Allar Lääne

Ecotoxicity of fine fraction from the Kudjape landfill

Master thesis in the field of landscape protection and preservation

Supervisors: Kaja Orupõld
Fabio Kaczala

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INTRODUCTION

The ability to use materials and energy to create desired environments has been a special trait of mankind as a species. Although the use of energy and materials has not always been most efficient, advancement in technology has improved it. The effects of inefficient material and energy usage are prominent to this day, the most recognizable in the accumulation of waste.

Organized waste disposal in form of landfills has been the main type when dealing with accumulated waste, since it is the easiest way to get rid of unwanted materials (Eggen et al., 2010; Kjeldsen et al., 2002). A landfill is basically a condensed space for waste (Verbel et al., 2007). But the unregulated piling of mixed wastes, especially in municipal landfills can have unexpected consequences. Hazards from older landfills to the environment can come in many forms, one of them being the leachate that is contaminated and exposed to the surroundings (Schwarzbauer et al., 2002).

Reclamation of landfilled materials and land by means of landfill mining has become an economically attractive method, since landfills contain large amounts of different resources, like metals, plastic etc. (Hogland et al., 2011). Resource shortage and environmental challenges have triggered the trend towards resource efficient, low carbon circular economies, to which landfill mining seems to be one good option (Jones et al., 2013).

It is also important to follow the trend to save energy during the process; the use of materials available on site for recultivation can be considered an option. These so called biocovers can be used to cover a mined landfill (Masi et al., 2014). One ingredient of the biocover can be fine fraction ($\varnothing<4$ mm) sieved from the excavated landfill since it constitutes large percentage of the mined material (Masi et al., 2014).

Fine fraction’s suitability as a component for the cover material has to be evaluated by testing its toxicity towards different types of organisms. Ecotoxicological tests can be carried out together with physicochemical analyses to assess the suitability of fine fraction as a possible landfill cover material (Masi et al., 2014).
The topic of the research is connected to the field of landscape protection and preservation, since landfills are quite distinguishable elements in the landscape in terms of size and composition. The problems and potential hazards deriving from closing landfills need attention in order to make the right decisions.

The aim of this research is to determine the ecotoxicity of fine fraction excavated from the Kudjape landfill, Saaremaa, Estonia and used as biocover material in there. In order to achieve that goal, ecotoxicological tests with three different types of organisms were carried out with the artificial leachate of the fine fraction. The results would be used in a toxicity classification system to determine a measure for the fine fractions overall ecotoxicity. To support the ecotoxicological tests, seed germination tests were also performed. For better interpretation of the results the physicochemical characterization of the prepared leachates was carried out.

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1. LITERATURE OVERVIEW

Throughout history landfills have been the most used type of organized waste disposal. The most common working principle of a landfill is to confine the waste material to a space as small as possible (by compacting the waste) and cover it with layers of soil (Verbel et al., 2008; Eggen et al., 2010). Older landfill sites can contain waste from different time periods as well as different sources. As the waste is piling up through continuous disposal, the lower layers begin to decompose and chemical reactions among the different chemicals residing in the waste take place, resulting in the formation of new compounds. This is due to the diverse nature of waste. New compounds, which have been added to some products to improve quality, are likely to become a potential source of harm to the environment when disposed of. And since there are so many of those added compounds, all of their effects towards nature as well as their interaction with each other are not yet completely understood (Eggen et al., 2010).

