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ENVIRONMENTAL HAZARD OF THE WASTE STREAMS OF ESTONIAN OIL SHALE INDUSTRY: AN ECOTOXICOLOGICAL REVIEW

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In Estonia there is the largest industrially-used oil-shale basin in the world. This review addresses the environmental hazard of the waste streams of oil-shale industry via solid waste- and water-path, mainly focusing on ecotoxicological risk due to open semicoke deposition inducing hazard to surrounding soils and groundwater. This is the first comprehensive ecotoxicological review of available data on oil shale industry pollution in Estonia as well as world-wide.

Introduction

Oil shale is fine-grained sedimentary rock containing relatively large amounts of organic matter. Oil shales are widely distributed all over the world; more than 600 deposits are known, and their prospective resources are estimated to be over 500 million tonnes [1]. On dry weight basis, oil shale consists of 10–60% of organics, 20–70% of carbonate minerals and 15–60% of sandy-clay minerals [2]. Estonian oil shale basin is the largest industrially used one in the world: the mining of oil shale in Estonia started in 1916 and reached its peak in 1980 when 31 million tonnes of oil shale per year were excavated [3].

As referred by Raukas [1] during last fifty years, power production in Estonia has almost fully been based on domestic oil shale: about 90% of the excavated Estonian oil shale is used as fuel in power plants. This has resulted in serious environmental problems. Over 90% of the water consumed in Estonia is used in oil shale mining and consumption. About 97% of air pollution and 86% of total waste come from the power industry. In addition to the use in power generation, about 10% of the excavated oil shale is used for producing oil and oil shale chemicals.

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Several papers [4–10] have mainly been concentrating on study of key pollutants and/or technological aspects of potential remedial options providing very valuable background information for this study.

The present study is the first comprehensive analysis of available data published on oil shale chemical industry pollution in Estonia, including all the results obtained by the ecotoxicology group of National Institute of Chemical Physics and Biophysics (NICPB) during past ten years. This review addresses the environmental impact of oil shale industry solid waste deposition in Estonia, focusing mainly on the toxicants leaching from semi-coke dumps to the surrounding soils and waters. This review will not tackle the aspects of air-born pollution.

Ecotoxicology in Environmental Hazard Assessment

The fate and hazard of toxicants in the environment depends on the source (air, solid waste, soil, wastewater) and nature of the toxicant (solubility, volatility, adsorption properties, biodegradability etc.), transfer route (via precipitation, leaching), exposed compartment (soil, sediments, surface water, groundwater) and biological targets (water and soil ecosystems, animals, humans). Almost in the whole world the assessment of environmental hazard is performed by chemical measurement of certain hazardous key pollutants (e.g., heavy metals, polyaromatic hydrocarbons, oil products) or certain integrated parameters (e.g., biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, total phosphorous) that are determined from the polluted soils and/or waters and compared to the legislatively set permitted level values (PLVs) for these compounds/parameters. The final set of these measured parameters depends on the information of the history of the pollution but also the money available for the analysis.

When aquatic samples are considered, the chemical analysis may underestimate the toxicity of the sample due to possible synergistic effects of chemicals in complex mixtures. Also, even with the most sophisticated techniques or due to the incomplete list of the pollutants to be analyzed, important toxicants may remain undetected. The toxic influence of pollutants could be relevantly measured only by (eco)toxicological tests (and not analytical techniques) that integrate all toxic signals for a certain test organism taking into account the possible effects of chemicals in complex mixtures. Historically the ecotoxicological testing started from aquatic media (wastewaters, groundwater, leachates) as water is the main vector for distributing of pollution considering the risk to human.

For the toxicity testing of soils, solid wastes, sediments and sludges there are four main approaches:

- application of terrestrial toxicity tests directly on solid matrix samples,
- application of aquatic toxicity tests on aqueous leachates of soils/ sediments/ solid wastes,

- application of aquatic toxicity tests on organic solvent leachates of soils/ sediments/ solid wastes,
- application on aquatic toxicity test organisms on suspensions (slurries) of soil/sediment/solid waste (i.e. direct contact assays).

Aquatic toxicity testing is performed using aquatic species, while for regulatory testing of ecotoxicological hazard of pure chemicals the following test species are used: fish (OECD Guideline 203), *Daphnia* (OECD Guideline 202), algae (OECD Guideline 201). For the evaluation of toxicity of polluted groundwater and leachates of soils and sediments mostly photobacterial tests with *Vibrio fischeri* (e.g., Microtox™) are used [11–13]. Microtox™ test (based on reconstituted freeze-dried (viable) luminous bacteria *Vibrio fischeri* where the reduction of light output of bacteria after their contact with toxic chemicals used as toxicity endpoint [14]) is widely used due to its sensitivity, high sample throughput and cost-efficiency. Currently a database of toxicity for more than 1000 chemicals tested with Microtox assay is available [15]. Moreover, toxicity data obtained with photobacterial assays correlate well with toxicity data obtained on daphnids, fish, other bacterial assays as well as *in vitro* animal cell cultures [16–18]. As no single test or species of living organism show uniform sensitivity to all chemical compounds, the battery of biotests with different sensitivity profiles is often recommended and used to assure adequate evaluation of the ecotoxicological situation [19]. Due to the complexity of ecosystems the ecotoxicological hazard assessment is more informative/predictive if the battery involves organisms of different trophic levels [20–22].

The environmental hazard assessment of solid phase environmental samples (soils, sediments, solid wastes) is more complicated than that of aqueous samples, as heavy metals [23, 24] and hydrophobic organic toxicants [25, 26] tend to be adsorbed by solid matrix and become less bioavailable. Due to the sorption of pollutants by the solid matrix and aging of pollution often false-positive results in terms of actual hazard could be obtained, i.e. the samples prove much less hazardous to biota than the chemical analysis predicts [26].

However, the protocols of state agencies used for legislative decision-makers rely mostly on analytical methods that involve vigorous extraction of soils and sediments either with organic solvents (e.g., to extract PAHs) or concentrated acids (to extract heavy metals). The aim is to remove all, or as much as possible, of the pollutant from the environmental sample. The concentrations obtained in this way are completely different from the fractions potentially hazardous to living organisms, i.e. bioavailable fractions of these chemicals, as bioavailability of a certain pollutant is its potential of uptake by living organisms depending on the nature and physicochemical properties of this toxicant, on the environmental matrix (groundwater, soil, sediment) and on the exposure route for the biological recipient and its physiology [27, 28]. The organisms used for ecotoxicological evaluation of soils are earthworms (OECD Guideline 207), soil enchytraeid worms *Enchytraeus*

albidus (ISO Guideline 16387), plants (OECD Guideline 208) and bacteria (OECD Guidelines 216 & 217).

For regulatory purposes, as it was noted for aquatic toxicity assays, the ecotoxicological soil assays are mainly applied for testing of the environmental hazard of new chemicals in accordance to the present European Union policy on hazard classification and landfill disposal of waste materials for tighter control over the release of contaminants into the environment. Hazardous Waste Directive 1991/689/EC defines a set of 14 properties to be used in waste hazard classification. According to that directive, ecotoxicity is one of the hazardous properties to be determined from wastes although as by now are no special testing requirements or specific limit values for the ecotoxicity of waste eluates.

However, assays with soil organisms, e.g., earthworms, collembolas, plants and bacteria have been widely used also for evaluation of hazard of polluted soils [24, 28–32]. The most relevant tests for evaluation of risks concerning the paths soil-plants, soil-microorganisms and soil-soil fauna are terrestrial assays with soil biota (see above). However, as these tests need a lot of laboratory space and time, this approach is not very widely used for environmental monitoring. Aquatic assays on water extracts of soils and/or sediments are often used for the hazard evaluation. The aqueous extracts are considered also to be more representative of the bioavailable part of the toxicants. Moreover, the water extractable fraction of toxicants predicts the potential risk of transfer of toxicants from polluted soils, sediments or solid wastes to the groundwater [33] that is a very important factor for distribution of pollution. In addition to commercially available photobacterial assays (e.g., Microtox), some additional low-cost small-volume aquatic toxicity tests with crustaceans, algae, protozoa and rotifers have been commercialized (e.g., ToxKits) and are often used as a battery [34].

Persoone [35] has developed Toxkits-technique on the basis of small-scale toxicity tests – microbiotests [19], where the test organisms (daphnids, rotifers, protozoa, algae) are stored in dormant and/or non-growing forms. The absence of the need for continuous cultivation/breeding of the test organisms makes toxicity testing easier and thus more cost-effective.

Aquatic test batteries have been used the for the analysis of the water-leachable toxicity of soils contaminated by explosives, oil and heavy metals [24, 36, 37], oil-shale industry wastewaters, solid waste and polluted soils [37, 38, 39, 40]. Aquatic test organisms may also be applied for the analysis of organic solvent (ethanol, methanol, dimethylsulfoxide etc.) extracts of soils and sediments [25, 39, 41, 42] to evaluate the potential toxic effect of hydrophobic pollutants strongly sorbed to solid environmental matrix. Usually the solvent extraction indicates the maximum toxic potential, i.e. the worst possible scenario.

In order to miniaturise terrestrial toxicity tests, the solid phase tests in suspensions using small test organisms as bacteria and algae have been designed. The use of soil/sediment suspensions (slurries) instead of exposure

to ′dry′ soils is the joint nominator for the contact microbiotests that use e.g., soil bacterial populations [30], photobacteria [36, 43], algae [44, 45], ostracods [46, 47] as well as recombinant luminescent sensor bacteria for heavy metals [24, 48, 49]. All these above mentioned studies have demonstrated that in case of direct contact even the particle-bound pollutants may become bioavailable as the toxicity in case of contact exposure (test organisms are incubated in soil suspensions) exceeds the toxicity of the aquatic extracts (test organisms are incubated in soil particle free extracts). The mechanisms for the increased bioavailability (desorption of pollutants due to the direct contact of organism with polluted soil particles) in case of contact exposure are not clear and need further studies.

The most relevant approach for the environmental hazard assessment is the combination of the chemical and ecotoxicological/biological methods that has not so far been often used, mostly due to the lack of respective legislation. However, there is a growing awareness among environmental toxicologists that for the meaningful environmental hazard assessment an interdisciplinary effort of biotesting and environmental chemistry measures, considering physicochemical, molecular, toxicological, physiological and ecological processes should be used [24, 32, 50–60].

Environmental Concerns of Oil Shale Processing

Oil shale deposits are found on all inhabited continents. Oil shale contains both a solid hydrocarbonous mixture (kerogen) and minerals. Kerogen when heated (retorted) yields combustible gases, shale oil, and a solid residue called with different names: spent shale, retorted shale, processed shale or semicoke. In Estonia, about 75–80% of kerogen (the organic part of oil shale) may be converted to oil. Usually the content of kerogen in kukersite (Estonian oil shale) is 30–45%. The major components of organic matrix are phenolic moieties with linear alkyl side-chains [61, 62]. The mineral part of oil shale consists of carbonates and sandy-clay minerals. The comparative composition of oil shale and semicoke is given in Table 1. Estonian oil shale is rich in sulphur, and in the retorting process more than 50% of it remains in the solid residue. In the fresh ash sulphur occurs mainly in the form of calcium and iron sulphides, and, to a smaller extent, as corresponding sulphates (formed mainly as a result of oxidation of other sulphur forms, see [5]). It is important to note that sulphide is the most toxic form of sulphur in the environment.

Semicoke deposits

Thermal processing of Estonian oil shale and refining of the products of its semicoking process (retorting) is accompanied by the formation of large amounts of different process waters and wastewaters containing phenols, tar and several other products, heavily separable and toxic to the environment.

Table 1. **General characteristics of oil shale and semicoke, mass %**

	Oil shale [64-67]	Semicoke [68]
Organics	28–43	5–10
Ash, %	35–46	65–75
CO_2 carbon, %	10–18	30
Total sulphur	1–2 (of kerogen)	1.5

The solid waste of the thermal treatment process, semicoke, is discharged from the retorts and disposed in an open dump. The mining and processing of 1000 million tonnes of oil shale in Estonia up to now has been accompanied by deposition of about 300 million tonnes of solid waste: 90 million tones of mining waste, 70–80 million tonnes of semicoke deposited in heaps covering about 180–200 ha near the towns of Kohtla-Järve and Kiviõli (oil and chemical industry waste), and 200 million tonnes of combustion ashes accumulated in 810 ha of plateaus near the town of Narva (power-generation waste). All these wastes are located in Ida-Viru County causing major environmental pollution sources in this region. In 2003 the share of total oil shale related waste to total waste was 73%, and the hazardous oil shale related waste to total hazardous waste was 95% in Estonia [63]. Although oil shale semicoke is produced in much lower amounts than combustion ashes, semicoke consists, in addition to minerals, up to 10% organics (Table 1) that may pose hazard to the environment due to leaching of toxic compounds as well as due to the self-ignition.

Open deposition of semicoke causes distribution of pollutants via air (dust) as well as via aqueous vectors (leaching by rainfall and snowmelt). Leachates from various spent shales have been studied by a number of investigators. Properties of spent shale vary widely with the retorting process, but in general they contain significant amounts of total dissolved solids, sulphate, carbonate, bicarbonate, and other inorganic ions, and lesser amounts of trace elements and organic compounds.

This review will not tackle the air pollution aspects and focuses on distribution of pollutants via solid waste- and water-path. It is important to note that groundwater is the main source of drinking water in the Estonian oil-shale region.

Water and oil-shale processing

The potential aqueous vectors of distribution of pollutants have been described by Kamenev et al. [8] as follows: water enters the technological process of oil shale thermal treatment from different sources: physical moisture in mined oil shale; water from oil shale semicoking process (process water); precipitation on the factory's territory; leakages in the cooling water system; used drinking water and washwater, etc.

