

PHYTOTOXICITY OF OIL SHALE SEMI-COKE AND ITS AQUEOUS EXTRACTS: A STUDY BY SEED GERMINATION BIOASSAY

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*Phytotoxicity of oil shale semi-coke has been studied by germination bioassay. Timothy (*Phleum pratense*) seeds were germinated on solid semi-coke and in its water extracts. Fresh semi-coke completely blocked seed germination but weathering significantly decreased its inhibition effect. The inhibitory effect of aqueous extracts was less than that of solid semi-coke, and it decreased as the liquid-to-solid ratio used for preparing the extracts increased. The inhibition of seed germination was mainly caused by high content of soluble salts in semi-coke. Good correlations were found between seed germination and electrical conductivity, and Ca^{2+} content of water extracts.*

Introduction

Up to the present, suitability of oil shale semi-coke for seed germination has been studied only rarely. However, taking into account the need to cover semi-coke dumping sites and the fact that covering semi-coke heaps by plants enhances bioremediation of semi-coke to a great extent [1], the question is currently of high interest. The few experiments carried out for growing grasses on semi-coke show that fresh semi-coke inhibits seed germination, i.e. the seeds sown on the fresh semi-coke do not germinate [2]. However, it has been also shown that the mixing of semi-coke with acidic sphagnum peat and weathering decrease the inhibition effect of semi-coke [2]. Trials on semi-coke heaps have revealed that seeds can easily germinate and grasses grow on semi-coke of several years of age, as well as its weathered mixture with peat. The limiting factors for plant growth on weathered semi-coke are water content and availability of nutrients [3].

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It is not clear what exactly causes phytotoxicity of oil shale semi-coke towards seeds. Oil shale semi-coke is of a most variable composition because of differences in the raw material and the technological processes involved in oil production. Thus, it contains inorganic and organic compounds in varying amounts. Of the organic compounds found in semi-coke, phenols and polycyclic aromatic hydrocarbons (PAHs) have inhibited seed germination in different studies [4–8]. It is also known that phenolic compounds are the main components of natural growth regulators [9–10]. However, it is doubtful that these compounds cause the inhibition of seed germination. Phenolic compounds are more biodegradable than the other organic compounds found in semi-coke [11–12], and therefore their influence on seed germination should be shorter than the 6–7 months found in the former studies [2]. The content of PAHs in the semi-coke is rather small, up to 13 mg/kg [13, 14].

Germination of the seeds can be influenced by high pH of semi-coke, which is higher than 12 in the case of fresh semi-coke and about 10 in the case of semi-coke weathered for several years in heaps [13]. As seeds should germinate better at neutral pH there is reason to believe that the inhibiting effect of semi-coke on seed germination is at least to some extent caused by its high pH. However, this alone cannot be the sole reason, since the inhibiting effect did not decrease appreciably when pH of semi-coke decreased after mixing with acidic peat [2].

The investigations published so far have paid no attention to the very high content of soluble inorganic compounds in oil shale semi-coke. It is known that semi-coke contains a high amount of sulphur (1.7–2.1%) in different forms and calcium oxide (8–14%) [13]. Ecotoxicological tests on the leachate from semi-coke heaps carried out by A. Kahru *et al.* [15] have revealed that the ecological risk is up to 25% caused by sulphur-containing compounds, mostly by sulphides.

It is also evident that high salinity of growth medium can inhibit germination [16–18], mainly by diminishing the water potential of the growth media which in turn hinders water entering into seeds [17, 19]. High concentration of calcium ions can raise osmotic pressure to a level toxic to seeds and plants [20]. Beside the influence on the water potential of the growth medium, a high concentration of ions may also be toxic to the seeds. For instance, it has been shown that the influence of high concentrations of sulphate ions during germination can kill seeds [21].

The aim of the current work was to study oil shale semi-coke by germination bioassay using timothy (*Phleum pratense*) seeds. Timothy seeds were chosen because timothy is widely spread in Estonia on natural as well as on cultivated grasslands and therefore may be of importance also for recultivating semi-coke heaps. The results of germination tests were compared with the results of chemical analysis in order to test for a correlation between the main chemical properties and phytotoxicological effect of semi-coke. Additionally, attention was paid to the determination of phytotoxicity of

aqueous extracts of semi-coke and its applicability for phytotoxicity assessment.