1.1 Landfill mining

Landfill mining has become an increasingly considered method for dealing with old or closed landfills. The reasons are mainly increasing costs of raw material and potential environmental problems (Kaartinen et al., 2013). A recent study conducted in Finland assessed the treatability, material mass balances and material recovery potential of municipal solid wastes (MSW) (Kaartinen et al., 2013). Also, the sorting efficiency in that study was examined through the comparison between manual sorting and full scale mechanical pre-treatment of wastes. Some of the MSWs were also assessed to evaluate their potential usability as solid recovered fuel. The finer MSW material from the landfill, which was situated in Kuopio, Finland, was tested for its possible landfill acceptability (using leaching). Results from that study Kaartinen et al., 2013) provided knowledge of the treatability of MSW in a full-scale mechanical process. Shredding, magnetic separation, drum-sieving and wind sieving of the coarsest material were
the steps taken, which produced ~30% of possible solid recovered fuel, mostly consisting of plastics. The finer particle sizes contributed to ~50% of the materials sampled. They consisted of the landfilled waste and the soil materials used for intermediate covers. Landfill acceptability of the fine fractions was found to be non-hazardous regarding leaching, except DOC levels (dissolved organic carbons), which may pose a problem in EU. The sampling and characterization methods were said to be applicable at other possible landfill mining sites. The results from the tests, however were site-specific and could not be generalized to fit other landfills (Kaartinen et al., 2013).

Another research conducted in Thailand aimed to determine the potential of excavated waste form a municipal landfill for recycling (Prechthai et al., 2008). In order to do that, composition and physico-chemical characteristics of waste from the landfill were determined, as well as heavy metal concentrations. The main emphasis was put on the toxicity of heavy metals, low molecular weight organic compounds and ammonia content in the waste, in order to assess the suitability to be used as compost. As a dominant species in the area of study, Oryza sativa L. (Linnaeus, 1753) was used in the seed germination tests for its high sensitivity to heavy metal toxicity. In order to determine the amounts of heavy metals during leaching, toxic characteristic leaching procedure (TCLP) was conducted on the non-recyclable waste fraction. By comparing the quality of waste with the quality standards, the potential for recycling of the excavated waste was evaluated.

The results showed, that the landfill had a heterogeneous nature from its varying composition and that it was undergoing methanogenic phase. The excavated waste mainly consisted of plastics with high calorific value. Approximately 69 % of the soil was extracted from the waste after screening (screen size 25 – 50 mm). Also, it was effective for separating fractions that had higher concentrations of heavy metals. Additionally, approximately 69 % of the waste fractions bigger than 50 mm had high plastic content that could be recovered. Lower heavy metal concentrations were present in larger waste fractions. The waste fractions below 25 mm did not meet the requirements to be used as compost, however, phytotoxicity of the waste was low, suggesting that the waste be used as compost for non-edible crops. The removal of glass, plastic, stone and metal from the material before use was strongly emphasized. The rest of the non-recyclable waste was determined suitable to be disposed into the municipal solid waste landfill.


1.2 Landfill leachate and ecotoxicity

The diversity and quantity of chemicals and compounds found nowadays in landfills pose a potential risk to the environment, especially since the probability of them making their way to the surroundings is high when buried underground. Also, the way they become pollutants differs. Some compounds become gaseous and are released into the air or are just airborne particulate matter (Slack et al., 2005), others are easily leached out from the solid waste with rainwater and can enter the soil, surface- or groundwater. Some compounds in a landfill have a high vapor pressure in room temperature and therefore belong to a class of VOC-s (Volatile Organic Compounds), which means they are easily released into the atmosphere. Measuring VOC-s in the air had taken a recent step forward, when Turkish researchers from the Materials Institute of TÜBİTAK Marmara Research Center successfully tested a new type of VOC sensor based on carbon nanotubes (Tasaltin & Basarir, 2013).

As previously mentioned, some compounds can leach out from the landfill and contaminate the soil, groundwater and surface water. The leachate that flows out of a landfill may contain, besides other harmful materials, certain organic compounds called xenobiotic organic compounds (XOC) (Kjeldsen et al., 2002). Not all of the existing XOC-s have been classified and not all of the effects of already classified XOC-s are fully understood. This is mainly due to the XOC’s low concentrations in the leachate and the limited possibilities provided by available chemical analyses (Matejczyk et al., 2010). A study in Denmark (Baun et al., 2004) discovered 55 different XOC-s in 10 Danish landfills, showing that they are numerous and need more research to get more data about them. Other components in the soil that can be harmful for the environment besides the different organic compounds may include for example heavy metals like zinc, chrome, lead, semimetals like arsenic etc. which are serious anthropogenic stressors for plants and other living organisms.