All effluents from oil shale thermal treatment by the Kiviter process currently used by AS Viru Keemia Grupp (Viru Chemistry Group, Ltd) are concentrated into three main wastewater streams:

1. process water containing mainly physically and chemically bound water liberated during semicoking. This first stream passes through a complicated treatment process for oil and phenol removal, and is led to the aerobic bio-treatment plant;
2. industrial wastewater containing effluents of different origin, collected from the territory of the factory. This second stream passes through the local purification unit, where the coagulation and flotation processes are used for pretreatment;
3. ash dump leachates collected in several ditches on the foot of the semicoke dump and containing different dissolved pollutants (phenols, mineral salts) from semicoke and other deposited solid wastes like oil pitch or fusses and waste sludges from local wastewater treatment plant. Also, till recently semicoke in open deposits was compacted with alkaline process waters containing phenols and tars.

Semicoke dump leachates are collected in the ditches and equalisation basin surrounding semicoke heaps, and the overflow is directed via local rivers Kohtla and Purtse to the Baltic Sea [69]. About 500,000 m^3 of leachates reached the environment annually [70]. As by 1996, semicoke dump leachates were considered too toxic to be directed for biopurification to the local wastewater treatment plant [69]. As for solid wastes of Galoter process used in the Narva oil plant [8], detailed ecotoxicological studies have not been performed. However, some minor biological effects on fish in the Narva River have been demonstrated [71].

Chemical Assessment of Oil Shale Waste Streams

Solid wastes and soils

The oil shale industry-related pollution cycle starts from deposition of solid waste (semicoke, ashes) whereas the risk for surrounding soils, water-bodies and groundwater is now mainly caused by leaching of the toxicants by water. Waste rock, oil shale combustion ashes, semicokes of different age and leachate-polluted soils were chemically analyzed for the **key pollutant levels** (PAHs, oil products, heavy metals and phenols) (Table 2) and also analyzed for ecotoxicity:

- Eight **surface soils** and **oil shale industry solid wastes** (E1-E8) were sampled in 1999 in Ida-Virumaa and in a presumably clean area (agri-cultural soil E9 as a presumably clean control) in north-western Estonia. Heavy metals (Cd, Cu, Zn, Ni, Cr, Pb, As) and total oil products were analyzed in Laboratory of Geological Survey (LGS, Tallinn, Estonia), total PAHs in the Institute of Chemistry (Tallinn, Estonia), total water-extracted phenols in Environmental Research Laboratory of Viru County

(Kohtla-Järve, Estonia), and organic matter in the Estonian Research Institute of Agriculture (Saku, Estonia).

- Three **semicoke** samples were collected in 2002 from Kiviõli and Kohtla-Järve within the framework of a project organized by Estonian Ministry of Environment "Environmental hazard assessment of semicoke" [73]. Total organic carbon (TOC) was analyzed by Tartu Environmental Research Ltd. (Tartu, Estonia), total PAHs, total oil products by the Estonian Environmental Research Centre (Tallinn, Estonia), and volatile (monobasic) phenols by EcoLabor Ltd. (Tallinn, Estonia).
- Seven additional **oil shale** semicokes and **combustion ashes** were sampled in 2002 within the framework of Estonian-Norwegian joint research project "Risk based environmental site assessment of the oil-shale industry in Estonia" [72]. Total PAHs (sum of 16), BTEX (benzene, toluene, ethylbenzene and xylenes) and phenols (sum of 9) were analyzed in Hydroisotop GMBH (Germany), and inorganic compounds (heavy metals, Ca, S, etc.) in ACME Laboratory (Vancouver, Canada).
- Data for 6 control soils were also included: 4 "negative controls" presumably not-polluted agricultural soils and 2 "positive controls", one soil polluted with heavy metals (T_{REF}) and one with PAHs (C_{pol}) sampled from other regions. The data on soils T_{017} and T_{REF} are taken from [45].

In the presumably clean control soils all the measured pollutant levels were below Estonian PLV_r. The PAH-polluted soil (C_{pol}) contained 800 times more PAHs than allowed for residential areas and heavy metal polluted soil T_{REF} contained Cd, Pb and Zn in amounts exceeding Estonian PLV_r 2.7-4 times. The levels of heavy metals in all solid samples from oil shale industrial region were lower than respective PLV_r (i.e. <5 mg/kg for Cd, <500 mg/kg for Zn and <300 mg/kg for Pb etc), and thus heavy metal data are not presented as there is presumably no hazard through soil/waste-water path.

Three fresh semicokes as well as all combustion ashes did not contain any of the measured key pollutants in hazardous concentrations, i.e. exceeding the PLV_r. Only in fresh semicokes E3 and V0 the oil products content (527 and 1760 mg/kg, respectively) exceeded the respective PLV_r. However, old semicokes as well as leachate-polluted soils contained PAHs up to 434 mg/kg and oil products up to 7231 mg/kg. Also the total content of monobasic phenols in soil E4 and E4a (13.3 and 43 mg/kg, respectively) exceeded the respective PLV_r. Relatively higher amounts of PAHs and oil products in the old semicokes and leachate-polluted soils but not in fresh semicoke are explained by the fact that semicoke heaps have been also used for dumping of other phenolic and oily waste of oil shale chemical industry as well as waste sludge of the local wastewater treatment plant (WWTP).

Table 2. **Characterization of solid wastes and soils: chemistry**

Samples		Description	Year of sampling	pH	Orgamic matter, %	Oil products, mg/kg dwt	SumPAH, mg/kg dwt	Total phenols, mg/kg dwt	Water-soluble sulphides, mg/kg	Ca %	S %	References
Estonian PLV for industrial area (PLV_i) , mg/kg dwt				6-9		5000	200	200				
Estonian PLV for residential area (PLV_r), mg kg dwt				6-9		500	20	20				
Agricultural soils, controls	E8	Oil shale region agricultural soil	1999	6.6	25	486	0.82	0.2[a]	n.a.	n.a.	n.a.	[37, 40]
	E9	Agricultural soil from Laitse	1999	6.5	5	248	2.5	n.a.	n.a.	n.a.	n.a.	[40]
	T_{017}	Negative control soil for heavy metal pollution (France)	2000	8.3	27	n.a.	n.a.	n.a.	n.a.	4	n.a	[45]
	C	Negative control soil for PAH pollution (France)	2000	8.5	12	n.a.	15.8	n.a.	n.a.	n.a	n.a	[39]
	T_{REF}	Heavy metal polluted soil[b] (France)	2000	7.9	32	n.a.	n.a.	n.a.	n.a.	3	n.a.	[45]
	C_{pol}	PAH polluted soil (France)	2000	8.1	38	n.a.	**17503**	n.a.	n.a.	11	n.a.	
Waste rock & ashes	E1	Waste rock from the pile at Kukruse	1999	**12.1**	0.67	321	0.14	0.5[a]	n.a.	n.a.	n.a.	[37, 40]
	KJ-A	Ashes from ash landfill in Kohtla-Järve	2002	**12.9**	1.2	0.083[c]	0.01	0	n.a.	18	1	[72]
	N-AF	Filter ashes from Baltic Power Plant	2002	**12.9**	0.02	n.a.	n.a.	n.a.	n.a.	19	2	[72]
	N-AC	Collector ashes from Baltic Power Plant	2002	**12.9**	0.02	n.a.	n.a.	n.a.	n.a.	26	1	[72]
Fresh semicoke	E3	Fresh semicoke from Kohtla-Järve	1999	**12.4**	10	**527**	17.4	0.6[a]	n.a.	n.a.	n.a.	[37, 40]
	KJ-F	Fresh semicoke from new Kohtla-Järve landfill	2002	**12.3**	7.3	0.25[c]	4.5	9.4	n.a.	17	1	[72]
	V0	Fresh semicoke from Kohtla-Järve	2002	**11.8**	14	**1760**	10.8	n.a.	422	n.a.	n.a.	[73]
	K0	Fresh semicoke from Kiviõli	2002	**12.1**	12	n.a.	n.a.	n.a.	1100	n.a.	n.a.	[73]
	KV-F	Fresh semicoke from Kiviõli new landfill	2002	**12.5**	12	0.095[c]	0.51	0.9	n.a.	21	1	[72]

Table 2. Continued

Old semicoke	V10	10-years[d] old semicoke from Kohtla-Järve	2002	**10**	13	**3600**	6.8	n.a.	62.6	n.a.	n.a.	[73]
	KV-M	20-years[d] old semicoke from Kiviõli	2002	**9.9**	7.8	0.056[c]	3.4	0.1	n.a.	20	2	[72]
	KV-O	40-years[d] old semicoke from Kiviõli	2002	8	11	0[c]	0.46	0	n.a.	18	0	[72]
	E7	40-years[d] old semicoke from Kiviõli	1999	7.2	18	240	0.13	0[a]	n.a.	n.a.	n.a.	[37, 40]
	E2	50-years[d] old semicoke from Kohtla-Järve	1999	7.2	13	**1044**	**39**	0.8[a]	n.a.	n.a.	n.a.	[37, 40]
Leachate-polluted soils	E4	from Kohtla-Järve	1999	**10.3**	18	**7231**	**240**	**13.3[a]**	n.a.	n.a.	n.a.	[37, 40]
	E4a	similar to E4	2000	**10.9**	10	**1348**	**35**	**43.0[a]**	n.a.	n.a.	n.a.	[39]
	E5	Kohtla River bank soil	1999	7.6	7	**640**	**434**	1.6[a]	n.a.	n.a.	n.a.	[37, 40]
	E6	Erra River bank soil	1999	6.8	2	**2334**	0.048	0[a]	n.a.	n.a.	n.a.	[37, 40]

All the values are total concentrations (mg/kg dry soil);
The levels exceeding the Estonian permitted limit values for residential areas (PLVr) and pH values exceeding 9 are in bold;
n.a. – not analyzed;
[a] monobasic phenols (PLV_r=10 mg/kg & PLV_i=100 mg/kg);
[b] 20 mg Cd/kg, 1050 mg Pb/kg and 1390 mg Zn/kg;
[c] measured as sum of BTEX;
[d] approximate age of the deposit

Chemistry of semicoke leachates

Main characteristics of semicoke leachates:

The reported chemical composition of the semicoke leachates varies depending on the amount of rain, sampling place, time before analysis (Table 3). Those leachates contain much sulphur compounds, are very alkaline (due to the high concentration of CaO in the semicoke) and are characterised by high COD and BOD. The COD/BOD ratio about 2 indicates that leachates are largely biodegradable. High concentrations of sulphates in leachates should be related to the retort process. Other sulphur compounds such as sulphides, thiosulphates and sulphites have also been reported in significant amounts [74]. The phenols in those leachates are mainly mono-basic. In spite of being considered as the major environmental problem in leachates, they contribute only to 0.07 – 40% COD. In fact, even if the leachates were toxic [74], we have also shown that toxic impact of phenolic compounds to the overall toxicity of leachates was only 7–50%, depending on the sample [75]. And thus, the COD, and probably also toxicity may be partly due to other reducing coumpounds such as sulphides formed from sulphate by anoxic conditions. Although combustion wastes of oil shale (as well as in semicoke) are often thought to contain toxic amounts of heavy metals (by confusion with incineration of municipal waste), heavy metal content in oil shale wastes is very low. Indeed, the level of heavy metals in leachates has also been shown to be very low and not contributing to toxicity of the leachates [72, 74].

Table 3. **Composition of the semicoke dump leachates**

Reference	[69]	[6, 7, 76]	[77]	[74]	[38]	[75]	[75, 78]	[8]	[72]
pH	12–13	8.5–12	9.3–12.3	10.3	7.8	11.0	12.3	n.a.	10.5–13.2
COD, mg/l	2000–4600	795–3090	674–3533	3070	n.a.	n.a.	967	n.a.	n.a.
BOD, mg/l	810–2700	400–1650	n.a.	n.a.	n.a.	n.a.	410	183	n.a.
Total phenols, mg/l	0.1–1.4	0–34	54	195	77.6	24.4	162	56.1	0–96
Monophenols, mg/l	n.a.	n.a.	4.7–21.8	195	59.7	24.1	152	n.a.	n.a.
Contribution of phenol to COD (%)[a]	0.07	2.6	3.6	15.1	n.a.	n.a.	40	n.a.	n.a.
COD/BOD	1.7–2.4	1.4–2.0	n.a.	n.a.	n.a.	n.a.	2.4	n.a.	n.a.
Total P, mg/l	27–45	n.a.	0.4–6.1	n.a.	n.a.	n.a.	0.03	3.9	n.a.
Total N, mg/l	27–45	n.a.	13.3–55	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sulfate, mg/l	900–1000	817	n.a.	1676	n.a.	n.a.	n.a.	261	n.a.
Sulfide, mg/l	<5	n.a.	n.a.	22.2	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. – not analyzed;

[a]Calculation of contribution of phenols to COD is based on:

$C_6H_5OH + 7\ O_2 \rightarrow 6\ CO_2 + 3\ H_2O$ (Mwt phenol = 94 g , for 224 g COD)

The deposition of other wastes to the semicoke dumps explains the presence of phenols (and other pollutants not characteristic to fresh semicoke) in the semicoke dump leachates. Indeed, in fresh semicoke the organics is mainly not water-extractable (bitumoids etc, [73]): the water-extractable fraction (measured as dissolved organic carbon, DOC) was very low, about 0.025% of organic carbon in the fresh semicoke [72].

Phenolic composition of semicoke leachates

The phenolic composition of leachates as well as total concentration of phenols in the leachates is very variable (Tables 3 & 4). The Estonian Ministry of Environment has composed a list of phenolic compounds that should appropriately describe the oil shale industry-related phenolic pollution. This set includes altogether 9 different phenolic compounds: 5 monobasic phenols (phenol, *p*-cresol; 2,4-dimethylphenol; 2,3-dimethylphenol; 3,4-dimethylphenol) and 4 dibasic phenols (resorcinol, 5-methylresorcinol, 2,5-dimethylresorcinol and 5-ethylresorcinol). Most phenols of this list have also been found in semicoke dump leachates (Table 4) [75, 79, 80]. Also other phenols may be found. For example, 15-60 mg/l 2,3,4-trimethylphenol, 0–10 mg/l 2.4-dibutylphenol, 24–243 mg/l pyrocatechol were mentioned [69].

