. 7).

There were high levels of individual variability in the germination index, from 20.3 to 28.8% in all tree stands at 105.0 days. Therefore, it is evident that germination of the pine seeds in the field under different conditions depended on both the conditions of the planting medium and the origin of the seeds. This is especially true of the conditions for seed formation and the genetic characteristics of the trees.

Seedling survival rate

The data from Fig. 8 enabled us to evaluate the influence of the different potting media and seed origin on seedling survival. Based on the survival data, it was found that the potting medium from SA-P provided the most favorable conditions for the seedlings from all tree stands.

Unfavorable seedling survival conditions were provided by the potting media from the areas with high and moderate levels of technogenic pollution. We found that these media produced the lowest seedling survival rates over the course of the trial, with a 20.0–30.0% decrease for seedlings from all stands.

Fig. 5 Root and hypocotyl lengths of seedlings

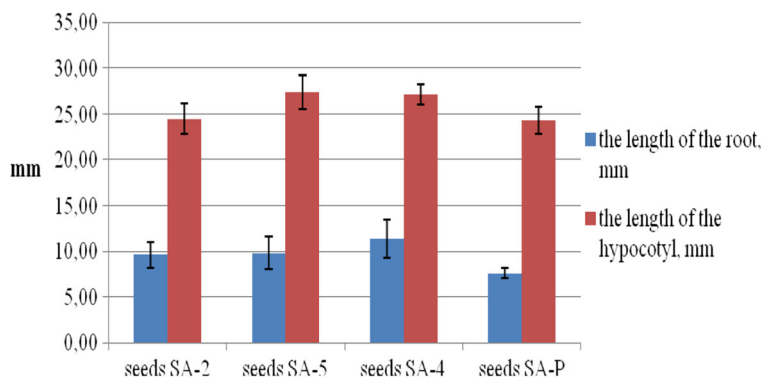
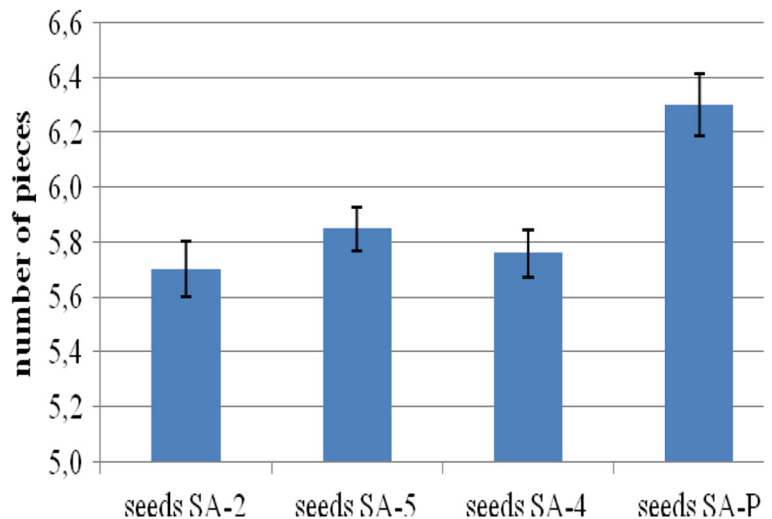


Fig. 6 Number of cotyledons

Seedling growth and development

We observed seedling development in the greenhouse conditions over two vegetative seasons (Fig. 9). During the first season, all seedlings were phenotypically homogeneous in height and in terms of passage through the development stages (germination, hypocotyl growth, cotyledon expansion, development and growth of epicotyl and juvenile needles, growth/height, and apical bud formation) (Fig. 9a–c).

By the 105th day of observation, the greatest number of seedlings was at the stage of growing epicotyls, as well as developing and growing cotyledons (Fig. 9c). Paired secondary needles appeared on the lower part of the shoots of some seedlings.

During the second vegetative season (Figs. 9d, e), various stages of individual seedling development and as a consequence, a variety of phenotypes, were observed. By the end of the second vegetative season (~470.0 days), a typical seedling was lignified in the lower part of the stem (hypocotyl); it also exhibited dry

juvenile needles, secondary needles in the upper part of the stem or in the upper and lower parts of the stem, and a whorl at the top. In some seedlings, one bud from the whorl had developed as a new sprout. The seedlings from seeds that germinated late in the first vegetative season did not achieve typical conditions by fall, and they had only juvenile needles and a lengthening hypocotyl or occasional secondary needles in the lower part of the hypocotyl.

