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Original article

Germination of Seeds and Growth of Young Crops on the Mine Dump Soil of Kišnica and Its Surrounding Area

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Abstract

The percentage of seed germination and growth of young plants of wheat crops (*Triticum vulgare* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) on the substrate of the mine dump of Kišnica mine and its surrounding area was studied. The growth of young plants was monitored for 25 days from sowing. The results obtained during the study showed that the percentage of seed germination and the growth of the studied plants varied depending on both the plant species and on the concentration of the pollutants in the used soil from different sites, as well as the distance of the sites from the pollutant emitters. The highest percentage of germination was observed in wheat seeds from the site Gračanica (3 km away from the emitter) and the smallest percentage of germination was found in barley seeds from the site Kišnica mine. The fastest growth was recorded in the wheat from the site Gračanica, and the greatest inhibition during plant growth was recorded in the corn from the site Kišnica mine pit where the height of stem was only 2.45% compared to the control. Toxic substances (heavy metals from the mine dump of Kišnica mine) had the inhibitory effect on the seed germination. Low concentrations of heavy metals in the soil from Gračanica stimulated the growth of plants that, after 25 days of growth, was stopped in all the plants growing on that soil. However, high concentrations of heavy metals inhibited seed germination and the growth of young plants on the substrate from Kišnica mine. The inhibition or stimulation of seed germination and the growth of young plants in the presence of heavy metals in the soil was caused by the inhibition or activation of the enzymatic system responsible for the metabolic processes during morphogenesis.

Keywords: Mine dump, Crops, Heavy metals, Germination, Growing of young plants.

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INTRODUCTION

Kosovo and Metohija is, morphologically considered, one of high regions of Serbia. At an altitude of 550 m near the village of Gračanica, there is an industrial giant, Kišnica mine, which deposits its emissions in mine dump, located near the dam of Gračanica Lake. The Gračanica Lake represents an artificial reservoir on the Gračanka River, and supplies water to the city of Priština and surrounding settlements. The Gračanka River feeds into Sitnica.

The waste of the Kišnica mine have been formed by processing lead and zinc ores and represent the most serious environmental problem of the Gračanica region and a constant threat to environment at large. Environmental damage is reflected in the pollution of air, watercourses and surrounding soil. About 11.2 million tons of waste is deposited in the mine dump, whose average composition is SiO_2 (30%), Al_2O_3 (6.5%), Fe (15%), S (16%), MgO (3%), CaO (1.5%), As (0.6%), Pb (0.6%), Zn (0.5%), Ag (17 grt^{-1}), Cu, Ca, Ni and other elements in low concentrations, indicating that heavy metals, which are very toxic, are in the largest amount in the waste.

Environmental pollution by toxic substances presents a major ecological problem of the modern time, and therefore the problem of human health (Kim et al., 2003). Most heavy metals accumulate in the top soil and in the long term their contaminations increase in the soil as a result of an increased absorption and accumulation in plants (Filipović-Trajković et al., 2012). Toxic substances in the environment cause morphological, biochemical, physiological and genetic changes to biological systems, primarily in plants. Also, the transport of heavy metals to the overhead parts of the plant depends on the phase during the digestion and development of the plant (Liu et al., 2005).

The negative effects of the harmful substances, primarily heavy metals, on plants appear in the first phase of morphogenesis, i.e. during germination of seeds, as well as during the vegetation period of growing and development of plants (Shafiq et al., 2008; Kumar and Jayaraman, 2014). Considering this, the correlation between the degree of inhibition and the concentration of toxic metals, primarily lead, was determined (Trajković, 1995; 2004).

The higher the lead concentration is, the germination gets reduced, while low lead concentrations cause increased seed germination. Lead leads to the reduction of the photosynthetic apparatus (Kastori et al., 1998), inhibits the growth of cells due to its effect on the metabolism and transport of auxin (Woolhose, 1983). In such a disturbed environment, the plants are still growing and form the main link in the food chain. Considering this, it was decided to conduct the testing of seed germination and growth of crop plants in the mine dump of the industrial zone of the Kišnica mine and its surroundings.