Table 4. **Phenolic composition of semicoke dump leachates**

Reference	[69][a]	[79]	[7]	[74]	[38]	[75]		[72]	
Sample	–	–	–	AHW3	AHW4	AHW1	AHW2	KJ CH1	KJ CH2
Monobasic phenols, mg/l									
Phenol	0	21.2	13.8	84.1	25.5	11.7	72.2	2	33.8
o-cresol	0–6.7	4.9	3.5	8.5	0	0	0	0.6	16.5
m-cresol	0	7.7	8.3	17.2	22.8	0	0		
p-cresol	0	9.6		70.4	0	11.1	73.9	0	8.6
2.6-DMP	0–16	3.1	n.a.	4.4	7.8	0.3	0	0	0
2.3-DMP	0		4.6[b]	2.8	2.9	0.5	3.2	0	16
3.4-DMP	0			7.7	0.84	0.5	2.5	0	6.5
Total monobasic	n.a.	46.5	30.4	195	59.8	24.1	151.8	2.6	81.4
Dibasic phenols, mg/l									
Resorcinol	0	0.77	1.2	0	0.6	0.12	3	0	0
5-MR	109–135	1.42	5.7	0	11.4	0.16	3.9	0	14.4
4.5-DMR	0	2	n.a.	0	5.8	0	3.4	n.a.	n.a.
2.5-DMR	97–220	n.a.	n.a.	0	0	0	0	n.a.	n.a.
Total dibasic	0	4.19	6.9	0	17.8	0.28	10.3	0	14.4
Total phenols	**126–171**	**50.7**	**37**	**195**	**77.6**	**24.4**	**162**	**2.6**	**96**

n.a. not analyzed;
[a] the sum includes also 3,5-DMP and 2,5-DMP;
[b] additionally 0–16 mg/l of 2,6-DMP, 15–60 mg/l of 2,3,4-trimethylphenol, 0–10 mg/l of 2,4-dibutylphenol, 24–243 mg/l of pyrocatechol

Semicoke leachates, surface- and groundwater chemistry

As water represents the major vector of transfer of pollutants, in the framework of Estonian-Norwegian joint research project "Risk-based environmental site assessment of the oil-shale industry in Estonia" [72] a special attention was paid to all waterbodies in the oil shale industry region. Twenty groundwaters, pore waters from semicoke heap (special boreholes drilled into semicoke deposits) and natural semicoke leachates from the ditches surrounding the heaps were collected. All these samples have been chemically and ecotoxicologically analysed. The content of DOC, phenols, BTEX and PAHs were determined in Hydroisotop GMBH (Germany), major and trace elements in ACME Laboratory (Vancouver, Canada). As a complement on impact of semicoke leachates on surface waters, data on two river waters (Kohtla and Purtse) are also presented (Table 5).

Concerning some aspects, leachates collected from ditches and from boreholes from Kiviõli and Kohtla-Järve waste dumps are quite similar. As shown previously, pH of leachates is very alkaline and their conductivity very high, indicating a leaching of mineral salts, as reflected by calcium and total sulphur in the leachates. However, in Kiviõli leachates PAHs, BTEX and phenols were not found in significant amounts but Kohtla-Järve leachates were contaminated by those pollutants. This could be due to the dumping of other wastes on Kohtla-Järve semicokes heaps. In fact, fresh semicokes have been shown to be free of these pollutants [73]. As a consequence, the upper layers of groundwaters proved more polluted in Kohtla-Järve region than in Kiviõli, particularly with BTEX. Some Kohtla-Järve sampling wells were also contaminated with phenols.

Wastewater treatment

Dephenolated water from the Kiviter process together with other industrial waters and domestic wastewaters are directly treated in the wastewater treatment plant by aerobic treatment. Chemical composition of influents and effluents and efficiency of biological treatment processes during different years of operation (1997–2003) are described in Table 6. Also the results of a laboratory-scale experiment on biodegradability made by Kamenev et al. are reported [8]. Table 6 shows that the contribution of phenols to COD in the case of mixed influents is also low like observed previously for semicoke leachates. However, the mixing of industrial and domestic waters led to a more favorable COD/BOD ratio for biodegradation processes yielding efficient COD removal. In addition, the BOD removal and the biodegradation of mono- and dibasic phenols in the WWTP as well as in laboratory-scale experiments was higher than 94% in most of the studies, indicating that the aerobic sludge of the WWTP is well adapted for the degradation of phenols.

Table 5. **Characteristics of natural waters, wastewaters and natural semicoke leachates**

	Sample	Origin	pH	Con-ductivity, µS/cm	DOC[a], mg/l	BTEX[b], µg/L	PAH[c], µg/L	monobasic phenols, mg/l	dibasic phenols, mg/l	total phenols, mg/l	Ca, mg/l	S, mg/l	Ref.
Groundwater PLV [81]			**6-9**			**100**	**10**	**0.1**	**0.1**	**0.2**			
River waters	Purtse	River Purtse	7.9	n.a.	n.a.	n.a.	n.a.	0	0	0.	n.a.	n.a.	[39]
	Kohtla	River Kohtla	7.8	n.a.	n.a.	n.a.	n.a.	0.	0	0	n.a.	n.a.	
River waters	7	River Purtse (+ Kohtla)	7.6	n.a.	536	n.a.	n.a.	0.05	0.75	0.8	n.a.	n.a.	[82]
	6	River Kohtla (end)	7.6	n.a.	591	n.a.	n.a.	1.14	4.16	5.3	n.a.	n.a.	
Leachates around Kohtla-Järve semicoke heaps	KJ-CH1	Ditch around ash heap	**13.2**	11240	50	25.9	0.32	**2.6**	0	**2.6**	65	192	[72]
	KJ-CH2	Ditch around semicoke heap	**13.3**	14160	530	**2229**	**19**	**82**	**14**	**96**	294	288	[72]
Leachates from boreholes in the semicoke dumps	RA-KJ5	Kohtla-Järve semicoke heap	**13.1**	18000	473	**19543**	**628**	**59**	**35**	**93**	1260	120	[72]
	RA-KJ6		**13.1**	15090	19	**1213**	**461**	**87**	0	**87**	1213	172	[72]
	RA-KV5	Kiviõli semicoke heap	**13.2**	12150	49	0	1.2	0	0	0	181	466	[72]
	RA-KV6		**10.5**	9950	11	1.8	0	0	0	0	56	972	[72]
Groundwaters	KJ-600	Vicinity of Kohtla-Järve semicoke and ash waste heaps	7.4	2950	77	**135**	7	**13**	**3.6**	**17**	42	15	[72]
	KJ-601		**11.5**	1424	55	48	0	0	0	0	3	70	[72]
	KJ-602		7.5	867	14	**111**	**24**	0	0	0	37	176	[72]
	KJ-608		7.5	1120	7.8	**141**	0	0	0	0	212	106	[72]
	KJ-610		7.3	1184	6.8	**220**	0	0	0	0	240	110	[72]
	KJ-622		**9.3**	4560	214	**167**	4	**14**	0	**14**	3	51	[72]
	RA-KJ2		7.6	597	14	91	0	**0.16**	0.01	0.16	34	23	[72]
	RA-KJ3		7.5	1904	100	**350**	0	**0.91**	0.00	**0.91**	5	61	[72]
	KV-6	Vicinity of Kiviõli semicoke waste heaps	7.2	700	3.3	0	0	0	0	0	41	13	[72]
	KV-9		7.3	968	1.9	0	0	0	0	0	111	7	[72]
	RA-KV1		**13.0**	10000	100	6.2	0.15	**2.2**	**0.2**	**2.5**	396	67	[72]
	RA-KV2		7.5	2450	35	9.5	0.16	0.02	0	0.02	566	522	[72]

n.a. – not analyzed; [a]dissolved organic carbon; [b]benzene, toluene, xylenes; [c]polyaromatic hydrocarbons

The values exceeding Estonian permitted limit values (PLV) for groundwater [81] and pH values exceeding 9 are in bold

Table 6. **Main characteristics of influent and effluent of the wastewater treatment plant (WWTP) of Kohtla-Järve**

		pH	COD, mg/l	BOD, mg/l	COD / BOD	monobasic phenols, mg/l	dibasic phenols, mg/l	total phenols, mg/l	Contribution phenol to COD %	% total phenol biodegradation	% COD removal	% BOD removal	Ref.
WWTP Kohtla-Järve	Influent	n.a.	n.a.	205	n.a.	7.5	18.9	26.4	n.a.	–	–	-	[83]
	First stage	n.a.	n.a.	155	n.a.	2.19	10.4	12.6	n.a.	52.3	n.a.	24.4	
	Second stage	n.a.	n.a.	39.4	n.a.	0.1	4	4.1	n.a.	84.5	n.a.	80.8	
WWTP Kohtla-Järve	Influent	7.0	n.a.	n.a.	n.a.	10.4	7.6	18	n.a.	–	–	–	[75, 84]
	Effluent	7.0	n.a.	n.a.	n.a.	0.25	0.44	0.69	n.a.	96.1	n.a.	n.a.	
	Influent	n.a.	951	376	2.5	3.9	18.0	21.9	13.9	–	–	–	[8]
	Effluent	n.a.	376	11	34	0.03	1.2	1.23	0.8	94.4	60.5	97.0	
Laboratory biological reactor	Influent	n.a.	4500	2400	1.9	11	95	106	2.35	–	–	–	[8]
	Effluent	n.a.	240–300	6–7	30–40	0.13–0.17	0.5–1.0	n.a.	n.a.	98.9	93.3	99.7	

n.a. – not analysed

Ecotoxicological Assessment of Waste Streams from Oil Shale Industry

As recommended in the Report on Oil Shales prepared by the Congress of USA [85], surface water monitoring in the oil shale regions should include also measurement of aquatic biota to determine the changes resulting from the industrial activities. The possible parameters include the concentrations of the pollutants themselves as well as the levels of "indicator" parameters (e.g., characteristics of the aquatic biota) that provide a measure of the potential environmental disturbance. Biological parameters are especially appropriate and useful because they reflect the stability and response of the ecosystem [86]. Aquatic organisms are natural monitors of water quality since they respond in a predictable manner to the presence of most types of pollutants. Changes may indicate problems that are not easily detected by direct measurements of water quality. For example, heavy metals and some organic compounds tend to concentrate in the biota. Their levels in the tissues of certain fish could help predict pollution concentrations that are not readily measurable in the water itself. Communities that could be monitored include invertebrates, fish, algae, and bacteria. However, such a monitoring is time consuming and requires not only quantification of specific species or genera, but also their identification and chemical analysis of the toxicants in organisms (body burden). Thus, laboratory-scale testing of acute toxic effects is adequate, cost effective and quantitative way for identification of the adverse impact of pollutants in waters. Recent development of solid phase tests allows also testing of solid wastes.

Ecotoxicological tests

Ecotoxicity of oil shale waste streams has been mainly studied by the group of ecotoxicology of NICPB, and results of these studies are summarised in the present review. Several toxicity tests with organisms of different trophic levels were used (Table 7). In addition to aquatic toxicity tests, Solid-Phase Flash-Assay (SPFA) was used for the measuring of particle-bound toxicity. Most of the toxicity tests were in form of ToxKits (Algaltoxkit F™, Protoxkit F™, Daphtoxkit F™magna, Rotoxkit F™ and Rotoxkit F™chronic) and were performed according to the respective standard operational procedures (SOPs). Also, altogether two different photobacterial strains: *Vibrio fischeri* NRRL-B 11177 (strain used in Microtox test by Azur Environmental, USA as well as in VF1500 and BioTox™ preparation by ThermoLabsystems, Finland) and *Photobacterium phosphoreum* strain FEI 162095 (registered in the Finnish Environment Institute) were applied. The latter preparation (misleadingly under name of "Biotox™ test") was first characterized in a paper by Kahru [16]. The problems with double use of the name of 'Biotox' first for *P. phosphoreum* and afterwards for *V. fischeri* have historical reasons and will

Table 7. **Characterization of biotests**

Test	Test organism	Toxicity endpoint (type of the test)	Exposure time	Guidelines
Microbiotest Assays				
Algaltoxkit F™	Microalgae *Selenastrum capricornutum*	growth inhibition (chronic test)	72 h	OECD 201, ISO 8692:1989
Protoxkit F™	Protozoa *Tetrahymena thermophila*		24 h	
Daphtoxkit F™ magna	Crustaceans *Daphnia magna*	mortality / immobilization (acute test)	48 h	OECD 202, ISO 6341:1996
Thamnotoxkit F™	Crustaceans *Thamnocephalus platyurus*		24 h	
Rotoxkit F™	Rotifers *Brachionus calyciflorus*	Survival (acute test)	24 h	
Rotoxkit F™ chronic	Rotifers *Brachionus calyciflorus*	Reproduction (short-chronic test)	48 h	
Charatox[a]	Macroalgae *Nitellopsis obtusa*	membrane depolarisation (acute test)	45 minutes	Laboratory test [87]
Photobacterial Assays				
Biotox™	Bacteria *Photobacterium phosphoreum FEI 162095*	luminescence inhibition (acute test)	15 minutes	DIN 38 421/34 and ISO 11348-2:1998
Microtox™, VF 1500	Bacteria *Vibrio fischeri* NRRL-B 11177			
SPFA	Bacteria *Vibrio fischeri* NRRL-B 11177	luminescence inhibition (acute test)	30 seconds	OECD protocol under development

[a]performed in Institute of Botany, Vilnius, Lithuania [78]

not be discussed here. However, the *V. fischeri* and Microtox and VF1500 all mean the same test organism: *V. fischeri* NRRL-B 11177.

Ecotoxicological testing: L(E)C50 and TU

The toxicity of aqueous samples and the soil/solid waste aqueous extracts was calculated from the concentration-effect curves using the regression analysis as LC50 (half-lethal concentration) or EC50 (half-effect concentration) and expressed as a concentration of the sample in %. For example: the LC50 = 25% means that the original wastewater if diluted 4-times (i.e. 25% in the test) kills half of the test organisms. The L(E)C50 values were converted to toxic units (TU) to obtain an expression of toxicity where higher TU means higher toxicity and not *vice versa* (as in case of LC50 or EC50 values). L(E)C50 values were converted to toxic units (TU) values as follows: TU = 100%/L(E)C50. In case of low toxicity, i.e. if less than 50% of test organisms were harmed during the exposure to undiluted sample, TUs were calculated as parts of 50% effect [40]. If less than 20% of test organisms were harmed during the exposure to undiluted sample, the sample was assigned for zero toxicity.