Materials and methods

The samples (13–14 kg) used in the experiments were taken from the semi-coke waste heap owned by Kiviõli Keemiatööstus Ltd. (Estonia). They were collected immediately after disposal.

Preparation of semi-coke water extracts

For the experiments aqueous extracts of semi-coke of different liquid-to-solid ratios ($L/S = 0.3/1$ – $10/1$) were prepared. The extracts were prepared basically as described in the European standard EN 12457-4:2002. Semi-coke was mixed with a certain amount of water and shaken continuously at room temperature in the dark. After 24 h the suspended solids were allowed to settle, and the eluate was filtered for the following analysis. Analysis of the aqueous extracts for pH, electrical conductivity (EC), sulphides, sulphates, total hardness, calcium ions and chemical oxygen demand (COD) were carried out using standard methods approved for water analysis [22].

Seed germination

The germination tests were carried out with timothy *Phleum pratense* c 'Tika' seeds purchased from Jõgeva Plant Breeding Institute, Estonia. To determine the germination in water extracts of semi-coke, 100 seeds in 4 or 5 replicates of each experiment were placed on Petri dishes (diameter 9 cm) and located in a growth chamber Termaks with controlled temperature and lighting. Extract (4 ml) was added to each dish; control dishes used only distilled water. The dishes were covered with lids. The conditions for seed germination in the growth chamber were: 3 days at thermoperiod 7 °C without photoperiod and 10 days at a thermoperiod of 30 °C (8 hours) and 20 °C (16 hours) and a photoperiod 16 hours and 5 hours in the dark [23]. Some experiments were also carried out with solid semi-coke. In these experiments the bottoms of the Petri dishes were covered with moistened semi-coke (particle size <4 mm), and 100 seeds were pressed gently into the sample surface. All experiments were continued for 13 days.

The numbers of germinated seeds (G and G_0 for test solution and control, respectively) were counted, and the lengths of the radicles (L and L_0 , respectively for test and control) were determined. The results were quantified as germination rate ($G\% = G/G_0 \times 100$) and germination index ($GI = G/G_0 \times L/L_0 \times 100$). For the statistical analysis of the experimental data the *t-test* (independent variables) and *Multiple regression* function of the Statistica 7 program (StatSoft, Inc.) were used.

Some experiments with semi-coke were performed as pot experiments under lab conditions. For this purpose the pots were filled with fresh semi-coke. Seeds (100) were sown and after two weeks the emerged plants were assayed. Plant growth data were compared with the results of chemical analysis in order to study the relationship between chemical and phytotoxicological properties of semi-coke. The content of organic compounds in semi-coke was analysed in the Estonian Environmental Research Centre using internationally accredited methods.

Results and discussion

Seed germination on oil shale semi-coke

Experiments revealed that fresh semi-coke is toxic to seeds, thus confirming the results of earlier studies [2]. The seeds did not germinate on fresh semi-coke nor were they able to germinate even in pure water after previous contact with it. At the same time, the results of the study indicated that the toxic properties of semi-coke decrease over time. The long-term experiments in pots showed clearly that the germination of seeds was feasible after eight months of aging of semi-coke under laboratory conditions. However, the germination of seeds was achieved only on the top layer of semi-coke. Even after nine months the seeds were not able to germinate on the material taken from the deeper layers of semi-coke (Table 1). A more thorough study of the top layer showed that already the layer down to 10 cm had different properties. The results in Table 1, indicating that germination in the top 10-cm layer was lower after nine months compared to that after eight months, can be explained by the considerable reduction in phytotoxicity of semi-coke in the thinner layer. The results presented in Table 2 also confirm that remediation takes place probably only in the uppermost five-cm layer.