Material and Methods

In order to achieve this goal, soil samples from different sites were used. Sampling sites were determined in the straight line from Kišnica, at different distances from the site of the mine dump, in

order to determine the possible correlation between the effects and the distance from the emitter. The first soil sample was taken from the mine dump of Kišnica mine, the second sample was from in Gračanica, 3 km from the source of pollution. These two samples served as test samples for seed germination and plant growth. In addition to the test samples, the soil was also taken at a distance of 10 km from the mine dump, from Lipljan, and this soil sample was used as a control. For the tests of seed germination and growth of young plants, field crops *Triticum vugare* L., *Hordeum vulgare* (perrenial barley), *Zea mays* L. (corn - commercial seed) were used. One hundred seeds were sown in each pot filled with the mentioned soil samples from the selected sites and placed under controlled conditions at room temperature of 22-25°C. The watering of the seeds was carried out with well water until the end of the experiment. After the 3^{d} day for wheat and barley, and the 5th day for corn, seeds were counted in all samples. The number of germinated seeds for each plant species was recorded, and the percentage of germinated seeds was calculated in relation to the control. The results are shown in tables and in the form of a histogram. In the further course of the experiment, the plant growth was observed with occasional watering and measuring of the stem height and the leaf length after the , 7th 11th,18th ,28th day of germination. The length of the plant roots was measured upon completion of the experiment (45 days after the germination).

Results and Discussion

The results obtained during the experiment showed that the percentage of seed germination and the growth of the plants tested varied, depending on the plant species, the concentration of pollutants from the soil and from the distance of the site from the emitter.

Table 1. Percentage of germinated seed of *Tritucum vugare* L. (wheat), *Hordeum vulgare* (perennial barley), *Zea mays* (corn) in soil from Lipljan, Gračanica and Kišnica

Crops						
Soil origin	Wheat		Barley		Corn	
	Number of germinated seed	% of germinated seed	Number of germinated seed	% of germinated seed	Number of germinated seed	% of germinated seed
Lipljan (control)	77	100	70	100	80	100
Gračanica	70	90.9	64	91.4	61	76.2
Kišnica	16	20.7	15	21.4	68	85

During the experiment, differences in germination of seeds, length of leaves, height of the stem and root length were observed in plants that grew in the soil of various sites. The fastest germination after 72 hours after the beginning of the experiment was recorded in wheat and barley in the soil originating from Lipljan, while the corn germination in the same soil occurred after 96 hours (Table 1).

The largest number of germinated seeds was recorded in wheat in the soil from Gračanica (70 seeds), which was 99% compared to the control. The smallest percentage of germination was recorded in barley in soil from Kišnica (15 seeds), which was 21.4% compared to control (Table 1).

Difference in germination of seeds between plant species was also observed. Namely, according to our results, the highest percentage of germination in barley seeds in the soil from Gračanica was recorded and it was 91.4% compared to the control.

These differences in seed germination are probably related to the lower concentration of pollutants in the soil from Gračanica compared to the soil from Kišnica as well as with the composition of seeds, i.e. seed coat which is less likely to transmit pollutants, especially heavy metals, which in this case behave as activators of the enzymes involved in seed germination. Increased or decreased germination in the investigated plants is probably genetically conditioned because some seeds are more resistant to high concentrations of pollutants, and some are less resistant or not resistant at all. Decreased germination in mine dump soil, compared to control, is associated with a weak acidic environment of the substrate, and the acidic environment is a significant prerequisite for faster penetrability of lead into seeds which inhibits the enzymatic system in the germination process (Trajković, 1995).

Inhibition of seed germination by lead is a consequence of its effect on metabolic processes, loss of seed vitality and energy reduction in the embryo (Mahmood et al. 2005; Jamal et al. 2006).

Đelić et al. (2017) proved that germination of barley seed and the increase of germs in conditions of stress caused by sodium salts depend on the type of salt and its concentration.