Classification of the toxicity of environmental samples: MaxTox index

The MaxTox classification system generally adheres to that proposed by Persoone et al. [88, 89] for determination of the degree of toxic contamination of natural freshwaters and groundwaters and for the ecotoxicological determination of the toxicity of all kinds of wastes prior to their release into aquatic environments without or after treatment, leachates/percolates from waste dumps and from polluted soils. The toxicity data of **environmental samples** obtained by test battery were used for the characterization and classification of the samples by MaxTox index showing the highest toxic signal of the battery and thus predicting the weakest point in the food web (Table 8).

Table 8. **Toxicity classification system adhering to Persoone et al. [88, 89]**

MaxTox value of the test battery		Classification	Designation
TU	L(E)C50		
<1 TU	L(E)C50 >100%	not toxic	☺
≥1-10 TU	L(E)C50=10%-100%	toxic	☠
≥10-100 TU	L(E)C50=1%-10%	very toxic	☠☠
≥ 100 TU	L(E)C50<1%	extremely toxic	☠☠☠

Toxicity and biodegradability of pure phenolic compounds

Most industrial organic chemicals (incl. aromatic hydrocarbons such as phenols) are thought to exhibit a narcosis mode of toxic action [90, 91] and thus additivity appears to be a reasonable assumption for risk assessment purposes of wastewater discharges [92–94]. Therefore, if the toxicity of individual phenolic compounds in mixture is additive, the chemical concentrations could be translated into toxicity data and thus used for evaluating the role of phenols in overall toxicity of a leachate.

The toxicity of monobasic phenols has been relatively widely studied [15]. However, there is very little data available on the toxicity of dibasic phenols (resorcinols). Consequently, the toxicity and biodegradability of 8 phenols from the list of Estonian Ministry of Environment (Table 9) was studied [84]. Toxicity was analyzed using a battery of microbiotests with species representing different trophic levels (photobacteria, micro-algae, protozoa, rotifers and crustaceans) and biodegradability of each phenol (2.5 mM) was studied with acclimated and not acclimated activated sludge to the phenolic compounds, whereas the efficiency of detoxification was evaluated by residual toxicity of the incubation medium using photobacteria.

Applying the criteria described in EC Directive (79/831, 7th amendment, 92/32), phenol, 2,4- and 2,3-dimethyl phenol and 5-methyl resorcinol were classified as ′toxic′ and *p*-cresol, 3,4-dimethyl phenol, resorcinol and

Table 9. **Toxicity, L(E)C50, mg/l of 8 phenolic compounds to aquatic multi-trophic test battery and classification of phenols according to toxicity and biodegradability. Modified from [84]**

	Photobacteria *V. fisheri* (Microtox)	Crustaceans *Daphnia*	Crustaceans *Thamno-cephalus*	Protozoa *Tetrahymena*	Algae *Selenastrum*	Toxicity ranking[a]	Bio-degradability[b]
Phenol	19	10	**8.3**	520	244	Toxic	fast
p-cresol	**1**	6.5	9.2	90	118	Very toxic	fast
2,4-dimethyl phenol	**3.7**	5.4	13	70	20	Toxic	moderate
2,3-dimethyl phenol	**5.3**	11	6	190	50	Toxic	moderate
3,4-dimethyl phenol	**0.39**	6.3	13	90	53	Very toxic	slow
Resorcinol	186	**0.3**	**0.2**	910	595	Very toxic	fast
5-methyl resorcinol	129	**3**	**2.5**	530	397	Toxic	fast
2,5-dimethyl resorcinol	95	**5**	**1**	470	9	Very toxic	slow

Lowest toxicity values (most sensitive test for each phenol) are indicated in bold;
[a] according to EC Directive 79/831, 7th amendment, 92/32;
[b] measured as relative detoxification time in activated sludge laboratory test [84] expressed as the time needed for the detoxification of the phenolic compound while incubated with the suspension of activated sludge. The detoxification was considered ′fast′ if it was comparable to that of phenol, ′moderate′ – 2-9 days for the municipal sludge and 5–15 days for the acclimated activated sludge. If the detoxification time exceeded 15 days for the acclimated sludge and 9 days for the municipal sludge it was classified as ′slow′. The different time-scales for the two activated sludges resulted from the different initial (inherent) toxicities of the sludges.

2,5-dimethyl resorcinol as ′very toxic′ (Table 4 and [84]). The most sensitive tests were crustaceans (*Daphnia* and *Thamnocephalus*) and photobacteria (*V. fischeri*) whereas the photobacteria were more sensitive towards monobasic phenols and crustaceans towards resorcinols. The higher toxicity of resorcinols to *Daphnia* compared to monobasic phenols was also shown by Trapido and Veressinina [95] and the higher toxicity of monobasic phenols to photobacteria compared to resorcinols by Kahru et al. [96]. As crustacean and photobacterial tests were most sensitive in the battery and exhibited different but complementary sensitivity patterns, they were included in the test battery for the ecotoxicological hazard prediction of oil-shale industry pollution in further studies. Also, algal test, although not sensitive to oil-shale phenols, was included in the battery as a representative of primary producers and sensitive test for heavy metals [45].

Phenol, p-cresol, resorcinol and 5-methyl resorcinol were most rapidly detoxified, 2,3- and 2,4-dimethyl phenol had moderate detoxification rate, while the slowest was the detoxification of 2,5-dimethyl resorcinol and

3,4-dimethyl phenol [84]. Phenol and *p*-cresol, the most abundant phenols in most of the semicoke dump leachates (Table 4), were both rapidly detoxified and thus could be considered easily biodegradable.

Water-extracted and particle-bound toxicity of solid wastes and soils

As solid wastes induce potential hazard for groundwater and surface waters, the **water-leachable toxicity** of solid samples was analyzed by a battery of aquatic tests applied on laboratory leachates of solid samples. In addition, **particle-bound bioavailable toxicity** in the case of contact exposure was analyzed by the difference between the results of Solid Flash assay (toxicity of the suspensions containing solid matrix was analysed) and Flash assay (corresponding particle-free aqueous extracts were analysed) (Table 10).

All the presumably **clean (agricultural) soils** proved ′not toxic′ in all the tests. The positive control soils T_{REF} containing high levels of heavy metals showed no water-extracted toxicity. The PAH-polluted soil C_{pol} showed relatively low water-extracted toxicity (MaxTox 1.7 TU) compared to its very high total PAH levels (17,503 mg/kg; Table 2). This could be explained by low bioavailability of particle-bound hydrophobic pollutants (e.g., PAHs) in aged soils [26] as well as particle-bound heavy metals via soil-water path [23, 45, 49].

In the **waste rock** sample from Kukruse (E1), pollutants were below hazardous levels, but due to alkaline pH almost all aquatic tests showed toxicity (MaxTox = 17.8). The water-extracted toxicity of **oil shale combustion ashes** was very high: MaxTox 27-50 TU for aquatic test battery and even 38-824 TU for SPFA. As these samples did not contain any measured key pollutants in hazardous level (Table 2), the high toxicity of these samples is probably caused by alkaline pH: the Microtox test showed 63-160-fold reduction of toxicity of water extracts after neutralization. However, even neutralized samples showed some toxicity in Microtox assay (1.6-2.5 TU; Table 10).

Fresh semicokes were all classified ′very toxic′ (MaxTox 17.8-97), whereas the SPFA was the most sensitive test of the battery in most cases showing the hazard to biota exposed by direct contact or ingestion. The very alkaline pH (about 12) may explain at least partly the toxicity of fresh semicoke as neutralized leachates were 2–126 times less toxic in Microtox test. But in 4 cases of 5 toxicity (2.9-4 TU) was still present in neutralized leachates. As the content of water-soluble sulphides in V0 and K0 was 42–110 mg/l in the aqueous extract (Table 11), it is possible that the residual toxicity of the neutralized leachates to photobacteria (2.9 and 3.7 TU, respectively; Table 10) was caused by sulphides. In fact, sulphides are very toxic for biota: EC50 for photobacteria 7 mg/l [96].

Table 10. **Water-extracted and particle-bound bioavailable toxicity of solid wastes and soils from the oil shale industry (in toxic units, TU)**

sample	pH	TU, Microtox (pH not adjusted)	Decrease of toxicity after neutralisation, times[a]	Charatox[b]	Algaltox[b]	Protox[b]	Daphtox[b]	Thamnotox[b]	Rotox[b]	Microtox pH neutral[b]	Flash solid[b]	Flash extract[b]	MaxTox, TU	Classification[c]
Agricultural and control soils														
E8	6.6	0	n.a.	0.9	0	0.8	0	0	0	0	0	0	0.9	☺
E9	6.5	0	n.a.	0.9	0	0.5	0	0	0	0	0	0	0.9	☺
T017	8.3	0	n.a.	n.a.	0	0	n.a.	0	n.a.	0	0	0	0	☺
C	8.5	0	n.a.	n.a.	0	0.8	0	0	n.a.	0	0	0	0.8	☺
T_{REF}	7.9	0	n.a.	n.a.	0	0	n.a.	0	n.a.	0	0	0	0	☺
C_{pol}	8.1	0	n.a.	n.a.	**1.7**	**1.6**	**1.1**	0	n.a.	0	0.7	0	1.7	☠
Waste rock and combustion ashes														
E1	**12.1**	47	>47	**12**	**5.2**	**1.5**	**5.2**	**18**	0	0	**6.4**	**5.3**	18	☠☠
KJ-A	**12.9**	253	160	n.a.	**11**	0.7	**13**	**50**	n.a.	**1.6**	**38**	**8.0**	50	☠☠
N-AF	**12.9**	234	123	n.a.	**9.4**	1.0	n.a.	**50**	n.a.	**1.9**	**614**	**286**	614	☠☠☠
N-AC	**12.9**	158	63	n.a.	**9.4**	**1.1**	**27**	**5.0**	n.a.	**2.5**	**824**	**313**	824	☠☠☠
Fresh semicokes														
E3	**12.4**	42	>42	**18**	0.4	**1.2**	**14**	**14**	**1.8**	0.0	**5.3**	**4.6**	18	☠☠
KJ-F	**12.3**	78	20	n.a.	**3.2**	0.7	**5.3**	**5.3**	n.a.	**4.0**	**97**	**5.0**	97	☠☠
V0	**11.8**	36	13	n.a.	**2.3**	0.0	**2.7**	**2.4**	n.a.	**2.9**	**85**	**10**	85	☠☠
K0	**12.1**	6	2	n.a.	**1.7**	1.0	**2.9**	**5.3**	n.a.	**3.7**	**89**	**8.0**	89	☠☠
KV-F	**12.5**	417	126	n.a.	**5.2**	0.8	**17**	**10**	n.a.	**3.3**	**58**	0.0	58	☠☠

Table 10. Continued

Old semicokes														
KV-M	**9.9**	3	1	n.a.	0.4	0.5	0	1.0	n.a.	**2.5**	**1.3**	0	2.5	☠
KV-O	8	0	n.a.	n.a.	0	**1.0**	0	0	n.a.	**2.5**	0	0	2.5	☠
E7	7.2	0	n.a.	0	0	0.6	**1.5**	0	**1.6**	0	**3.5**	0	3.5	☠
V10	**10.0**	3	1	n.a	0	0	0	0	n.a	**2.4**	**4.0**	0	4.0	☠
E2	7.2	0	n.a.	**2.9**		**1.2**	0	0	**1.3**	0	0	0	2.9	☠
Leachate polluted soils														
E4	**10.3**	n.a.	n.a.	**8.7**	0	**1.3**	**7.5**	0.6	**2.3**	**1.5**	**5.6**	**3.0**	8.7	☠
E4a	**10.9**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0	n.a.	n.a.	**30**	**3.0**	30	☠☠
E5	7.6	0	n.a.	**1.2**	0	**1.1**	**1.1**	0	**2.2**	0	**5.3**	0	5.3	☠
E6	6.8	0	n.a.	0.9	0	0.4	0	0	0	0	**1.2**	0	1.2	☠

[a] reduction of toxicity (folds) after adjustment of pH to neutral (toxicity was measured by Microtox test);
[b] test battery used for classification by MaxTox, the highest TU value for each sample (MaxTox) was used for toxicity ranking.;
[c] ☺ not toxic, ☠ toxic, ☠☠ very toxic, ☠☠☠ extremely toxic; n.a. – not analyzed
Test results showing toxicity (TU ≥ 1) and pH values exceeding 9 are in bold

Table 11. **Comparison of laboratory leachates of semicokes of different age. Data compiled from Table 10 and [72, 73]**

Sample	Origin	Approxi-mate age, years	pH	Ca, mg/l	Sulfide, mg/l	Conduc-tivity, µS/cm	MaxTox, TU
E3	Kohtla-Järve	0	12.4	n.a.	n.a.	n.a.	17.8
KJ-F	Kohtla-Järve	0	12.3	1145	n.a.	4440	97
V0	Kohtla-Järve	0	11.8	n.a.	42	n.a.	85
K0	Kiviõli	0	12.1	n.a.	110	n.a.	89
KV-F	Kiviõli	0	12.7	14441	n.a.	5600	58
V10	Kohtla-Järve	10	10.0	n.a.	6.2	n.a.	4
KV-M	Kiviõli	20	9.9	144	n.a.	1123	2.5
KV-O	Kiviõli	40	8.0	26	n.a.	357	2.5
E7	Kiviõli	40	7.2	n.a.	n.a.	n.a.	3.5
E2	Kohtla-Järve	50	7.2	n.a.	n.a.	n.a.	2.9

n.a. – not analyzed

Old semicokes of different age (10–50 years of deposition) were remarkably less toxic than fresh ones and were classified as ′toxic′ (MaxTox 2.5-4 TU). The decrease in toxicity of semicoke during open deposition is probably a result of leaching of Ca and resulting decrease in alkalinity (Table 11), and probably also of evaporation (if volatile) or degradation of organic toxicants. It is reasonable to suggest that also other water-leachable toxicants will be washed out from semicoke to surrounding soils and upper groundwater layers. The amount of sulphides in laboratory leachates of 10-year-old semicoke (V10) was 6.2 mg/l being remarkably lower than in fresh semicokes, 42–110 mg/l. The old semicokes were only slightly alkaline (pH up to 10), and thus the neutralization did not reduce the toxicity of their water extracts to photobacteria (Table 10).