Table 1. Germination of timothy seeds in semi-coke (sample 1) in pot experiments

Time of semi-coke weathering, months	Germination rate, %	Semi-coke pH
0	0 ^a	12.66
2	0 ^a	12.63
3	0 ^a	nd
4	0 ^a	nd
5	0 ^a	nd
7	34 ^a	nd
8	56 ^a	8.62 ^a
9	31 ^b	8.62 ^b
	0 ^c	11.10 ^c
	0 ^d	11.77 ^d

^a – top layer, ^b – layer 0–10 cm; ^c – layer 10–20 cm, ^d – layer 20–30 cm, nd – not determined

Table 2. Timothy seed germination on semi-coke samples having undergone different treatments

Semi-coke sample	pH	Germination rate*, %	Germination index*, %	Notes
6	12.73	0	0	fresh
6	12.71	0	0	rinsed, L/S=1 l/kg
6	12.61	0	0	rinsed, L/S=2 l/kg
5	10.45	52 ± 4	10 ± 1	rinsed, L/S=10 l/kg
4	8.30	30 ± 19	6 ± 6	aged 6 months in lab under wet conditions
6	12.60	18.5 ± 6.5	0	rinsed, L/S=30 l/kg
6	11.84	61.5 ± 3.5	19 ± 0.1	rinsed, L/S=60 l/kg
6	nd	51 ± 7	3 ± 0.3	rinsed, L/S=30 l/kg, dried in lab for 1 week
6	nd	75 ± 1	26 ± 1	rinsed, L/S=60 l/kg, dried in lab for 1 week
6	11.86	65.5 ± 10.0	27 ± 5	weathering in lysimeter 6.5 months, top layer (0–5 cm)
	12.10	2 ± 1	0	weathering in lysimeter 6.5 months, layer 5–10 cm
	12.66	1.5 ± 0.9	0	weathering in lysimeter 6.5 months, layer 10–15 cm
	12.65	0	0	weathering in lysimeter 6.5 months, layer 15–20 cm

nd – not determined; * – data are the means of four replications ± standard deviation

Compared to laboratory conditions, remediation of semi-coke in natural conditions was faster, and germination of seeds was possible there even after only four months of weathering.

It was observed that pH of semi-coke decreases with time. Under laboratory conditions pH in the top layer of semi-coke, where the germination of seeds was achieved, decreased after eight months from 12.66 to 8.62. The latter can be considered suitable for plant growth. The pH of semi-coke in deeper layers was significantly higher than optimal for the seeds or plants. The reduction in phytotoxicity of semi-coke in the top layer can be explained by the decrease in pH due to the contact with atmospheric CO₂. However, the results obtained so far indicate that the high pH is not the main reason for the inhibition of seed germination. The results show that one of the reasons for the reduction in phytotoxicity is leaching of toxic compounds with water. Comparison of the results in Table 2 reveals that rinsing semi-coke with water improves the germination of seeds more than pH decrease alone. The seeds can germinate on solid semi-coke after treatment with water (Table 2). The results show considerable reduction in the inhibiting effect of semi-coke rinsed with water under laboratory conditions, or left outside and rinsed with natural rain water.

Besides pH, changes in the content of organic compounds were investigated and their relationship to seed germination studied. The results showed that the phenol content in semi-coke had changed, but there was no decrease

in oil products or in the content of polar organic compounds (Table 3). The content of organic compounds in different layers did not differ significantly (Table 3) and therefore they cannot be responsible for the inhibition of seed germination to any significant extent. This is also confirmed by the results of experiments with pure substances, which showed that concentrations toxic to seeds are much higher than those found in the semi-coke samples [24].

Table 3. Organic compounds in semi-coke (sample 1)

Compound	Content, mg/kg				
	Fresh semi-coke	1 month old semi-coke	9 months old semi-coke		
			0–10 cm	10–20 cm	20–30 cm
Resorcinol	6.22	4.46	1.3	<0.5	<0.5
5-methylresorcinol	<0.5	<0.5	<0.5	<0.5	<0.5
2,5-dimethylresorcinol	1.58	3.15	<0.5	<0.5	0.96
Phenol	26.7	29.5	1.97	1.28	2.35
<i>p</i> -, <i>m</i> - cresol	12.2	12.5	1.65	1.06	2.70
<i>o</i> -cresol	1.26	1.25	0.23	<0.1	0.31
2,3-dimethylphenol	1.56	1.99	0.23	<0.1	0.16
2,6-dimethylphenol	3.30	3.43	<0.1	<0.1	0.27
3,4-dimethylphenol	1.85	2.4	<0.1	<0.1	<0.1
3,5-dimethylphenol	3.04	1.49	0.2	<0.1	<0.1
Oil products	465	333	533	254	395
Polar hydrocarbons	4058	6410	5900	5110	5900