Table 2. Stem height and leaf length during the growth of young wheat plants in soil originating from Lipljan, Gračanica and Kišnica

Days of growth	Soil site	Wheat (*Triticum vulgare* L.)			
		Stem height (cm)	% of stem height compared to control	Leaf length (cm)	% of leaf length compared to control
7th day after sowing	Lipljan (control)	9	100	7	100
	Gračanica	11	122	7.7	110
	Kišnica	No germination	/	No growth	/
11th day after sowing	Lipljan (control)	13	100	10	100
	Gračanica	18	138	12	120
	Kišnica	1.5	11	7	70
18th day after sowing	Lipljan (control)	20	100	12.9	100
	Gračanica	24	120	17.5	135
	Kišnica	17.5	87.5	8.8	44
25th day after sowing	Lipljan (control)	27.3	100	24.3	100
	Gračanica	27	98.9	18.6	76.5
	Kišnica	21.5	78.8	13.5	55.6

The authors state that all of the used concentrations of the tested salts have acted inhibitive to the observed parameters. Barley was not tolerant to sodium salts. The length of root and hypocotyls are a good indicator of the studied salts. Based on the total toxicity to the percentage of germination, the authors listed the studied salts as such: NaCl <Na_2SO_4 <$NaHCO_3$ <Na_2CO_3.

Pollutants not only affect germination, but also plant growth, especially during the first few days after germination. Thus, the obtained results for plant growth immediately after germination show differences in the length of the leaves, the height of the stem and the length of the root.

The length of the leaves in wheat, barley and corn was measured 7, 11, 18 and 25 days from the emergence of the first leaf after the beginning of the experiment. From the obtained results it can be noted that the average length of leaves after the first measurement in wheat from the soil of Gračanica was 7.7 cm which is 10% higher than the control (Table 2).

Table 3. Stem height and leaf length during the growth of young barley plants in soil from Lipljan, Gračanica and Kišnica

Days of growth	Soil site	Barley (*Hordeum vulgare* L.)			
		Stem height (cm)	% of stem height compared to control	Leaf length (cm)	% of leaf length compared to control
7th day after sowing	Lipljan (control)	6.9	100	4.3	100
	Gračanica	10	122	5.8	134
	Kišnica	No germination	/	No growth	/
11th day after sowing	Lipljan (control)	12	100	9.5	100
	Gračanica	15	125	11.5	121
	Kišnica	6.2	51	7.1	74
18th day after sowing	Lipljan (control)	12.5	100	9	10
	Gračanica	22	176	12	133
	Kišnica	9.8	78	8.6	95.5
25th day after sowing	Lipljan (control)	19.5	100	15.	100
	Gračanica	26.8	137	15	98
	Kišnica	13.7	70.2	11.5	75

The average length of barley leaves sown in the soil from Gračanica was 5.8 cm, which is 34% more compared to the control. The average leaf length from the same soil was 6 cm higher than the control (the soil from Lipljan), in which the corn was in the initial phase. However, after 11 and 18 days after the beginning of plant growth, similar phenomena were observed, i.e. the growth of barley, wheat and maize plants in the soil from Gračanica was higher compared to the control. The length of wheat leaves after 12 days was 12 cm, of barley 11.5 (Table 3) and of corn 7.8cm (Table 4). After 18 days, in wheat it was 17.5 cm, in barley 12 cm, and in corn 17 cm (Tables 2, 3 and 4).

Table 4. Stem height and leaf length during the growth of young corn plants in soil originating from Lipljan, Gračanica and Kišnica

Days of growth	Soil site	Corn (*Zea mays* L.)			
		Stem height (cm)	% of stem height compared to control	Leaf length (cm)	% of leaf length compared to control
7th day after sowing	Lipljan (control)	Beginning growth phase	/	Beginning growth phase	/
	Gračanica	35.5	/	6	/
	Kišnica	No germination	/	No germination	/
11th day after sowing	Lipljan (control)	5.5	100	6	100
	Gračanica	6.8	123	7.8	130
	Kišnica	Weak growth	Weak growth	Weak growth	Weak growth
18th day after sowing	Lipljan (control)	13.3	100	12.5	100
	Gračanica	21.9	164	17	136
	Kišnica	Weak growth	Weak growth	Weak growth	Weak growth
25th day after sowing	Lipljan (control)	40.7	100	32.3	10
	Gračanica	23	59.8	19.3	59.8
	Kišnica	1	2.45	Leaf didn't develop	Leaf didn not develop