Semicoke from an old Kiviõli dump (E7; about 40 years old) showed toxicity in some biotests (Table 10, [37]) but did not contain any of the measured key pollutants above PLV_r. This heap is now used as sightseeing place for local people and tourists. Also, the old waste-rock heap near the village Kukruse (E1) is in public use for motor sports.

Toxicity of leachates, surface and groundwaters

The toxicity of two river waters (Kohtla and Purtse), influent and effluent of Kiviter WWTP, 6 natural leachates sampled from ditches surrounding semicoke heaps, 4 semicoke heap pore waters and 12 groundwaters (mostly from upper horizons and at maximum distance of 500 m from semicoke dumps) was studied with a battery of tests (algae, crustaceans, protozoa, rotifers and photobacteria, Table 12).

Table 12. **Toxicity (TU) of natural waters, wastewaters and groundwaters from oil shale industry region**

Sample	pH	Microtox pH not adjusted	Impact of unfavorable pH [a]	Impact of phenols to toxicity, %	Charatox[b]	Algaltox[b]	Protox[b]	Daphtox[b]	Thamnotox[b]	Rotox[b]	Microtox pH neutral[b]	MaxTox, TU	Classification[c]
River waters													
Purtse	8	n.a.	n.a.	n.a.	n.a.	0	0	0	0	0	0	0	☺
Kohtla	8	n.a.	n.a.	n.a.	n.a.	0	0	0	0	0	0	0	☺
Wastewater treatment plant													
Effluent	7	n.a.	n.a.	n.a.	n.a.	0	0	**3.3**	0	0	0	3.3	☠
Influent	7	n.a.	n.a.	n.a.	n.a.	0	**7**	**14.7**	**3**	0	**14**	14.7	☠☠
Semicoke leachates													
AHW1	**11**	**6**	n.a.	n.a.	**14**	0	**3**	**2**	**2**	**2**	n.a.	14	☠☠
AHW2	**12**	**154**	n.a.	n.a.	**22**	0	**63**	**63**	**48**	**63**	n.a.	154	☠☠☠
AHW3	**10**	**77**	n.a.	n.a.	n.a.	0	**15**	**19**	**10**	**17**	n.a.	77	☠☠
AHW4	7.5	n.a.	n.a.	n.a.	**9**	**5**	**22**	**17**	**6**	**7**	**67**	67	☠☠
KJ-CH1	**13.2**	**159**	174	18	n.a.	**16**	0	n.a.	**20**	n.a.	1	20	☠☠
KJ-CH2	**13.3**	**265**	1	9	n.a.	**22**	**1**	n.a.	**48**	n.a.	**345**	345	☠☠☠
Pore waters of oil shale industry waste deposits													
RA-KJ-5	**13.1**	**436**	1	11	n.a.	**63**	**50**	n.a.	**27**	n.a.	**304**	304	☠☠☠
RA-KJ-6	**13.1**	**412**	1	8	n.a.	**1**	n.a	n.a.	**100**	n.a.	**449**	449	☠☠☠
RA-KV-5	**13.2**	**103**	21	0	n.a.	**12**	**2**	n.a.	**26.7**	n.a.	**5**	26.7	☠☠
RA-KV-6	**10.5**	**4**	1	0	n.a.	**1**	n.a	n.a.	**3**	n.a.	**4.4**	4.4	☠
Groundwaters													
KJ-600	7.4	n.a.	n.a.	37	n.a.	**3**	**10**	n.a.	**6**	n.a.	**24**	24	☠☠
KJ-601	**11.5**	**21**	17	0	n.a.	**3**	1	n.a.	**4.9**	n.a.	**1**	4.9	☠
KJ-602	7.5	n.a.	n.a.	0	n.a.	0	1	n.a.	0	n.a.	0	1.0	☺
KJ-608	7.5	n.a.	n.a.	n.a.	n.a.	0	0.9	n.a.	0	n.a.	0	0.9	☺
KJ-610	7.3	n.a.	n.a.	n.a.	n.a.	0	**1**	n.a.	0	n.a.	0	1.0	☺
KJ-622	9.3	**29**	1	11	n.a.	**2**	**3**	n.a.	**4**	n.a.	**31.3**	31.3	☠☠
RA-KJ-2	7.6	n.a.	n.a.	n.a.	n.a.	0	**1.2**	n.a.	0	n.a.	0	1.2	☠
RA-KJ-3	7.5	n.a.	n.a.	0	n.a.	0	0.9	n.a.	0	n.a.	0	0.9	☺
KV-6	7.2	n.a.	n.a.	n.a.	n.a.	1	0	n.a.	0	n.a.	0	1.0	☺
KV-9	7.3	n.a.	n.a.	n.a.	n.a.	0	0	n.a.	0	n.a.	0	0.4	☺
RA-KV-1	**13**	**169**	124	11	n.a.	**18**	**4**	n.a.	**31.8**	n.a.	**1**	31.8	☠☠
RA-KV-2	7.5	n.a.	n.a.	n.a.	n.a.	0	**1**	n.a.	1	n.a.	0	1.0	☺

[a] reduction of toxicity (folds) after adjustment of pH to neutral (Microtox test);
[b] tests included in test battery for toxicity classification,
[c] ☺ not toxic, ☠ toxic, ☠☠ very toxic, ☠☠☠ extremely toxic
n.a. – not analyzed
Test results showing toxicity (TU ≥ 1) and pH values exceeding 9 are in bold

Semicoke leachates (6 samples; Tables 5 and 12) were very alkaline (pH up to 13) and contained 2.6–195 mg phenols/l. The pollutant levels as well as

toxicity of these leachates (MaxTox 14-345 TU) varied. They were classified from ′very toxic′ to ′extremely toxic′. For the analysis of impact of phenolic compounds to net toxicity of environmental samples, the phenolic composition (HPLC) and the toxicity of the wastewater (or leachate) to Microtox bacteria were measured. The toxicity of pure phenolic compounds to Microtox bacteria was taken from [96] (EC50, mg/l: *o*-cresol 16.3; *m*-cresol 5.5; 2,6-DMP 10.6 and 2,3,5-TMP 7.5) and [84] and used for the calculations of the impact of phenols to the net toxicity of the (waste)water or leachate. Additive toxicity of individual phenols in mixture to *V. fischeri* was assumed. Phenols accounted for only 9–18% of the net toxicity of these semicoke leachates (Tables 5 and 12). The alkalinity also contributed to toxicity, but is definitely not the only reason as neutralisation reduced toxicity to Microtox test bacteria in the case of KJ-CH1 (174-fold) but not in the case of KJ-CH2 (Table 12).

In order to evaluate the impact of phenols on the toxicity of leachates to different test organisms, the toxicities of semicoke leachates (AHW1-AHW3; Table 12) and of their synthetic phenolic analogues (SPA) were simultaneously analyzed with a battery of tests. The contribution of phenols to the net toxicity of wastewaters was calculated from the difference [39]. Natural leachates were up to 75 times more toxic than their synthetic phenolic analogues, i.e. about 1% of the toxicity of some samples was “explained” by phenols [39]. Thus, phenols are apparently not the only toxicants as it was already stated for the leachate-polluted soils.

The **pore waters** obtained from special boreholes **in semicoke dumps** in Kohtla-Järve (RA-KJ-5 & 6) as well as in Kiviõli “operating” semicoke heap (RA-KV-5 & 6) were also studied. The ecotoxicological test battery classified Kohtla-Järve heap pore waters as ′extremely toxic′ (MaxTox 304 and 449 TU, respectively). The PLVs for groundwater were exceeded for PAHs, BTEXs and phenols 63, 195 and 480 times (Table 5), respectively. Despite of the relatively high levels of BTEX (up to 19.5 mg/l) and PAHs (up to 0.6 mg/l; Table 5), these concentrations are not toxic in ecotoxicological assays. The Microtox EC50 values for most water-soluble PAHs naphthalene is 1.9 mg/l and phenanthrene 0.48 mg/l [58]. The toxicity of BTEX compounds in Microtox assays is even lower: the EC50 value for benzene is 531 mg/l, toluene 33 mg/l and xylene 97 mg/l [16]. Only 8–11% of the toxicity of these samples was explained by phenols. Alkalinity of pore waters from Kohtla-Järve semicoke heap was not responsible for their toxicity (Table 12 and 5). Thus, the toxicity of these pore waters must be caused by other contaminants, e.g., originating from oil pitch (fusses) that have to be analysed for their biological effects in the future. The Kiviõli semicoke pore waters were by an order of magnitude less toxic than that of Kohtla-Järve: MaxTox 4.4 and 27 TUs, respectively. Despite of the high toxicity, the sample RA-KV-5 did not contain any key pollutants in hazardous concentrations (Table 5). Also, alkalinity (pH 13.1) of this sample did not explain all the toxicity, as after the neutralization the toxicity was considerably reduced (21 times), but the sample still remained toxic (5 TU).

The **groundwater** is the main source of drinking water in this area, and the leaching from semicoke heaps poses hazard to the groundwater. Therefore, the concentrations of key pollutants in groundwaters have been monitored [97]. The present study classified 8 of 12 groundwaters ′not toxic′, the MaxTox values lower or slightly above 1 TU (Table 12). Four groundwaters were toxic, 2 of them (KJ-601 and RA-KV-1) mostly due to alkalinity as after neutralisation their toxicities were reduced 17 and 124 times, respectively. In 3 toxic groundwaters phenols accounted for up to 37% of the net toxicity (Table 12). Thus, most of the groundwaters, although sampled from upper aquifer layers and close to semicoke dumps, were not polluted and toxic. However, when the toxicity was established, it was not totally explained by the pollutants measured (phenols, BTEX) as well as alkalinity. Thus, the full list of toxicants present in oil shale wastewaters and polluted groundwater has to be found out in the future.

As expected by available chemistry data (Table 5), both **river water samples** (Kohtla and Purtse) were classified ′not toxic′.

The **influent** directed to the local wastewater biotreatment plant (18 mg/l phenols, containing also wastewaters of the oil shale chemical industry) was not toxic for rotifers, but ′very toxic′ to *Daphnia magna* (14.7 TU) and *V. fischeri* (14.5 TU) (Table 10). The **treated effluent** still contained 0.7 mg/l phenols, i.e. more than allowed according to HELCOM (0.5 mg/l) and it was still toxic to *Daphnia magna* (3.3 TU). The reasons for residual toxicity could be residual resorcinols (0.42 mg/l resorcinol) [75] as *Daphnia* is very sensitive towards resorcinol (LC50=0.3 mg/l).

Biodegradability of the leachate toxicants in the activated sludge system

In order to solve the problem of semicoke leachate, treatment by activated sludge process could be proposed. Thus, the toxicity of semicoke dump leachate containing 195 mg phenols/L (Table 2) to two different activated sludges (acclimatized and not acclimatized to phenolic wastewater) was evaluated by measuring the adenosine triphosphate (ATP) content of the sludge after exposure of the sludge to leachate for 60 minutes. In parallel, the toxicity of the leachate to photobacteria was measured [96]. Leachate was relatively non toxic to activated sludges, especially to the acclimatized sludge. The respective 60-min EC50 value for leachate (the concentration of leachate, %, which decreased the ATP level of activated sludge compared to the not-exposed control by 50% after being in contact with activated sludge for 60 minutes) was 20–30% for both, not acclimatized sludge and photobacteria whereas for acclimatized sludge the toxicity was 2–3 times lower (i.e. 50–60%). Therefore, the biopurification of this leachate AHW using an adapted activated sludge process was considered as feasible [96].

In further studies, the detoxification efficiency of the acclimated activated sludge consortium and phenol-utilizing bacterial strains *Rhodococcus sp.*, *Pseudomonas sp.* and *Kurthia sp* isolated previously from the activated sludges of Kohtla-Järve WWTP acclimated towards phenolic compounds were studied [38]. The two leachates studied contained 78 and 195 mg phenols/L and were toxic to photobacteria, daphnia and rotifers (LC50 1-9%). The acclimated activated sludge consortium proved remarkably more powerful in removal of toxicity of semicoke dump leachates than the pure cultures of phenol-degrading strains (*Rhodococcus sp.*, *Pseudomonas sp.* and *Kurthia sp)*: relative detoxification times were 2–3 days (activated sludge) versus > 7 weeks (pure bacterial strains). Compared with an artificial laboratory leachate of a similar phenolic and sulphide composition (Table 3), detoxification of natural leachate compared to its "phenolic analogue" was slower (≥3 days versus 2 days) being most probably inhibited by inorganic (e.g., sulphuric) compounds present in the leachate. Also, the presence of toxic recalcitrant organic compounds in the leachate (missed by chemical analysis) that were not readily biodegradable even by activated sludge consortium should not be excluded [38]. Thus, it showed again that the biotreatment of the wastewaters and leachates of semicoke dumps in the activated sludge process could be feasible for the detoxification of its phenolic constituents [38, 96].

The biodegradation efficiency of phenol, dimethylphenols and cresols in semicoke dump leachates was also studied by Heinaru et al. [10] in micro-cosms with 4 phenol-degrading species originating from oil-shale region. Their results were different from those of Kahru et al. [38]: complete degradation of phenol and 3.4-DMP was observed within 2 days, cresols within 10 days but there was no significant degradation of other dimethyl-phenols during 30 days. These apparent discrepancies may be explained by the major differences in experiments. In our system *V. fischeri* was used as a "sensor" to report the detoxification of the test medium due to biodegrada-tion of the phenolic compound by activated sludges or other phenol-degrading bacteria. For example, contrary to Heinaru et al. [10] we reported that 3,4-DMP was detoxified slowly (Table 9). It could be partly due to the fact that photobacteria are extremely sensitive towards 3,4-DMP (EC50 <0.5 mg/l) and thus the toxicity was still present even if 99.8% of the compound is degraded. The efficiency of activated sludge process in removal of phenols has been studied by Munter et al. [4], who also showed that monobasic phenols were more efficiently removed than resorcinols: the concentration of resorcinols in the effluent remained even as high as 35–40 mg/l. These data are in accordance with [84]: the detoxification rate of 2,5-dimethyl resorcinol in the activated sludge system was slow.