Investigation of water extracts of semi-coke by seed germination bioassay

Table 4 presents the results of the trials with extracts of semi-coke obtained from different samples by using the most often applied liquid-solid (L/S) ratio (10 l/kg) in waste characterization. It can be seen that the seed germination rates in semi-coke water extracts (L/S=10) were between 72 and 97% compared to germination in water. More than the germination rate, the radicle length was influenced by water extracts of semi-coke. The developing radicles were much shorter and more branched than those growing in control experiments in water where the average radicle length was 28 ± 7 mm. The great variation in radicle length also caused considerable variation in germination indexes as can be seen in Table 4. The chemical parameters in Table 4 show that samples of semi-coke differ quite considerably, although it was not possible to find any significant correlation between chemical parameters and the results of germination studies.

The effects of the semi-coke water extracts obtained by applying different L/S ratios in preparing them are given in Table 5. The germination rate and radicle length depended strongly on the utilized L/S ratio in preparing the extracts (Fig. 1). The germination rate in water extracts of semi-coke

differed significantly ($p < 0.001$) from that in water in the case of $L/S \leq 2$. More than germination, the L/S ratio influenced radicle length. If compared with water, the radicle lengths were shorter in all water extracts studied. Decreasing the L/S ratio (< 1.8) used to prepare the extracts from semi-coke increased the negative effect on the seeds, and an inhibition rate similar to that with solid semi-coke samples was obtained. Radicles on the emerged seeds in water extracts were not observed at L/S ratios < 1.8 .

Table 4. Influence of semi-coke water extracts ($L/S=10$ l/kg) on germination of timothy seeds

Semi-coke sample	Germination rate*, %	Radicle length*, mm	Germination index, %	pH	EC, mS/cm	COD, mg/l	Ca ²⁺ , mg/l	S ²⁻ , mg/l	SO ₄ ²⁻ , mg/l
2	86 ± 8	7.2 ± 1.9	50	12.81	nd	680	nd	309	nd
6	82 ± 7	10.4 ± 1.7	38	13.03	7.56	396	1235	102	804
6	98 ± 6	16.3 ± 1.3	50	12.89	8.31	330	1034	344	156
7	83 ± 6	6.8 ± 0.4	16	12.71	8.44	767	1262	202	250
8	72 ± 6	6.4 ± 1.2	13	12.80	7.99	584	1467	152	742
9	85.5 ± 7	10.2 ± 2.5	24	12.72	7.21	549	1235	128	367
10	81.5 ± 12	6.1 ± 2.5	13	12.50	6.25	1154	1158	249	304
13	97 ± 11	25.9 ± 0.6	71	12.60	6.92	780	1010	140	100

nd – not determined, * – data are the means of four replications ± standard deviation

Table 5. Influence of semi-coke (sample 6) water extracts on timothy seeds

L/S, l/kg	Germination rate*, %	Radicle length*, mm	Germination index, %	pH	EC, mS/cm	COD, mg/l	Ca ²⁺ , mg/l	S ²⁻ , mg/l	SO ₄ ²⁻ , mg/l
0.8	2 ± 2	0	0	12.70	16.50	nd	nd	nd	nd
1.1	15 ± 6	0	0	12.75	14.48	nd	nd	nd	nd
1.5	38 ± 5	0	0	12.79	13.72	nd	nd	nd	nd
1.8	41 ± 5	1.5 ± 0.7	2	12.91	12.86	2160	1824	69	97
3.2	89 ± 3	4.8 ± 1.1	13	12.92	10.82	1360	1455	361	128
4.6	100 ± 7	8.6 ± 0.7	27	12.91	9.32	940	1154	255	111
7.5	95 ± 4	14.3 ± 1.7	42	12.84	8.99	615	1082	113	112
14.5	98 ± 6	16.3 ± 1.3	50	12.89	8.31	330	1034	344	156

nd – not determined, * – data are the means of four replications ± standard deviation

The after-germination of seeds in water showed that the water extracts with L/S ratio of 1.1 and 1.5 inhibited germination reversibly, i.e. germination of the seeds exposed to the semi-coke water extract was later possible in water and the emerged seeds also showed radicles. The stronger water extract ($L/S = 0.8$) had toxic non-reversible effect on seeds, after-germination in water was not achieved. The latter was similar to the effect of fresh

semi-coke, in which case it was not possible to germinate the seeds even afterwards.