In the soil from the Kišnica, the plants of the corn were dormant, i.e. the growth was even slower (Table 4). These changes in growth probably occurred due to the activation of the phytohormonal factors, under the influence of low concentrations of heavy metals in the tested soils. But after 25 days since the beginning of growth, high differences in growth of the leaves in plants from Lipljan soil (control). Thus, the length of the leaves was much higher in comparisons to the tests, i.e. wheat 24.3 cm, barley 15.3 cm, and corn 32.3 cm. In Tables 2, 3 and 4, it can be clearly noticed that after 25 days of growth, there has been inhibition of growth in all plants in soils from Gračanica and Kišnica. The inhibition in growth is most likely due to the high density of plants, i.e. to the competition for light, mineral matter and water. Some morphological changes in the leaves during plant growth were also noted. These changes were related to the color of the leaves and the size of leaf plates.

Also, it was noted that the wheat leaves grew the best in the soil from the contaminated area of Kišnica. The changes that were also detected in the length of the leaf were, also, noticed in the height of

the stem. Thus, the highest stem after 25 days from the beginning of growth was in control plants from the soil from Lipljan (Tables 2, 3 and 4). However, the largest inhibition of growth was recorded in the substrate of the Kišnica mine dump, after 25 days of growth, with the height of the stem at just 1 cm on average (Table 4). This was probably due to the sensitivity of the cytoplasm of the cells of these crop to heavy metals that inhibit growth. The toxic effect of heavy metals (Cd, Fe, Ni and Zn) on seed germination, root growth and leaf length in *Rhobinia pseudoacacia* L., depends on the type and concentration of metals. (Đelić et al., 2017). The lethal concentration of heavy metals was 100 mol/m^3. Since the germination of seeds was higher than 50% in solutions of 1 mol/m^3, the authors stated that the species *Rhobinia pseudoacacia* L. is tolerant to these concentrations of heavy metals, indicating the possibility of using this plant in the phytoremediation of soils containing these metals. Trajković et al, (2008) stated that ornamental plants react differently to the presence of lead acetate. Thus, the growth and development of *Datura arborea* was rapid at low concentrations, and so was the growth of *Tagetus patula* at a concentration of 2×10^2, while *Begonia tuberhibrida* resulted in total inhibition of growth and development in both concentrations.

After 45 days of growth, the length of root of plants was measured, whereby the obtained results showed that there were differences in root length and they depended on the species of plants and on the site. The longest roots were measured in barley in the soil from Gračanica (8.1 cm), while the shortest roots were found in plants of corn from Kišnica (0.8 cm) (Figure 1).