Traditionally, physico-chemical analyses have been the main methods for measuring the toxicity of leachates from landfills. With those analyses, the pH, electrical conductivity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), as well as other methods for measuring organic compounds can be used to determine the toxicity of leachate samples (Schwarzbauer et al., 2001). But since a lot of the organic compounds residing in a landfill are still unknown and some of the effects and behavior of known compounds are also not fully understood, chemical and physical analyses do not give proper and conclusive answers
whether a landfill can really be considered harmful or not. Therefore it is reasonable to use methods that study the effect of a leachate towards the environment.

Evaluating ecotoxicity, the toxic effect towards the ecosystem, including plants, microorganisms etc. would be an alternative in order to get a more general overview of effects towards the surrounding areas of a landfill. Certain tests with different kinds of plants and microorganisms are performed. To find out how well ecotoxicological tests correlated with physical and chemical tests, a group of scientists in Spain conducted experiments on leachate waters from four landfills (Pablos et al., 2011). Their main goal was to find out if there was a connection between the results of the widely used physical and chemical tests and the less commonly used ecotoxicological tests. When the results were different, it would mean that ecotoxicological tests would not be required on leachate samples that show high numbers in physical or chemical analyses, deeming the leachate already as hazardous. On the other hand, it is possible to carry out ecotoxicological tests on samples that do not show any high results in the physical or chemical tests but may still be hazardous.

To find the levels of ecotoxicity in the leachate sample from a landfill, the sample is tested on different kinds of organisms. One way of doing so is to evaluate the effects of the sample on organisms, which have different levels of sensitivity. It means that the species used in the experiment react differently towards the test substances, some may show signs of inhibition, others nothing at all. Also the living habitat (soil, water etc.) should be different, as to show the ecotoxic effect on different parts of the ecosystem. One of those kind of experiments was conducted in Sweden (Ribe et al., 2012), in which pine bark was evaluated as a treatment for landfill leachates. They tested the leachate for toxicity on water fleas (Daphnia magna) (Straus, 1820), on bioluminescent bacteria (Vibrio fischeri) (Beijerinck, 1889), on algae (Pseudokirchneriella subcapitata) (Korshikov) and on water plants (Lemma minor) (Linnaeus, 1753). The results showed that although pine bark removed effectively metal contaminants from the leachate, it did not change the toxicity noticeably (Ribe et al., 2012).

Studies regarding the components leaching out of landfills and posing a possible hazard have been carried out in many countries. One of those studies was carried out by a research team from Norway and Germany with the goal of investigating the levels of emerging organic compounds in untreated landfill leachates (Eggen et al., 2010). Tests were performed on samples collected from three differently engineered landfills and were carried out in both water- and particle phases to get an overall knowledge about the components distribution patterns.
Suitable treatment processes for removing hazardous compounds from untreated leachates were also investigated. Many kinds of compounds were identified in the samples, most prominently perfluorinated compounds (PFC) and legacy compounds - contaminants that have been left in the environment by sources that are no longer exist (Hutchinson et al., 2013). Many of the identified compounds had higher concentrations in the leachate than in the solids. They also were found to be persistent and thus might pose significant removal challenges in the treatment. Due to the qualitative and quantitative variation of composition of the pollutant mixtures, a combined treatment protocol that includes different processes was recommended in the end (Eggen et al., 2010).

Another leachate toxicity research was carried out by researchers in Malaysia (Fauziah et al., 2013), where the toxicity of leachate from sanitary landfills was tested on the climbing perch (Anabas testudineus) (Bloch, 1792). Leachate physic-chemical characteristics were also measured. The conducted toxicity tests involved three stages. Acclimatization, in which the fish were kept in an aquarium for 14 days with the water flushed daily. The range finding test was next, where five different concentrations were used to determine the suitable one. The short-term definitive test was last, in which the narrowed range concentration leachate was used. Discoloration was an effect observed due to the loss of coloring pigment, the cause being ammonia poisoning. Other observations included: behavioral changes, unusual leaping action, swimming disorder, loss of equilibrium and in other activities, which are most probably due to a neurotoxin effect caused by leachate exposure. It was concluded that leachate from the landfills was toxic to A. testudineus based on high mortality rate and behavioral changes.