It was found that seedling hypocotyl length was dependent on the conditions of the planting medium (Fig. 10). The seed genitures from all tree stands can be described as a linear relationship as follows: as the level of the technogenic pollution of the planting media decreased, the index increased. Moreover, the differences between the experimental variants were significant ($p < 0.05$).

Figures 11 and 12 show the seedling height index and its dependence on the planting medium at the end of the first and second vegetative periods, respectively. The diversity of seedling height for seeds from the same tree

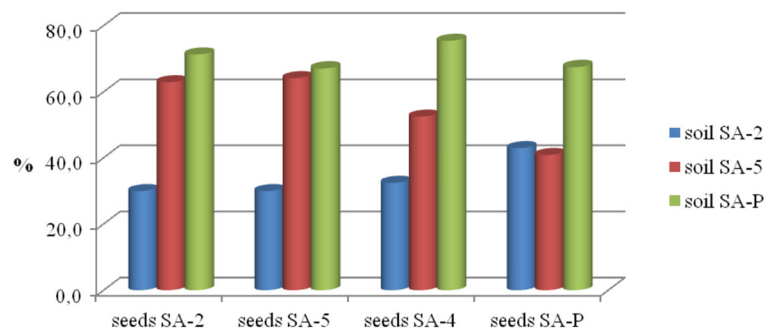
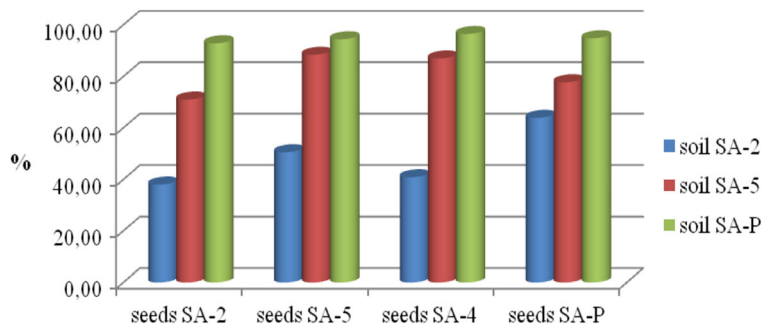
Fig. 7 Seed germination rates in pot trials

Fig. 8 Seedling survival rates



stand but under different planting media conditions varies by 1.5 to 2.0 times. The differences between the experimental variants (background conditions vs. technogenic pollution) were significant ($p < 0.05$).

As shown in Fig. 13, seedling root collar diameters were significantly different (by 1.4 to 1.9 times) for the different planting media conditions. The highest diameter index values at the root neck were detected for seeds from all stands in the experiment using the SA-P planting medium, whereas the lowest values were for the experiment using soil from the areas with high levels of technogenic pollution.

The dependence between the index of seedling root collar diameter and the level of technogenic pollution of the soil in the vegetative experiment could be described by linear functions, which showed high determination coefficients:

$$SA-2 : y = 0.300x + 0.770, R^2 = 0.750$$

$$SA-5 : y = 0.415x + 0.560, R^2 = 0.979$$

$$SA-4 : y = 0.245x + 0.843, R^2 = 0.952$$

$$SA-P : y = 0.235x + 1.007, R^2 = 0.982$$

Overall, the morphometric characteristics of the seedlings in the vegetative experiment were observed to be dependent on the experimental soil conditions. As a rule, higher index values were observed in seedlings planted in the control planting medium, whereas the lowest index values were identified for those areas with high pollution levels. In addition, we found that the seedlings from different SAs were diverse in their morphometric characteristics under homogeneous planting media conditions. Therefore, morphological differences are most likely determined by genetic characteristics and seed formation conditions.

Discussion

Significant differences in seed field germination, sprout survival, seedling length at various growth stages, and diameter of the seedling root collar were discovered between the trial variants for the pot trial using growing media from the polluted areas and the control site. It was observed that the differences between the trial variants depended on seed origin and the level of soil pollution.