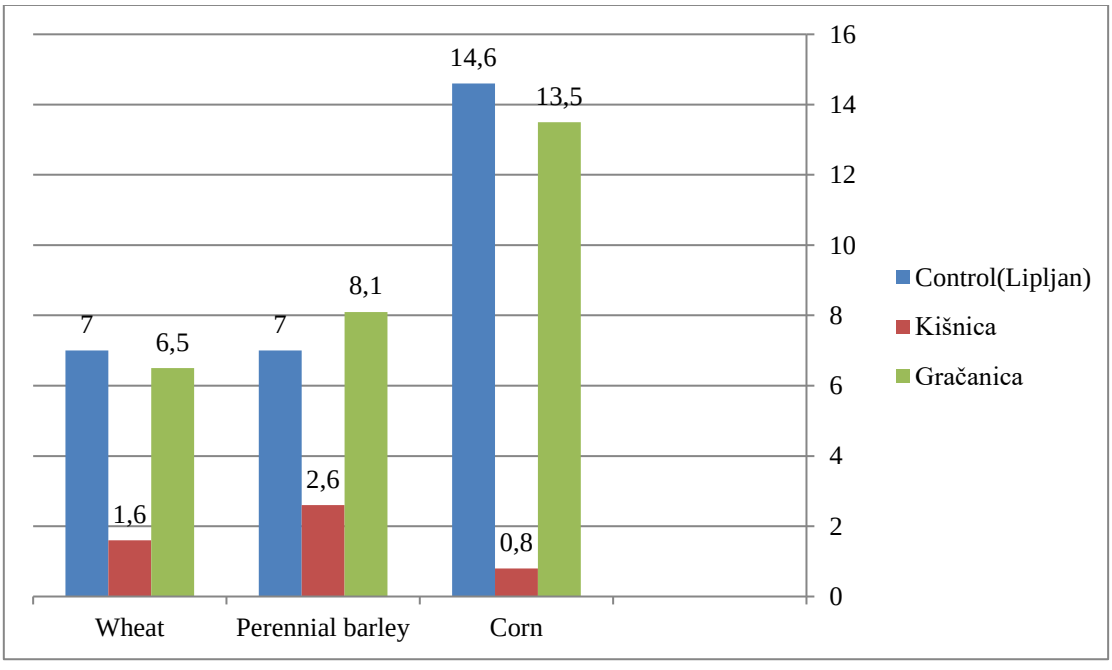

Figure 1. The length (cm) of the roots of wheat, barley and corn during the vegetation for 45 days from germination of seeds in the soil from the Kišnica mine dump and in the soil from Gračanica and Lipljan (control)

The experiment was completed after 45 days from the beginning of the growth because the plants in the soil from Kišnica dried up. The drying of plants was probably due to soil contamination. The pollutants were absorbed by the plant cells and caused the destruction of the cells' cytoplasm. A mixture of organic pollutants stimulates the enzymatic system responsible for the processes of germination and growth of vegetable crops (Trajković et al., 2004; 2005).

Conclusion

Considering the development of the industry, anthropogenic sources of heavy metals have become significant pollutants and their encroachment into the soil should be prevented or at least controlled, so that in the soil-plant-man system the end user get biologically safe food. The atmosphere is an important medium for the transport of metals from various sources, often for hundreds of kilometers from their emitters.

Based on the results obtained during the research on the influence of various pollutants from the soil of the Kišnica mine dump on the germination and growth of crops (wheat, barley and corn), it can be concluded that toxic substances, primarily heavy metals, could have inhibitory or stimulating effect. High concentrations of heavy metals in the soil stimulate growth, leaf length, and the stem height of plants in the soil from Gračanica. The length of leaves of crops from the soil of Gračanica after 18 days of growth were 135% in wheat, 133% in barley and 136% in corn compared to the control. After 25 days from the beginning of the experiment, the growth of leaves of crops in control (Lipljan) intensified. The same changes were observed in the height of the stems. The effect of the low concentration of heavy metals in the growth of plant crops was disturbed after 25 days after the beginning of the experiment in all plants from the soil of Gračanica. The growth inhibition most likely occurred due to the density of plants, ie, inter-species relationships. The high concentrations of heavy metals inhibit seed germination and the growth of young crop plants in the sample from the mine dump of the Kišnica mine.

The inhibition or stimulation of seed germination in the presence of heavy metals in the soil represents the inhibition or activation of the enzymatic systems responsible for the metabolic processes during seed germination. The increased activity of the enzymatic system responsible for germination processes was adaptation to the environmental conditions relative to control, which is a defense mechanism. Morphological modification of growth (depending on the quantity of heavy metals in the soil) was also registered in plants cultivated in different soil, which manifested as:

- reduction of stem height in wheat, barley and corn in the soil from the mine dump of the Kišnica mine compared to the control, and stimulation of their growth in the soil originated from Gračanica;

- reduction of leaf length in wheat, barley and corn in the soil from the Kišnica mine dump compared to control, and stimulation of their growth in soil from Gračanica

- reduction of root growth in wheat, barley and corn in the soil from the mine dump of the Kišnica mine compared to control, and stimulation of root growth of wheat, barley and corn in the soil from Gračanica

The soil is a vital ecological and agricultural resource and should be protected from further degradation. By accelerating the increase of the human populations on Earth, food demands are rising. However, an increase of anthropogenic sources of heavy metals can reduce the yield and quality of crop plant products. In the soil, heavy metals are a major problem, since many of them are stable for hundreds and thousands of years, and therefore these soils require a special way of use. The waste of the Kišnica mine is deposited near the dam of Gračanica Lake, the Gračanka River and Sitnica through which toxic substances spread into large areas, indicating even more harmful pollution from the mine dump of the Kišnica mine.

These results will contribute to a better clarification of the potential presence and toxicity of certain pollutants in the investigated mine dump of the Kišnica mine and can be used for biological monitoring for the purpose of early detection of pollution.

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