One way of determining the risk of a landfill area, specifically the ground areas becoming contaminated is to use the ECRIS system (Ecotoxicological Classification Risk Index for Soil) Senese et al., 2010). This index is based on leachate samples taken from landfills and the system is based only on organic compounds. Researchers (Senese et al., 2010) collected leachate samples from landfills in northern Italy and analyzed them for different organic compounds. Then a list of the 60 most common organic compounds found in the samples was created and a value was given to each of those 60 previously mentioned constituents. That value should describe the potential hazard the corresponding compound has. Different characteristics of the compounds were taken into account, such as the flux from soil to water, flux from soil to air, aquatic toxicity (tests with Daphnia magna, certain species of fish etc.) and bioaccumulation to name a few. An estimated number was generated for each compound that way and a clearer
view of the potential of a landfill becoming a source of serious pollution was achieved. Other risk assessment studies have also been carried out, one of them in Italy, where an integrated strategy to evaluate toxicity of leachate was performed. Chemical analyses, risk assessment guidelines and in vitro assays were used. Results from the risk models suggested that the leachate had important toxic effects on fish and rodents because of ammonia and inorganic constituents (Baderna et al., 2011).

Landfill leachate characteristics may change in time and to study and confirm these changes happening, researchers investigated different physico-chemical parameters of landfill leachates in Poland (Kulikowska & Klimiuk, 2008). The purpose of the study was to determine the possible effects of age on the major components in leachate from Polish municipal landfills. Chemical and biochemical oxygen demand, nitrogen, phosphorous, heavy metals, mineral compounds and BTEX (benzene, toluene, ethylbenzene, and xylenes – volatile organic compounds found in petroleum) were investigated for the research. The main pollutants in leachate were organic compounds and ammonia. With the increasing age of the landfill, COD had decreased and ammonia had increased. The other parameters in the leachate, i.e. phosphorus, calcium, chlorides, magnesium, heavy metals, sulfate, dissolved solids and BTEX, showed only seasonal variations and weren’t dependent on the age of the landfill. The pH was considered high, COD was considered low, as well as heavy metal concentrations and the BOD5/COD ratio. Taking those results into account, the landfill was found to be characterized by methanogenic conditions. The variation of the main parameters, organics and ammonia, in time was suggested to have important implications in leachate management. The final conclusion from the research was that the right treatment technology could only be considered when the characteristics of the leachate were understood completely.

A study conducted in Cartagena, Colombia (Verbel et al., 2008) was trying to find out if there were any relationships between the composition of leachates from a landfill with the toxicity. Leachate characterization included measuring the concentrations of metals (Cd, Cu, Hg, Ni, Mn and Pb) and parameters such as pH, conductivity, COD and hardness. The data was then compared with the mortality data from toxicity tests with Artemia franciscana (Leach, 1819). Using multivariate analysis, the toxicity was found to be dependent on Cd concentrations and COD, however, independently from toxicity, the dose-response curve correlated with Ni concentrations. Thus it was deducted that Ni can increase the sensitivity of A. franciscana to other contaminants.
1.3 Ecotoxicological tests and data interpretation

Ecotoxicological tests have been used to assess the environmental risk and determine the actual biological impacts of toxicants on living organisms (Parvez et al., 2006). Different types of animals can be used together for comparison of toxicity test results and, when integrated with physico-chemical data, identification of the most toxic compounds is possible (Farre & Barcelo, 2003).

Choosing the right organisms for the toxicity tests is also important, depending on the substance that is tested. A well known genus for toxicity tests is Artemia, which has been used to determine toxicity for a wide range of substances.