The results of the study show that seeds formed under control conditions (SA-P) weighed more and had more

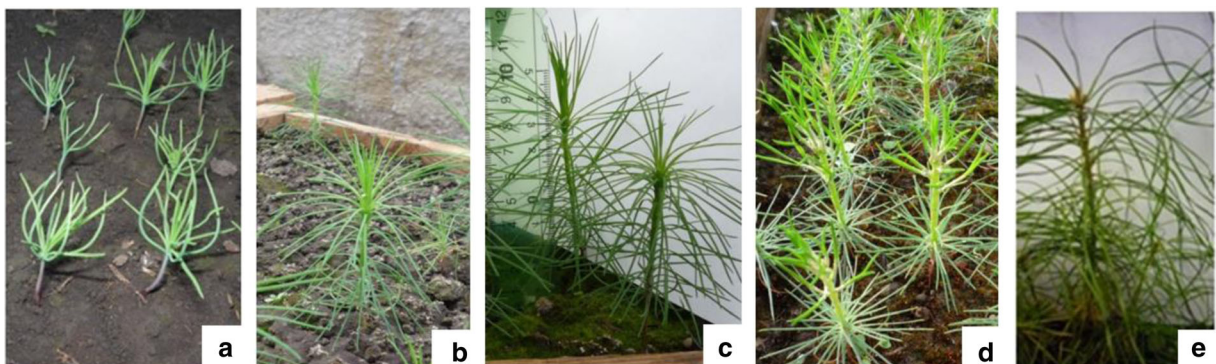
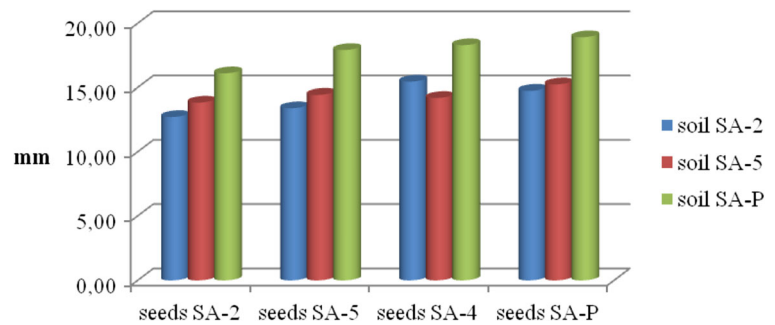


Fig. 9 Pine sprouts and seedling development in the pot trial

Fig. 10 Seedling hypocotyl length

seed lobes than those formed under higher levels of technogenic pollution caused by magnesite production emissions.

When planted under favorable conditions (growing media variant SA-P), pine seeds from the areas of technogenic pollution showed approximately the same levels of field germination and sprout survival as seeds from SA-P. However, the levels of seed sprout length and seedling length for SA-P were significantly greater than those for the areas of technogenic pollution. It is worth noting that the SA-P seeds were also characterized by higher levels of the studied seed/seedling characteristics than those for the strongly polluted growing medium SA-2. Thus, SA-P seedling survival in growing medium SA-2 was greater than both SA-2 and SA-5 seedling survival in the same medium by 1.2 to 1.7 times. By the end of the first growing season, SA-P seedling length in growing medium SA-2 was 1.3 times higher, and by the end of the second growing season it was 1.2 to 1.3 times higher. At the same time, the diameter of the collar root was 1.2 to 1.4 times greater in comparison with that of seeds from the other tree stands. Meanwhile, in comparison with the SA-P growing media, significant decreases in field germination for growing media SA-2 and SA-5 were seen for SA-P seeds. Based on the levels of the studied characteristics, seeds from SA-4 were frequently found to fall between

the SA-P seeds and the seeds from areas with high and medium technogenic pollution levels.

The seedlings for the stands growing under control conditions or in areas with weak technogenic pollution were found to be more susceptible to technogenic pollution in the planting medium than were seedlings for the areas with strong and medium technogenic pollution.

By contrast, SA-2 and SA-5 seeds had relatively high germination levels in growing media SA-5; indeed, they were almost equal to their germination levels in the growing medium from the control site. The most unfavorable conditions for seed germination, sprout growth, and seedling growth were found for SA-2. The differences from the other trial variants were revealed only at the stages of seed germination and seedling formation. The differences in germination levels of seeds from the same origin in growing media SA-P and SA-2 were 1.6 to 2.4 times, and by the end of the trial from 2.3 to 2.4 times in the meanings of seedling length. So a negative value of the «technogenic planting media pollution» factor for the development of Scots pine seedlings significantly decreases in the scale «strong–medium–weak pollution».