Despite of that these phenolic compounds could be still considered to be of low environmental hazard due to their rapid biodegradability/detoxification in activated sludge test (Table 12) as well as in soil [98]. The degradability of phenolic compounds both in stabilization ponds as well as in batch lagooning

processes has been studied by Orupõld et al. [6, 7, 76]. Also, natural attenuation potential was shown for Ida-Viru County Rivers, continuously polluted with phenolic compounds of semicoke leachate due to the presence of the phenol-degrading strains, mainly pseudomonads [77, 99, 100].

Among the methods of removal of phenols (biodegradation, chemical oxidation with O_3 or mixture of O_3/H_2O_2) aerobic biotreatment has been considered the most feasible option for the treatment of wastewaters and leachates of semicoke heaps [101, 102]. However, these oxidative methods are relatively expensive [4] and may lead to the formation of the toxic by-products from phenol, dimethylphenols and cresols. In the case of dimethylphenols the complete detoxification was not achieved [95]. The feasibility of the biological methods to mineralize high-strength phenolic mixture (containing phenol, cresols and dimethylphenols) has also been shown by Brenner et al. [103]. It should be mentioned that by now the wastewater treatment facilities of VKG have been renovated, and, to our current knowledge, a part of the semicoke leachates is treated in this plant.

Fate and toxicity of phenolic pollutants in solid wastes and soils

The potential transfer of pollutants from semicoke heaps to soils is illustrated by water-extracted toxicity of leachate-polluted soils (MaxTox 1.2-30 TU; Table 8). Although these soils contained relatively high amounts of oil products and also PAHs (Table 2), the water-extracted toxicity of these soils was not explained by these pollutants, mostly due to their low water-solubility. For comparison, the levels of PAHs in oil-shale region samples were much lower than in Cpol but the water-extracted toxicity was higher. As for the leachate-polluted soils, very low level of water-extracted phenols (<0.2 mg/kg) [98] compared to high level of phenols in the leachates that are polluting these soils (up to 195 mg/l; Table 5) was especially surprising. Theoretically these very low concentrations of phenols in leachate-polluted soils may be explained: 1) by sorption/aging of the phenols; 2) by natural attenuation, i.e. biodegradation by indigenous bacteria, and 3) by degradation of phenols during the extraction procedure itself.

The **sorption** of phenols by soil matrix is possible as suspensions of soil E4a studied in the SPFA were ′very toxic′, 30 TU, while the respective particle-free extracts were of much lower toxicity (3 TU; Table 10). Thus, the following conclusions were reached [98]: The SPFA detected water-extracted toxicity that was not explained by phenols, PAHs and oil products as much more contaminated soils (E6 and E2) showed no toxicity in this assay. Low concentration of phenols in the soils of the oil-shale region is most probably the reflection of both **natural attenuation** (biodegradation by indigenous bacteria) and pollution aging. The “aging” has been demonstrated for hydrophobic organic pollutants (e.g., PAHs): they are adsorbed by soil particles, become sequestered and of reduced bioavailability. The example of

decrease of hazard due to aging of pollution was also demonstrated by analysis of soil of Erra river bank (E6) that contained 2334 mg oil/kg but showed practically no water-extracted toxicity (MaxTox 1.2 TU (Table 10). The latter is surprising because for more than 50 years, until 1979, the oil shale chemical industry wastewaters were directed through this river. Despite of the fact that the water-extracted toxicity was not detected in this sample, the Solid-Phase Flash-Assay showed the presence of sorbed toxicants in this sample. All leachate-polluted soils showed the presence of particle-bound bioavailable toxicity (Table 10). Theoretically, particle-bound oil products and phenols may contribute to this toxicity but the presence of other hydrophobic pollutants characteristic to oil shale industry is not excluded.

It needs to be stressed that the phenolic compounds present in the polluted soils and waters are organic compounds that are relatively easily biodegradable by the indigenous ("local") microflora. **Biodegradation during the extraction procedure** was studied in a leachate polluted model soil E4a containing 43 mg/kg monobasic phenols, 1348 mg/kg oil products and 35 mg/kg PAHs [98]. Only 5.8% of phenols was water-extracted, whereas about 50% of the leached amount was biodegraded by the soil microorganisms during 24 h of extraction. Phenol and cresols were biodegraded by 80%, but the concentration of dimethylphenols practically did not change.

Comparison of chemical and ecotoxicological evaluations

There is not much information available in the literature on the correlation between the chemical concentrations of pollutants and biological adverse responses to the contaminants in the soil environment. Mostly these studies have been associated with the following of the efficiency of bioremediation/ biodegradation of soils contaminated with oil [104], PAHs [33], pentachlorophenol [105], explosives [52] and hazard evaluation of solid wastes [50, 60, 106]. In most of these studies the direct correlation between the concentration of the pollutants and the toxicity was not found. Quite often the toxicity or mutagenicity increased during the biodegradation process, probably due to the formation of more toxic and/or more polar metabolites compared to the parental compound(s) by the soil microbes during the biodegradation [52, 105]. **In the case of oil shale waste deposits and soils,** the chemical and ecotoxicological data of samples are summarized in Table 13.

Table 13 shows that the aqueous extracts of presumably **clean control soils** (agricultural soils) were also not toxic in the biotests used. The **negative control soils** polluted by heavy metals or PAHs also did not show much water-extractable toxicity as these pollutants strongly sorb to solid matrix. The solid wastes of oil-shale industry and soils showed both, water

Table 13. **Comparison of chemical and toxicological evaluations of solid wastes and soils**

	Pollutants and number of samples where PLV_r was exceeded	Max Tox, TU	Classification by toxicity[a]	Particle-bound toxicity[b]	Most sensitive tests
Control soils					
Agricultural soils (4) HM-polluted soil (1) PAH-polluted soil (1)	– Zn, Cd, Pb PAHs, Cd	0–0.9 0 1.7	☺ ☺ ☺ – ☠	0/4 0/1 0/1	 Algae
Oil shale industry solid wastes and soils					
Waste rock (1)	–	17.8	☠☠	0/1	Crustaceans[c] algae
Combustion ashes (3)	–	50–824	☠☠ – ☠☠☠	3/3	SPFA, crustaceans[c]
Fresh semicokes (5)	oil products (2)	18–97	☠☠	4/5	SPFA, crustaceans[c], algae
Old semicokes (5)	PAHs (1), oil products (2)	2.5–4	☠	3/5	SPFA, crustaceans[c], photobacteria
Leachate-polluted soils (4)	PAHs (3), oil products (3), phenols (2)	1.2–30	☠ – ☠☠	4/4	SPFA, algae

[a]☺ not toxic, ☠ toxic, ☠☠ very toxic, ☠☠☠ extremely toxic; [b] positive samples/total number of samples analyzed, [c]*Daphnia magna* or *Thamnocephalus platyurus* tests

extractable as well as particle-bound bioavailable toxicity, although not all samples contained analysed key pollutants in hazardous amounts.

Oil shale combustion ashes and in general also fresh semicoke did not contain heavy metals, oil products, PAHs and phenols in hazardous levels (i.e. exceeding the PLV_r) but showed water-extracted toxicity, mostly due to unfavorable pH and probably also due to sulphides. It is important to note that fresh semicoke was classified as hazardous waste according to acute toxic hazard via solid waste-water path [37] together with the findings of high TOC content (12–14%) and high pH (pH 10-13) [73]. Old semicokes (being washed by rain) were practically neutral and considerably less toxic. In old semicokes and especially in leachate-polluted soils the concentrations of oil products and PAHs were relatively high but did not explain the water-extracted toxicity of samples. Solid-Phase Flash Assay detected the particle-bound toxicity in all leachate-polluted soils. Theoretically, oil products and phenols may contribute to this toxicity but the presence of other pollutants specific to oil shale industry is not excluded. Also, accumulation of hydro-

phobic pollutants in the leachate-polluted soils as well as river sediments could pose a problem.

The **most sensitive tests** for the analysis of water-extracted toxicity of oil shale industry solid wastes and soils were crustaceans (both, *D. magna* and *T. platyurus*) and algae. SPFA was the most sensitive test for 11 oil-shale region samples of 18 out of ones, and particle-bound toxicity was detected in 14 samples indicating that this test is a valuable tool for screening of oil shale industry pollution (Table 13). Due to the simplicity of use, small sample volumes and short exposure time, Microtox assay proved very informative for the analysis of impact of alkalinity (Table 10).

In the case of semicoke leachates and other aqueous samples, the impact of phenolic and sulphuric compounds, heavy metals and alkalinity to the net toxicity of one leachate was evaluated using luminescent photobacteria [74]. In this highly contaminated leachate (195 mg phenols/l, 1700 mg sulphate/l and 22 mg sulphide/l, 0.5 mg total heavy metals/l, Table 3) the toxicity was mainly attributed to phenolic and sulphuric compounds whereas the main contributors were *p*-cresol (58% of toxicity), sulphide (22%), 3,4-dimethyl-phenol (8.5%) and phenol (5.6%). This leachate had very low buffering capacity and concentration of heavy metals in the leachate was very low (total 0.5 mg/l), and thus toxicological impact of alkalinity and heavy metals was considered negligible. It should be mentioned, however, that in the case of other leachates and polluted groundwaters (Table 14) the alkalinity had high impact to the net toxicity of the leachate.

Table 14 shows that the **semicoke dump leachates** contained 3–195 mg phenols/l, were very alkaline (pH up to 13) and ′very toxic′ to ′extremely toxic′. The **pore waters of semicoke heap** contained up to 93 mg/l phenols, up to 19 mg/l BTEX, up to 0.6 mg/l PAHs and were ′toxic′ to ′extremely toxic′.

Study of the **groundwaters** of the upper layer of aquifer from the vicinity of semicoke dumps showed that 8 out of 12 samples could be considered not hazardous. However, some groundwaters contained up to 17 mg phenols/l and were very toxic, while the toxicity was caused not only by phenols and alkalinity, but there must be other toxicants of concern as well.

The **most sensitive tests** for the analysis of aqueous sample toxicity were crustaceans and luminescent photobacteria. Thus, even if the samples meet the criteria of the respective environmental law, there still may be a hazard to the biota that could be demonstrated by applying relevant ecotoxicological assays.

Table 14. **Comparison of chemical and toxicological evaluations: aqueous samples**

	Determined key pollutants and number of samples where $PLV_{groundwater}$ was exceeded	*pH*	*Total phenols, mg/l*	*Impact of phenols,%* [a]	*Impact of pH* [b]	*MaxTox* [c]	*Toxicity* [d]	*Most sensitive test*
2 river waters	Phenols (0)	7–8	0	n.a.	n.a.	0	☺	
1 WWTP effluent	Phenols (1)	7	18	7	n.a.	3.3	☠☠	Crustaceans[e]
1 WWTP influent	Phenols (1)	7	0.7	n.a.	n.a.	14.7	☠	Crustaceans[e]
6 semicoke natural leachates	Phenols (6), BTEX (1), PAH (1)	7–13	3–195	4–50 9–18	1–174	14–345	☠☠–☠☠☠	Crustaceans[e], protozoa, photobacteria
2 Kohtla-Järve semicoke pore waters	Phenols (2), BTEX (2), PAH (2)	13	87–93	8–11	1	304–449	☠☠☠	Photobacteria
2 Kiviõli semicoke pore waters	Phenols (0), BTEX (0), PAH (0)	10–13	<0.01	11	1–21	26.7–4.4	☠–☠☠	Crustaceans[e], photobacteria
8 groundwaters around Kohtla-Järve semicoke heaps	Phenols (4), BTEX (6), PAH (1)	7–13	0–17	0–37	1–17	0.9–24	☺–☠☠	Photobacteria, crustaceans[e]
4 groundwaters around Kiviõli semicoke heaps	Phenols (1), BTEX (0), PAH (0)	7–12	0–2.5	11	124	1–31.8	☺–☠☠	Crustaceans[e]

[a] calculated from the toxicity of individual phenols and the overall toxicity of the sample (Microtox test; see 2.8), [b]reduction of toxicity (folds) after pH adjustment to neutral (Microtox test); [c] MaxTox – [d]☺ not toxic, ☠ toxic, ☠☠ very toxic, ☠☠☠ extremely toxic;
[e] *Daphnia magna* or *Thamnocephalus platyurus* tests

Proposal of a test battery for hazard assessment of oil shale waste streams

The use of test batteries for the hazard assessment of soils, sediments and solid wastes is a relatively new, but rapidly developing approach. Several criteria have to be taken into account to select ecotoxicological assays:

- the trophic level of the test organism and its sensitivity,
- recognition of the test by international standardization organizations,
- simplicity of the test, its commercial availability of the test organisms,
- the necessary equipment and the running costs

According to the summarising tables given above, the following (reduced) test battery for the ecotoxicological hazard assessment of solid-phase samples *via* soil-water path could be proposed:

- *Daphnia magna* mortality assay,
- *Tetrahymena thermophila* growth inhibition assay,
- *Selenastrum capricornutum* growth inhibition assay
- and *Vibrio fischeri* luminescence inhibition assay (Microtox test).

For the evaluation of **particle-bound bioavailable toxicity of soil** suspensions, Solid-Phase Flash-Assay (SPFA) is also recommended.