Influence of water extracts of semi-coke on seed germination

It has been proposed [2, 25] that the negative influence of semi-coke on seeds can be caused by high pH. The tests carried out in this study revealed that this is not the main reason for inhibition of seed germination. The various water extracts tested, although having similar pH values, showed totally different effects on seed germination and radicle growth (Table 5).

Semi-coke contains a considerable amount of soluble inorganic compounds (Tables 4 and 5). Concentration of calcium ions in water extracts was high, and the concentration of magnesium ions ca 3-5% of the calcium value. Also, the high concentration of sulphur-containing compounds is worth mentioning. This results in a high concentration of different ions and therefore in a high electrical conductivity of the water extracts prepared from it. The EC value in soil is an important parameter for evaluating the appropriateness of growth media to seed germination or plant growth. Salinity effects are considered mostly negligible in saturated extracts of soils [26], with EC of 2 mS/cm at 25 °C or less [27, 28], but those effects become important with increasing EC values. EC values > 4 mS/cm in soil water can have a negative influence on full-grown plants [29]. If the EC of soil water increases, the water potential decreases, and therefore water absorption by seeds slows down [30]. This can slow germination, but it can also result in the dormancy of seeds, which stops after their placement into water [16].

By taking into account the data in Table 5, the above-mentioned increasing negative effect of water extracts can be explained by an increasing concentration of different ions in them (increasing values of EC). The results of the statistical analysis of the data presented in Table 5 show that germination index is significantly correlated with the EC and calcium content, indicating that these parameters could cause the inhibition of seed germination and radicle elongation (Table 6). The EC correlated well ($r = 0.99$, $p < 0.001$) with the content of calcium ions in the studied extracts; the correlation of concentration of sulphur-compounds with EC was weak.

Table 6. The correlations between germination rate and radicle length, and chemical characteristics of water extracts

	EC	Total hardness	Calcium ions
Germination rate	-0,89**	-0,89**	-0,93**
Radicle length	-0,64	-0,94***	-0,92*

* $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$

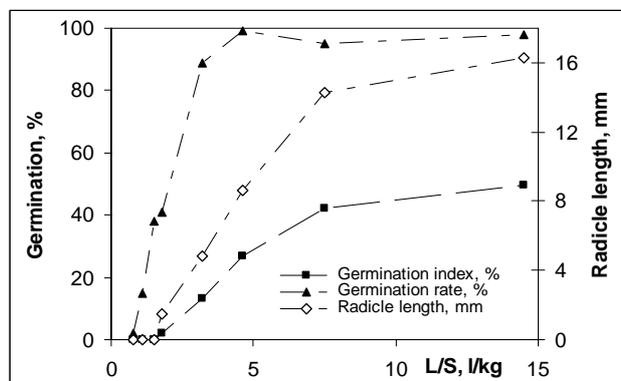


Fig. 1. Correlation between liquid/solid ratio used in preparation of water extracts and its effect on seed germination

Since the level of EC value having no negative effect on seeds was exceeded in all the water extracts studied, extracts of different electrical conductivities were prepared and investigated in an extra series of tests (Figures 2 and 3).