Therefore, Scots pine seed germination was sensitive to growing media conditions, i.e., high pH levels due to magnesite pollution. Other results were obtained for

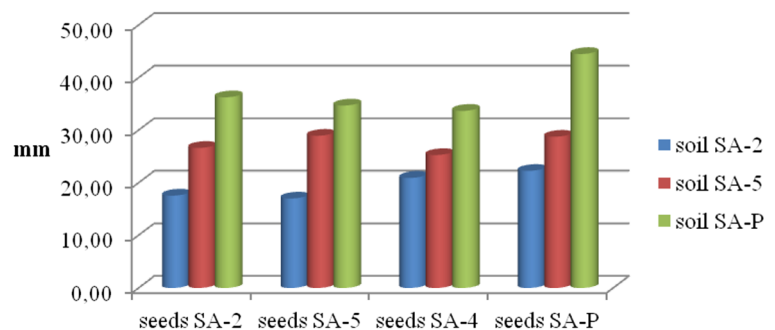
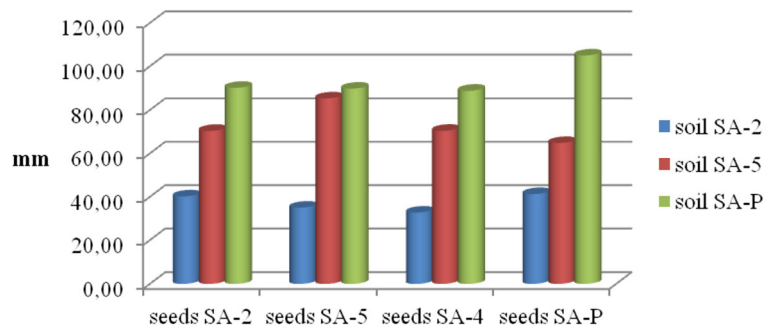
Fig. 11 Seedling height index at the end of the first vegetative period

Fig. 12 Seedling height index at the end of the second vegetative period



laboratory and pot trials using solutions and growing media polluted with heavy metals (Lapiro and Lebedeva 2009; Ivanov et al. 2013). These authors noted an absence of the influence of pollution on seed sprouting and germination. A possible reason provided by the authors was the sustainability of the upswelling and piping period to the heavy metals. We also found a negative influence of magnesite pollution in the growing media at the later stages (post germination) of sprout and seedling growth in all trial variants. In addition, the extent of the differences between the trial variants (SA-P vs. polluted growing media) increased with the age of the seedlings. Thus, the difference in the sprout hypocotyl lengths of the seeds from the different site was 1.3 times between SA-P and SA-2; the differences found in the 2-year-old seedlings between these areas were between 2.3 and 2.4 times. A negative effect of heavy metal salts on plant sprout growth was noted by Lapiro and Lebedeva (2009) and Ivanov et al. (2013).

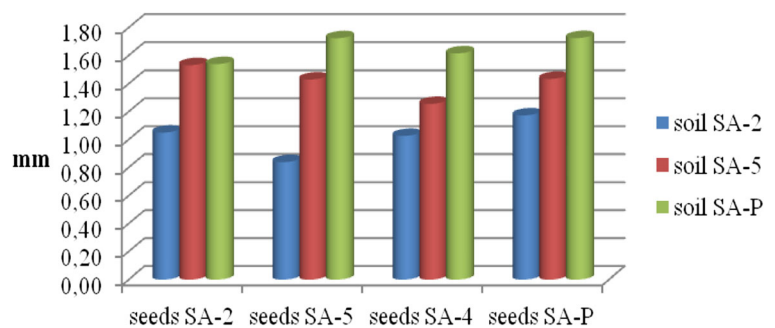
According to literature data, the maternal tree habitat greatly influences seed quality and seed reproduction. A negative effect on generative system development could be caused by (i) overwetting and drought (Pimenov et al. 2014; Velisevich 2017), (ii) weather conditions in years when the generative structures were initiated and developed (Nekrasova 1983; Pukkala et al. 2010), (iii) the sum of positive temperatures during summer in the North of

Eurasia (Fedorkov 2007), (iv) species biological features (Lyanguzova 2011), and (v) genetic traits of the populations (Ayari et al. 2016; Hisamoto and Goto 2017).