This test battery is a multitrophic one containing bacteria (destructors), algae (primary producers) and crustaceans and protozoa (consumers). It involves acute tests (Microtox, SPFA, *Daphnia* test) as well as short-chronic tests (*Selenastrum* and *Tetrahymena* growth assays). The proposed battery is not including Charatox, Rotox chronic and Thamnotox tests in spite of their use in the reported studies. It should be noted that, even if Charatox is relatively sensitive, it requires a special equipment. Rotox short-chronic test is difficult to perform and is of equal sensitivity of another short-chronic test, Protox [40]. Moreover, both test organisms are representatives of a consumer level of food-web. Thamnotox is a very sensitive assay and easy to perform. It may be used in large screening studies instead of *Daphnia*, as both are crustacean tests and of comparable sensitivity. However, for the proposed battery *Daphnia* is suggested as a test more recognized among ecotoxicologists. *Daphnia* and algal tests are OECD standard methods with toxicity data available in several databases (e.g. ECOTOX Database [107]. Microtox is one of the tests most widely used for ecotoxicological studies, as it is cheap and easy to perform. The toxicity data for various chemicals for Microtox test are easily available [15]. In the current review Microtox and *Daphnia* were two most sensitive aquatic toxicity assays. For the detection of particle-bound toxicity, the Solid-Phase Flash-Assay is suggested. This test is very rapid and cost-efficient in terms of reagents and disposables, and it also proved to be the most sensitive test in analysis of polluted soils and solid wastes of oil shale industry (Tables 8 and 11).

Conclusions

Oil shale combustion ashes and fresh semicoke did not contain heavy metals, oil products, PAHs and phenols in hazardous levels but showed water-extracted toxicity, mostly due to unfavorable pH and probably also due to sulphides. Fresh semicoke was classified as hazardous waste according to acute toxic hazard via solid waste-water path. Old semicokes were practically neutral and considerably less toxic due to rain washing. On the other hand, semicoke heaps have been historically used for dumping of different wastes (e.g., oil pitch, waste sludge) that explains the presence of phenols and other pollutants in the natural leachates and pore waters of semicoke heaps. Semicoke dump leachates as well as semicoke heap pore waters were very alkaline and ′very toxic′ to ′extremely toxic′. In old semicokes and especially in leachate-polluted soils the concentrations of oil

products and PAHs were relatively high, but as a result of the low solubility of oil and PAHs in water their total levels did not explain the water-extracted toxicity of samples. A particle-bound toxicity was always shown in all leachate-polluted soils. Theoretically, oil products and phenols may contribute to this toxicity, but the presence of other pollutants specific to oil shale industry is not excluded. Also, accumulation of hydrophobic pollutants in the leachate-polluted soils as well as river sediments could be harmful for ecosystems. Some groundwater samples of the upper layer of aquifer from the vicinity of semicoke dumps were very toxic due not only to phenols and alkalinity.

The results presented in the present review further prove that the risk assessment (especially for solid-phase samples) should not be based only on chemical analysis. The total concentrations of measured pollutants in the sample do not always predict the adverse effects to the biota, as these pollutants may be adsorbed by solid matrix and thus not to be bioavailable (i.e. the pollutants may not occur in toxic "form"). On the other hand, toxicity can be higher than predicted by chemical evaluation, as even the most complete chemical analysis can miss important toxicants.

This review shows the usefulness and need for combined use of chemical and biological methods for meaningful environmental risk assessment of oil shale industry waste streams. These ecotoxicological assays would be valuable also for a better monitoring of the wastewater treatment process and its efficiency.

Finally, according to EC REACH (Registration, Evaluation and Authorisation of Chemicals) Program the ecotoxicological assays (e.g., *Daphnia* and *Selenastrum)* are required for the registration of new industrial chemicals with a priority to those produced in large quantities. Both daphnia and algal tests are time-consuming and relatively expensive for pre-screening new chemical derivatives. As Estonian oil shale represents an important national source of new valuable chemicals and mixtures, luminescent bacteria are most suitable test organisms for screening of "chemical libraries". The application of photobacterial tests at the screening level of safety of chemicals should be seriously considered [18], as this could save a lot of money, manpower and lives of experimental animals and thus, contribute to the Three 'R's concept (replacement, reduction, refinement) introduced by Russel & Burch in 1959 [108].

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REFERENCES

1. *Raukas, A.* Oil shale industry and sustainability – governance through dialogue // Oil Shale. 2005. Vol. 22, No.1. P. 3–4.
2. *Bauert, H., Kattai, V.* Kukersite oil shale. // Geology and Mineral Resources of Estonia / A. Raukas, A. Teedumäe (eds.). Tallinn: Estonian Academy Publishers, 1997. P. 313–327.
3. *Kattai, V., Lokk, U.* Historical review of the kukersite oil shale exploration in Estonia // Oil Shale. 1998. Vol. 15, No. 2 (Special). P. 102–110.
4. *Munter, R., Trapido, M., Veressinina, J., Preis, S., Pikkov, L.* Detoxification of phenolic and carcinogenic compounds in the leachate from Kohtla-Järve dump // Proceedings of Estonian Conference on Ecology / Tartu, Estonia: Greif, 1994. P. 199–203.
5. *Mölder, L. Elenurm, A., Tamvelius, H.* Sulphur compounds in a hydraulic ash-disposal system // Proc. Estonian Acad. Sci. Chem. 1995. Vol. 44, No. 2–3. P. 207–211.
6. *Orupõld, K., Ohlsson, A., Henrysson, T.* Batch trials to simulate biological treatment in lagoons of leachate from oil-shale ash heaps // Oil Shale. 1997. Vol. 14, No. 4 (Special Issue). P. 476–487.
7. *Orupõld, K., Tenno, T., Henrysson, T.* Biological lagooning of phenols-containing oil shale ash heaps leachate // Water Res. 2000. Vol. 34. P. 4389–4396.
8. *Kamenev, I, Munter, R., Pikkov, L., Kekisheva, L.* Wastewater treatment in oil shale chemical industry // Oil Shale. 2003. Vol. 20, No. 4. P. 443–457.
9. *Roots, O.* Polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in oil shale and fly ash from oil shale-fired power plant in Estonia // Oil Shale. 2004. Vol. 21, No. 4. P. 333–339.
10. *Heinaru, E., Merimaa, M., Viggor, S., Lehiste, M., Leito, I., Truu, J., Heinaru, A.* Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area // FEMS Microb. Ecol. 2005. Vol. 51, No. 3, 1. P. 363–373
11. *Symons, B. D., Sims, R. C.* Assessing Detoxification of a Complex Hazardous Waste, Using the Microtox Bioassay // Arch. Environ. Contam. Toxicol. 1988. Vol. 17, No. 4. P. 497–505.
12. *Huling, S. G., Pope, D. F., Matthews, J. E., Sims, J. L., Sims, R. C., Sorensen, D. L.* Land treatment and the toxicity response of soil contaminated with wood preserving waste // Remediation. 1995. Vol. 5. P. 41–56.
13. Rozkov, A., Vassiljeva, I., Kurvet, M., Kahru A., Preis, S., Kharchenko, A., Krichevskaja, M., Liiv, M., Käärd, A., Vilu, R. Laboratory Study of Bioremediation of Rocket Fuel-Polluted Groundwater // Water Res. 1999. Vol. 33, No. 5. P. 1303–1313.

14. *Bulich, A. A.* A practical and reliable method for monitoring the toxicity of aquatic samples // Process. Biochem. 1982. Vol. 17. P. 45–47.
15. *Kaiser, K. L. E., Devillers, J.* Ecotoxicity of Chemicals to *Photobacterium phosphoreum* // Handbooks of Ecotoxicological Data. Vol. 2 / J. Devillers (ed.). Singapure : Gordon and Breach Science Publishers, 1994.
16. *Kahru, A.* In vitro toxicity testing using marine luminescent bacteria *Photobacterium phosphoreum*: The BiotoxTM test // ATLA. 1993. Vol. 21, No 2. P. 210–215.
17. *Kahru, A., Borchardt, B.* Toxicity of 39 MEIC chemicals to bioluminescent photobacteria (the Biotox^{TM} test): correlation with other test systems // ATLA. 1994. Vol. 22, No. 3. P. 147–160.
18. *Kahru, A.* Ecotoxicological tests in non-ecotoxicological research: contribution to 3Rs. Use of luminescent photobacteria for evaluating the toxicity of 47 MEIC reference chemicals// ALTEX, 2006. (in press).
19. *Blaise, C.* Microbiotests in aquatic ecotoxicology: characteristics, utility, and prospects // Environ. Toxicol. Water Qual. 1991. Vol. 8. P. 145–155.
20. *Dutka, B. J., Jones, K., Kwan, K. K., Bailey, H., McInnis, R.* Use of microbial and toxicant screening tests for priority site selection of degraded areas in water bodies // Water Res. 1988. Vol. 22, No. 4. P. 503–510.
21. *Wängberg, S-A., Bergström, B., Blanck, H., Svanberg, O.* The relative sensitivity and sensitivity patterns of short-term toxicity tests applied to industrial wastewaters // Environ. Toxicol. Water Qual. 1995. Vol.10. P. 81–90.
22. *Blaise, C.* Microbiotesting: An expanding field in aquatic toxicology // Ecotox. Environ. Saf. 1998. Vol. 40, No. 1–2. P. 115–119.
23. *François, M., Dubourguier, H. C., Li, D., Douay, F.* Prediction of the solubility of heavy metals in agricultural topsoils around two smelters by the physico-chemical parameters of the soils // Aqua. Sci. 2004. Vol. 66. P. 1–8.
24. *Kahru, A., Ivask, A., Kasemets, A., Põllumaa, L., Kurvet, I., Francois, M., Dubourguier, H. C.* Biotests and biosensors in ecotoxicological risk assessment of field soils polluted with zinc, lead and cadmium // Environ. Tox. Chem. 2005. Vol. 11. (in press).
25. *Bispo, A., Jourdain, M. J., Jauzein, M.* Toxicity and genotoxicity of industrial soils polluted by polycyclic aromatic hydrocarbons (PAHs) // Organ. Geochem. 1999. Vol. 30, No. 8 (2). P. 947–952.
26. *Alexander, M.* Aging, bioavailability and overestimation of risk from environmental pollutants // Env. Sci. Technol. 2000. Vol. 34, No. 20. P. 4259–4265.
27. Committee on Bioavailability of Contaminants in Soils and Sediments. Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications. - The National Academic Press, Washington, D.C., USA, 2003.
28. *Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N.* The bioavailability of chemicals in soil for earthworms // Ecotox. Environ. Saf. 2004. Vol. 57, No. 1. P. 39–47.
29. *Dorn, P. B., Vipond, T. E., Salanitro, J. P., Wisniewski, H. L.* Assessment of the acute toxicity of crude oils using earthworms, Microtox, and plants // Chemosphere. 1998. Vol. 37, No.5. P. 845–860.
30. *Rönnpagel, K., Liss, W., Ahlf, W.* Microbial bioassays to assess the toxicity of solid-associated contaminants // Ecotox. Environ. Saf. 1995. Vol. 31, No. 2. P. 99–103.

31. *Vaajasaari, K., Joutti, A., Schultz, E., Selonen, S., Westerholm, H.* Comparisons of terrestrial and aquatic bioassays for oil-contaminated soil toxicity // JSS. 2002. Vol. 2, No. 4. P. 194–202.
32. *Juvonen, R., Martikainen, E., Schultz, E., Joutti, A., Ahtiainen, J., Lehtokari, M.* A Battery of toxicity tests as indicators of decontamination in composting oily waste // Ecotox. Environ. Saf. 2000. Vol. 47, No.2. P. 156–166.
33. *Hund, K., Traunspurger, W.* Ecotox-evaluation strategy for soil bio-remediation exemplified for a PAH-contaminated site // Chemosphere. 1994. Vol. 29, No. 2. P. 371–390.
34. *Persoone, G., Janssen, C., De Coen, W.* New Microbiotests for Routine Toxicity Screening and Biomonitoring. – New York, Dordrecht, London, 2000.
35. *Persoone, G.* // Microscale Aquatic Toxicology. Advances, Techniques and Practice / P. G. Wells, K. Lee, C. Blaise (eds.) Boca Raton, FL, USA: CRC Lewis, 1998.
36. *Põllumaa, L., Kahru, A., Eisenträger, A., Reiman, R., Maloveryan, A., Rätsep, A.* Toxicological investigation of soil with solid-phase flash assay: comparison with other ecotoxicological tests // ATLA. 2000. Vol. 28, No.3. P. 461–472.
37. *Põllumaa, L., Maloveryan, A., Trapido, M., Sillak, H., Kahru, A.* Study of the environmental hazard caused by the oil-shale industry solid waste // ATLA. 2001. Vol. 29, No. 3. P. 259–267.
38. *Kahru, A., Reiman, R., Rätsep, A.* The efficiency of different phenol-degrading bacteria and activated sludges in detoxification of phenolic leachates // Chemosphere. 1998. Vol. 37, No. 2. P. 301–318.
39. *Kahru, A., Põllumaa, L., Reiman, R., Rätsep, A.* Microbiotests for the evaluation of the pollution from the oil shale industry // New Microbiotests for Routine Toxicity Testing and Biomonitoring / G. Persoone, C. Janssen, W. De Coen (eds.). New York, USA: Kluwer Academic/Plenum Publishers, 2000. P. 357–365.
40. *Põllumaa, L., Kahru, A., Manusadzianas, L.* Biotest- and Chemistry-Based Hazard Assessment of Soils, Sediments and Solid Wastes // JSS. 2004. Vol. 4, No. 4. P. 267–275.
41. *Kelsey, J. W., Kottler, B. D., Alexander, M.* Selective chemical extractants to predict bioavailability of soil-aged organic chemicals // Environ. Sci. Technol. 1997. Vol. 31, No.1. P. 214–217.
42. *Liste, H. H., Alexander, M.* Butanol extraction to predict bioavailability of PAHs in soil // Chemosphere. 2002. Vol. 46, No. 7. P. 1011–1017.
43. *Lappalainen, J., Juvonen, R., Vaajasaari, K., Karp, M.* A new flash method for measuring the toxicity of solid and colored samples // Chemosphere. 1999. Vol. 38, No. 5. P. 1069–1083.
44. *Baun, A., Justesen, K. B., Nyholm, N.* Algal tests with soil suspensions and elutriates: A comparative evaluation for PAH-contaminated soils // Chemosphere. 2002. Vol. 46, No. 2. P. 251–258.
45. *Aruoja, V., Kurvet, I., Dubourguier, H. C., Kahru, A.* Toxicity testing of heavy metal polluted soils with algae *Selenastrum capricornutum*: a soil suspension assay // Environ. Toxicol. 2004. Vol.19, No.4. P. 396–402.
46. *Chial, B., Persoone, G.* Cyst-based toxicity tests XIV application of the ostracod solid-phase microbiotest for toxicity monitoring of river sediments in Flanders (Belgium) // Environ. Toxicol. 2002. Vol. 17, No. 6. P. 533–7.