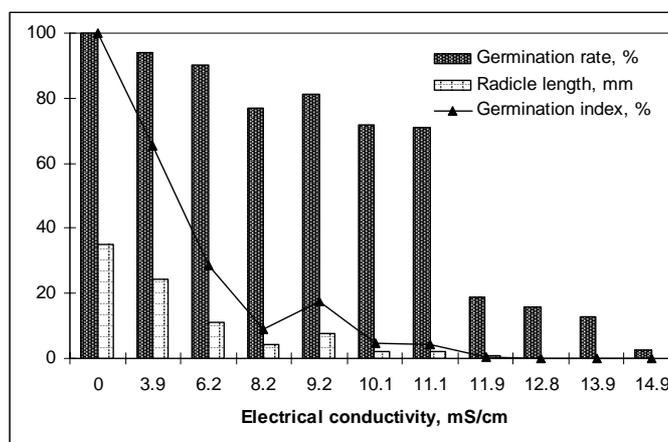


Fig. 2. Correlation between electrical conductivity of semi-coke water extracts and seed germination

Figure 2 shows that radicle length and germination index deduced from it decreased sharply with increasing electrical conductivity of semi-coke water extracts. Germination and radicle elongation in semi-coke water extracts differed from that in water already at electrical conductivity of 3.8 mS/cm. A sharp decrease in germination was observed at the EC value around 11 mS/cm; values >15 mS/cm inhibited germination completely. The latter

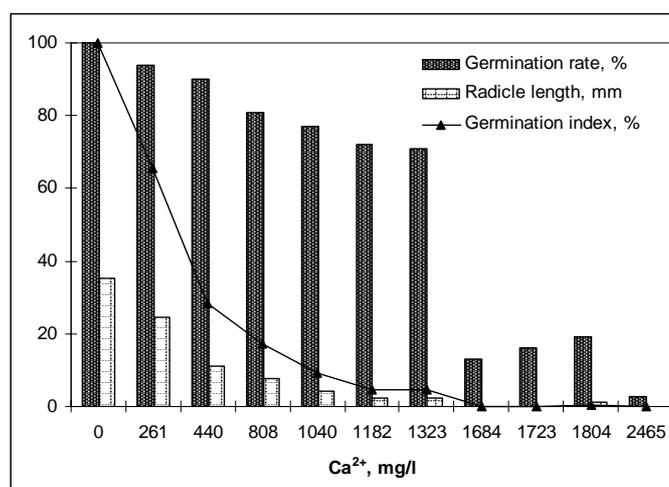


Fig. 3. Correlation between calcium ion content of the aqueous extracts of semi-coke and seed germination

value was also the limit at which seeds did not germinate even after replacing them into pure water. Radicle length, more than germination, was influenced by the increasing EC values. A 50-% decrease in radicle length was seen at the EC value of 4.8 mS/cm, and there was no radicle growth at EC values higher than 11 mS/cm. The conditions for germination can be best characterized by germination index. A decrease in germination index of ca 50% was observed in the case of EC around 5 mS/cm, and it fell to zero at an EC value around 12 mS/cm. These values can be characterized by calcium ion concentrations of approximately 400 and 1800 mg/l, respectively (Fig. 3).

Besides the good correlation between germination parameters and EC values of semi-coke water extracts, a good correlation with calcium content was also noted (Table 6, Fig. 3). It is well known that calcium is an essential plant nutrient but that, in excess, it can restrict plant communities on calcareous soils. When excessive calcium ions are present in the rhizosphere solution, plants may suffer calcium toxicity, which may prevent germination of seeds and reduce plant growth rates [31]. However, information on toxic effects of calcium ions is limited.

Conclusions

Fresh semi-coke completely blocked the germination of timothy seeds. The results of different tests showed that the inhibition of germination found at applying semi-coke samples from a correctly operated technological process is caused mainly by a high content of soluble salts. The investigation of

semi-coke water extracts and their influence on seeds revealed that much of calcium and other ions causing high electrical conductivity of the solutions can be washed out from semi-coke. The results coincide with the results with washed semi-coke samples which inhibited seeds less than the fresh semi-coke. We could also expect the negative effect of pH, especially on the radicle growth, which turned out to be more sensitive than germination rate. Therefore, our study also confirmed that germination index is a better parameter for estimating inhibition than germination rate as it integrates the effects of any environmental stress on both germination and seedling growth stages.

We also conclude that experiments with water extracts of solid samples may not reflect the actual inhibiting effect of the growth media, especially if germination inhibition is a result of salinity. In this case, changes in germination index with dilution of the extract would be expected, and applying higher dilutions in preparing the extracts for the test, as has been done in several cases, might yield misleading results.

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