The physiology, reproductive processes and general use of the genus Artemia in modern ecotoxicological tests were reviewed by researchers from Portugal and Belgium (Nunes et al., 2006). The review itself provides an overview of advantages, characteristics and general physiological features of the genus and distinct species of Artemia as test organisms in ecotoxicological tests, using reference material.

The downsides from the use of Artemia included, according to Persoone (Persoone & Wells, 1987), are greater resistance to chemical exposure due to increased resistance towards high salinity conditions, the fact that they don’t live in most of the marine ecosystems and many failed experiments with Artemia. As for the arguments for the use of Artemia in toxicity tests, a wide geographic distribution, ease of growth and maintenance in laboratory conditions, short life cycle, high reproductive capabilities, resistance to manipulation and the sizable knowledge regarding the species, are brought forth.

The general feature most well known for Artemia is the uncommon adaptability to extreme conditions (strong adaptability to hypersaline environments), believed to be the main cause for lesser sensitivity (Triantaphyllidis et al., 1998). However, Artemia is still considered to be one of the best examples of organisms suited for laboratory use provided one keeps strictly to laboratory protocols and given methodologies (Nunes et al., 2006). As a drawback, however, studies generally consider Artemia to be a less sensitive species for ecotoxicological tests (Okamura et al., 2000; Guerra, 2001).
Another well studied organism that is used in ecotoxicological tests is the rotifer *Brachionus plicatilis* (Müller, 1786). The potential applications, for which the rotifer can be used, from environmental management of eutrophication, pollution, petroleum compounds and cholera to wastewater treatment, transfer of useful substances and even tracking climate change (Kostopoulou et al., 2012).

*B. plicatilis* is considered to be more sensitive as for example *A. salina*, as a research for the toxicity of five aldehydes to those two organisms were examined, in which *B. plicatilis* was generally more sensitive than *A. salina* (Taylor et al., 2005).

The use of the bioluminescent bacteria *Vibrio fisheri* (Beijerinck, 1889) in toxicity tests is also quite widespread (Hernando et al., 2003). In order to find out the correlation of toxicity test data with other organisms with *V. fisheri*, it was tested together with several other organisms (various species of fish, the water flea *Daphnia* sp. (Müller 1785), *Artemia* sp. and *Chlorella* sp. (Beijerinck, 1890)) on several hundreds of chemicals (Kaiser, 1998). The results showed that there was quite a lot of correlation between the data from *V. fisheri* and several other aquatic species, providing a possibility to make predictions of one end point from another, but that would work only well with compounds that have a simpler chemical structure.

When evaluating the results from different kinds of toxicity tests, it is preferable to make conclusions based on all tests together. Therefore it is good to use a classification system that takes all the data into account and with a certain algorithm classifies the datasets. One of those classification systems is The Toxicity Classification System for Wastes Discharged into the Aquatic Environment (Persoone et al., 2003). Several types of toxicity classification systems have been worked on by scientists in different countries with the main purpose of attributing a comparable hazard score to polluted environments and toxic wastewaters (Persoone et al., 2003; Senese et al., 2010) In collaboration with researchers and groups from 10 European countries, a new toxicity classification system was developed (Persoone et al., 2003). The scoring systems working principle is that by ranking the wastewater in 5 classes of increasing toxicity and calculating a weight factor for the each toxicity class, it gives a generally strong result. This classification system was first applied in 2000 by the collaborating groups that tested samples from river-, ground-, drinking-, mine- and sediment pore waters as well as industrial effluents, soil and waste dump leachates. It is easier to apply and more cost efficient, making it suitable for regular monitoring. The toxicity classification system itself is based on different types of
Ecotoxicological tests. Because it requires testing only on undiluted samples of wastewaters that may flow into the aquatic environment, the system can be used for data indicating low toxicity.