47. *Chial, B. Z., Persoone, G., Blaise, C.* Cyst-based toxicity tests. XVIII. Application of ostracodtoxkit microbiotest in a bioremediation project of oil-contaminated sediments: sensitivity comparison with *Hyalella azteca* solid-phase assay // Environ Toxicol. 2003. Vol. 18, No. 5. P. 279–83.
48. *Ivask, A., Virta, M., Kahru, A.* Construction and use of specific luminescent recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium, zinc, mercury and chromium in the soil // Soil Biol. Biochem. 2002. Vol. 34, No. 10. P. 1439–1447.
49. *Ivask, A., François, M., Kahru, A., Dubourguier, H. C., Virta, M., Douay, F.* Recombinant luminescent bacterial sensors for the measurement of bioavailability of cadmium and lead in soils polluted by metal smelters // Chemosphere 2004. Vol. 55, No. 2. P. 147–156.
50. *Lambolez, L., Vasseur, P., Ferard, J. F., Gisbert, T.* The environmental risks of industrial-waste disposal – an experimental approach including acute and chronic toxicity studies // Ecotox. Environ. Saf. 1994. Vol. 28, No. 3. P. 317–328.
51. *Toussaint, M. W., Shedd, T. R., van der Schalie, W. H., Leather, G. R.* A comparison of standard acute toxicity tests with rapid-screening toxicity tests // Environ. Toxicol. Chem.1995. Vol. 14. P. 907–915.
52. *Jarvis, A. S., McFarland, V. A., Honeycutt, M. E.* Assessment of the effectiveness of composting for the reduction of toxicity and mutagenicity of explosive-contaminated soil // Ecotox. Environ. Saf. 1998. Vol. 39, No. 2. P. 131–135.
53. *Saterbak, A., Toy, R. J., Wong, D. L., McMain, B. J., Williams, M. P., Dorn, P. B., Brzuzy, L. P., Chai, E. Y., Salanitro, J. P.* Ecotoxicological and analytical assessment of hydrocarbon-contaminated soils and application to ecological risk assessment // Environ. Toxicol. Chem. 1999. Vol. 18, No. 7. P. 1591–1607.
54. *Blok, J.* Probability of biodegradation, a novel concept for improving chemical classification and risk assessment. Ecotox. Environ. Saf. 2000. Vol. 47, No.3. P. 221–230.
55. *Ahtiainen, J., Valo, R., Järvinen, M., Joutti, A.* Microbial toxicity tests and chemical analysis as monitoring parameters at composting of creosote-contaminated soil // Ecotox. Environ. Saf. 2002. Vol. 53, No. 2. P. 323–329.
56. *Kördel, W., Terytze, K., Hund-Rinke1, K.* Mobility and bioavailability of pollutants: International methods standardization // JSS. 2003. Vol. 3, No. 4. P. 235–236.
57. *Fent, K.* Ecotoxicological effects at contaminated sites. Toxicology. 2004. Vol. 205, No. 3. P. 223–240.
58. *Loibner, A. P., Szolar, O. H. J., Braun, R., Hirmann, D.* Toxicity testing of 16 priority polycyclic aromatic hydrocarbons using Lumistox® // Environ. Toxicol. Chem. 2004. Vol. 23, No. 3. P. 557–564.
59. *da Silva, N.-H., Robert, L., Steiner, G., Smith, B.* Residual toxicity after biodegradation: interactions among benzene, toluene, and chloroform // Ecotox. Environ. Saf. 2004. Vol. 57, No. 2. P. 162–167.
60. *Vaajasaari, K.* Leaching and exotoxicity tests as methods for classification and assessment of environmental hazard of solid wastes. PhD Thesis, Tampere University of Technology. – Tampere, 2005.
61. *Fomina, A. S.* Chemical composition of Baltic oil shale kerogen // United Nations Symposium on the Development and Utilization of Oil Shale Resources, Tallinn, August 26 – September 4 / Tallinn: Tallinna Polütehniline Instituut, 1968.

62. *Lille, Ü.* Current knowledge on the origin and structure of Estonian kukersite kerogen // Oil Shale. 2003. Vol. 20, No. 3. P. 253–263.
63. Environment in Figures 2003 // Statistical Office of Estonia. Tallinn, 2004. [in Estonian].
64. *Kogerman, P.* On the chemistry of the Estonian oil shale "Kukersite": a monograph. – Tartu, 1931.
65. *Holopainen, H.* Experience of oil shale combustion in ahlstrom pyroflow CFB-boiler // Oil Shale. 1991. Vol. 8, No. 3. P. 194–209.
66. *Aunela-Tapola, L. A., Frandsen, F. J., Häsänen, E. K.* Trace metal emissions from the Estonian oil shale fired power plant // Fuel Process. Technol. 1998. Vol. 57, No. 1. P. 1–24.
67. *Urov, K., Sumberg, A.* Characteristics of oil shales and shale-like rocks of known deposits and outcrops. Monograph // Oil Shale. 1999. Vol. 16, No. 3 (Special Issue, ISSN 0208-189X). P. 1–64.
68. *Koel, M.* Estonian oil shale // www.kirj.ee/oilshale/Est-OS.htm. 1999.
69. Vesi Hydro Ltd. Waste water treatment in the Kohtla Järve area. Stage one: Waste water pilot plant study. Report to Finnish Ministry of the Environment. – Helsinki, 1993.
70. VKG. Viru Keemia Grupp. http://www.vkg.ee, 10.10.2004
71. *Tuvikene, A., Huuskonen, S., Koponen, K., Ritola, O., Mauer, Ü., Lindström-Seppä, P.* Oil shale processing as a source of aquatic pollution: monitoring of the biologic effects in caged and feral freshwater fish // Environ. Health Perspect. 1999. Vol. 107, No. 9. P. 745–752.
72. Estonia, the oil shale industry. Risk based environmental site assessment of landfills. Norwegian Geotechnical Institute Report. - Oslo, Norway, 2004.
73. Environmental hazard assessment of semi-coke // Estonian Environmental Research Centre Report. Tallinn, 2003. [in Estonian]. (http://www.envir.ee/jaatmed/poolkoksi_uuring.pdf).
74. *Kahru, A., Kurvet, M., Kurvet, I.* Study of the toxicological impact of different components of ash-heap water // Oil Shale. 1997. Vol. 14, No. 4 (Special Issue). P. 469–475.
75. *Kahru, A., Põllumaa, L., Reiman, R., Rätsep, A.* Predicting the toxicity of oil shale industry wastewater by its phenolic composition // ATLA. 1999. Vol. 27, No. 3. P. 359–366.
76. *Orupõld, K., Henrysson, T.* Biological treatment of oil shale ash heap leachate containing phenolic compounds by biofilm reactors with suspended carriers // Res. Environ. Biotech. 1999. Vol. 2. P. 219–233.
77. *Truu, J., Talpsep, E., Heinaru, E., Heinaru, A.* Self-purification processes in Estonian rivers receiving sewage from oil shale processing industry. Large Rivers 13 (3–4) // Arch. Hydrobiol. Suppl. 2002. Vol. 141, No. 3–4. P. 459–469.
78. *Manusadzianas, L., Balkelyte, L., Sadauskas, K., Blinova, I., Põllumaa, L., Kahru, A.* Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests and correspondence between toxicity and chemical-based indices // Aquat. Toxicol. 2003. Vol. 63, No. 1. P. 27–41.
79. *Heinaru, E., Talpsep, E. J., Linnas, A., Heinaru, A.* Metabolic and genetic diversity of phenol-utilizing bacteria as an encancer of natural biodegradation in polluted waters // Oil Shale. 1997. Vol. 14, No. 4 Special. P. 454–468.

80. *Sooba, E., Jauregui, O., Galceran, M. T., Tenno, T.* Determination of phenols by liquid chromatography with electrochemical and UV detection // Oil Shale. 1997. Vol. 14, No. 4 (Special Issue). P. 544–553.
81. Maximum limits of hazardous substances in soil and ground water. Regulation No. 58 // Environmental Ministry of Estonia, RTL, 1999, 105, 1319. [in Estonian].
82. *Talpsep, E., Heinaru, E., Truu, J., Laht, T., Heinaru, A.* Functional dynamics of microbial populations in waters contaminated with phenolic leachates // Oil Shale. 1997. Vol. 14, No. 4 (Special Issue). P. 435–453.
83. *Castaldi, F. J.* Single sludge activated sludge biological treatment of carbon, nitrogen, and phosphorus in combined municipal and industrial wastewaters from the Kohtla-Jarve region of Estonia // Oil Shale. 1997. Vol. 14, No 4 (Special Issue). P. 488–508.
84. *Kahru, A., Põllumaa, L., Reiman, R., Rätsep, A., Liiders, M., Maloveryan, A.* The toxicity and biodegradability of eight main phenolic compounds characteristic to the oil-shale industry wastewaters: a test battery approach // Environ. Toxicol. 2000. Vol. 15, No. 2. P. 431–442.
85. An Assessment of Oil Shale Technology, Congress of the United States, Office of Technology Assessment, Washington, D.C., 1980. (http://www.wws.princeton.edu/~ota/disk3/1980/8004_n.html).
86. Committee on Bioavailability of Contaminants in Soils and Sediments. 2003. Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications. The National Academic Press, Washington, D.C., USA.
87. *Manusadžianas, L., Vitkus, R., Sakalauskas, V.* Wastewater toxicity assessment using the electrophysiological response of a charophyte *Nitellopsis obtusa* // Environ. Toxicol. Water Qual. 1995. Vol. 10. P. 49–56.
88. *Persoone, G., Goyvaerts, M., Janssen, C., De Coen, W., Vangheluwe, M.* Cost effective acute hazard monitoring of polluted waters and waste dumps with the aid of Toxkits. Final Report. Commission of European Communities, Contract ACE 89/BE 2/D3. 1993.
89. Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., Kolar, B. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters // Environ. Toxicol. 2003. Vol. 18, No. 6. P. 395–402.
90. *Bradbury, S. P., Lipnick, R. L.* Structural properties for determining mechanisms of toxic action // Environ. Health Perspect. 1990. Vol. 87. P. 181–182.
91. *Blum, D. J., Speece, R. E.* The toxicity of organic chemicals to treatment processes // Water Sci. Technol. 1992. Vol. 25. P. 23–31.
92. *van Wijk, R., Postman, J., van Houwelingen, H.* Joint toxicity of ethyleneamines to algae, daphnids and fish // Environ. Toxicol. Chem. 1994. Vol. 13, No. 1. P. 167–171.
93. *Broderius, S., Kahl, M. D., Hoglund, M. D.* Use of Joint Toxic Response to define the primary mode of toxic action for diverse industrial organic chemicals // Environ. Toxicol. Chem. 1995. Vol. 14, No. 9. P. 1591–1605.
94. *Chen, C. Y., Chiou, Y. S.* Toxicity of binary mixtures of organic chemicals // Environ. Toxicol. Water Qual. 1995. Vol. 10, No. 2. P. 97–106.

95. *Trapido, M., Veressinina, J.* Use of the Daphnia magna test to estimate the toxicity of the ozonation by-products of phenols and chlorophenols // ATLA. 1999. Vol. 27, No. 3. P. 425–431.
96. *Kahru, A., Kurvet, M., Külm, I.* Toxicity of phenolic wastewater to luminescent bacteria Photobacterium phosphoreum // Water Sci. Technol. 1996. Vol. 33, No. 6. P. 139–146.
97. Maves Ltd. Monitoring of organic compounds in groundwaters of north-eastern industrial area. Report for Estonian Environmental Research Centre. – Tallinn, 2000.
98. *Kahru, A., Maloverjan, A., Sillak, H., Põllumaa, L.* Toxicity and fate of phenolic pollutants in the contaminated soils associated with the oil-shale industry // Environ. Sci. Pollut. Res. 2002.Vol. 1 (Special Issue). P. 27–33.
99. *Heinaru, E., Truu, J., Stottmeister, U., Heinaru, A.* Three types of phenol and p-cresol catabolism in phenol- and p-cresol-degrading bacteria isolated from river water continuously polluted with phenolic compounds // FEMS Microb. Ecol. 2000. Vol. 31, No. 3. P. 195–205.
100. *Truu, J., Talpsep, E., Velder, E., Heinaru, E., Heinaru, A.* Enhanced bio-degradation of oil shale chemical industry solid wastes by phytoremediation and bioaugmentation // Oil Shale. 2003. Vol. 20, No. 3 (Special Issue). P. 421–428.
101. Kettunen, R. H., Rintala, J. A. Sequential anaerobic-aerobic treatment of sulphur rich phenolic leachates // J. Chem. Tech. Biotechnol. 1995. Vol. 62, No. 2. P. 177–184.
102. *Kettunen, R., Pulkkinen, E. M., Rintala, J. A.* Biological treatment at low temperatures of sulfur-rich phenols containing oil shale ash leachate // Water Res. 1996. Vol. 30, No. 6. P. 1395–1402.
103. *Brenner, A., Chozick, R., Irvine, R. L.* Treatment of a high-strength mixed phenolic waste in an SRB // Water Environ. Res. 1992. Vol. 64. P. 128-131.
104. *Dorn, P. B., Salanitro, J. P.* Temporal ecological assessment of oil contaminated soils before and after bioremediation // Chemosphere. 2000. Vol. 40, No. 4. P. 419–426.
105. *McGrath, R., Singleton, I.* Pentachlorophenol transformation in soil: a toxicological assessment // Soil Biol. Biochem. 2000. Vol. 32, No. 8–9. P. 1311–1314.
106. *Rojickova-Padrtova, R., Marsalek, B., Holoubek, I.* Evaluation of alternative and standard toxicity assays for screening of environmental samples: selection of an optimal test battery // Chemosphere.1998. Vol. 37, No. 3. P. 495–507.
107. ECOTOX Database // http://www.epa.gov/ecotox/, 12.09.2004
108. *Russel, W. M. S., Burch, R. L.* The principles of Humane Experimental Technique. – London: Methuen, 1959

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