Under the conditions of environmental technogenic pollution, the qualitative and quantitative parameters of the pollution may also have an effect on plant reproduction (in addition to the factors stated above). According to literature data, sulfur and lead can form complexes with exchangeable calcium, magnesium, potassium, and sodium in soils, thereby reducing their availability for root uptake by plants (Mikhailova et al. 2007, 2015). It is known that decreased pH generally results in the increased migration of pollutants from soils, leading to an increase in sulfur, fluoride, and heavy metal uptake into the tissues of woody plants (twigs, leaves, etc.) (Mikhailova et al. 2015). On the contrary, under conditions where soil solution pH increases, a decrease in the mobility of many biogeochemical elements in the soil is observed and soil nutritional disturbances are observed (Menshikov and Ivshin 2006). Thereby, biogeochemical disturbances in forest ecosystems ultimately lead to changes in the nutrient status of major producers (woody plants) and reductions in their morphostructural characteristics and overall growth (Mikhailova and Shergina 2011; Trowbridge and Bassuk 2004).

Moreover, it is worth noting that pollutants can accumulate in various plant parts. Thus, a strong

Fig. 13 Root collar diameter of the seedlings at the end of the second vegetative period



correlation has been found between the levels of SO₂, HF, and aerosols with heavy metals in polluted atmospheric air and the accumulation of related elements such as sulfur, fluorine, lead, cadmium, and mercury in pine needles (Mikhailova et al. 2003). Indeed, the accumulation of heavy metals, sulfates, polynuclear aromatic hydrocarbons, and fluorides in needles, branches, bark, and roots has been reported for coniferous trees (Menshikov and Ivshin 2006; Sazonova et al. 2011; Mendoza et al. 2015; Chropenova et al. 2016; Skripalschikova et al. 2016; Lyanguzova 2017) and established as a probable reason for decreases in needle life, as well as anatomical, morphological, and physiological destruction of palisade organs and tree vitality in general.

Many authors have reported increases in the disturbance of male and female pine generative systems in areas of technogenic pollution (Anikeev et al. 2000; Makhniova et al. 2003; Tretyakova and Noskova 2004; Kalashnik et al. 2008; Mohnachev 2014; Sadakova and Kolyasnikova 2014; Vasilevskaya and Petrova 2014; Korshikov et al. 2015; Chropenova et al. 2016; Mohnachev et al. 2016; Makhniova 2016; Makhniova et al. 2017). High levels of heavy metals and other elements might also be a factor in increasing abortive pollen frequency in plants (Sadakova and Kolyasnikova 2014; Reshetova et al. 2015; Chropenova et al. 2016). Pollutants have also been found in plant seeds (Seregin and Ivanov 2001; Lyanguzova 2011), although these authors specify that the degree of accumulation is significantly lower than that in other plant parts. This is associated with root intake being the primary route of uptake into the plant, as well as the presence of barriers that may prevent accumulation in the reproductive organs (Lyanguzova 2011).

It is known that pine populations growing under conditions of environmental technogenic pollution are characterized by chromosomal instability and a high frequency of genetic mutation (Muratova and Zubareva 1990; Romanovsky 1997; Micieta and Murin 1997). The frequency of pine seed mutation increases under the influence of industrial pollution (Bakhtiyarova et al. 1995; Kalchenko et al. 1993), and to some extent this is already specified at seed germination (Geraskin et al. 2008).

Thus, according to literature data and the results of our research, seed formation and sprouting, together with seedling formation, growth, and development,

depend on the genetic characteristics of the tree and the seed forming conditions, i.e., the type and level of technogenic pollution in the planting medium. However, the level of soil technogenic pollution predominantly contributes to the formation and growth of sprouts and seedlings, i.e., their morphometric characteristics. Most likely, under natural conditions, the level of soil technogenic pollution is a particularly strong factor in restricting plant seed reproduction.

Conclusions

The influence of technogenic pollutants in growing media on the relationship between soil and plants was studied comprehensively over an extended period. In this research, we determined the following:

1. Scots pine trees growing in areas with different levels of technogenic pollution caused by emissions from magnesite production yield different levels of seed production, seed growth, and seed survival; these levels are commensurable with those observed under laboratory and field germination conditions using favorable planting media.
2. The formation, development, and vegetative growth of pine seed sprouts and seedlings in pot trials depended on the pollution levels in the planting media, the seed forming conditions, and the modal tree genetic characteristics.
3. The seedlings were characterized by differences in their morphometric characteristics and vital capacity under different potting media conditions. The negative effect of technogenic pollution in the potting media on seedling development was significantly linearly correlated with the level of pollution.
4. The genotype of seeds from the tree stands in control areas and areas of weak technogenic pollution was more sensitive to planting media containing technogenic pollution than was the genotype of seeds from areas with high and medium levels of technogenic pollution.

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