Ecotoxicological tests can be used to evaluate landfill cover materials, especially when using material from the landfill itself. A recent study from Italy tested the suitability of organic residues, or fine fraction (ø<4mm) from landfill mining (Masi et al., 2014). Several options about the usage of the obtained material were considered: to store it in temporary storages, to make “bio-soils” that could be used in geoenvironmental applications, to use it as cover material in excavated landfills instead of soil or in environmental remediation activities. Physico-chemical analyses together with acute and chronic bioassays were carried out. In order to evaluate the toxicity of heavy metals on plants, variable growth substrates were used. The germination test was conducted on *Lepidium sativum* (Linnaeus, 1753) and root elongation test on *Vicia faba*. A new kind of chronic test with *Spartium Junctium* was also developed and carried out. In the results, it was concluded that the tested material can be used for formation of “‘bio-soils’” (fine fraction mixed with soil) and all the other possible ways, even as compost for cultivation of non-edible crops, but for that further study was needed.

Efficient and feasible bioassays to assess the ecotoxicological risks deriving from soil pollution are a necessity. One way to test the soil is to use the soil plate bioassay, which is a test that observes seed germination and seedling growth under controlled conditions over a specific time period. It is performed directly on the soil sample placed inside a Petri dish (Boluda et al., 2011).

Determining the potential dangers a landfill could present to its surroundings is a challenge that needs to be accepted. New ways of thinking as well as technological advancement help overcome the difficulties and the rewards are deeper knowledge about how different chemicals and compounds interact with the environment as well as cleaner ecosystems. Using new methods together with old ones to create systems helps to fill the gaps that technical limits have created so far.
2. MATERIALS AND METHODS

2.1 Sample collection and leaching of fine fraction

Samples from 4 different locations and 4 different depths on the Kudjape landfill (Saaremaa, Estonia) were collected for analyses (Figure 1). The landfill itself lies about 2 km south-east from the nearest town, Kuressaare and has an area of 5.6 ha, from which the dumping area is 4.2 ha. The landfill was closed in 2009, until then it had been a municipal waste landfill from the 1970s. The samples were collected by an international team (from 5 countries) consisting of students and academic staff (Bhatnagar et al., 2013). The fieldwork and sample collection was part of an international cooperation project named “Closing the Life Cycle of Landfills - Landfill Mining in the Baltic Sea Region for Future” in which has the participation of different countries from the Baltic Sea region and is established at the Linnaeus University, Kalmar, Sweden.
The sampling was done with excavator with bucket of 1 m³ from 4 different spots and four depths. Material with a particle size below 40 mm was sieved out. The material was further sieved to particle size <10 mm, which was the fine fraction used for laboratory analysis. It was kept in PVC zipper storage bags until the analysis. Samples labeled after the number of the hole and depth, for example the fine fraction from hole no. 1 and layer no. 1 would be labeled H1B1. The other sample bags were labeled in a similar fashion.

To reduce the number of samples needed for analysis they were mixed by layers as well as by sampling spots. For example, 4 samples from hole no. 1 were mixed together, composing sample H1, 4 samples mixed from the second point composed sample H2 etc. The same was done with the other locations. Afterwards, samples from the same depths were mixed together in the same fashion. The end result was 8 samples: H1 - H4 and L1 - L4 (Figure 2).

Leaching with liquid to solid ratio (L/S) 10 L/kg was conducted in a laboratory and followed the Nordtest method (NT ENVIR 005) standard, which is the standard for solid waste and granular inorganic material.

First, 20 g of the mixture from each of the 8 samples were heated in an oven at 105 °C for 1 hour to determine the moisture content. The samples were weighed before and after the oven with the difference of weight being the moisture content. Considering this, the samples could be weighed and mixed with deionized water for the leaching (jar test). For L/S 10 100 g of (dry) solid sample was mixed with 1 liter of deionized water.

![Figure 2. Mixing of the 16 samples by layers and by holes resulting in 8 samples for analysis (marked bold)](image-url)
The device used for the leaching was the flocculator Stuart SW6. The samples were put into 1 L beakers and stirred for 24 hours. After 24 hours, the samples were allowed to settle, the supernatant was decanted and stored in 50 mL and 15 mL tubes. One 50 mL tube from each sample as well as the 15 mL tube were stored in a freezer, the other two 50 mL tubes from each sample were stored in a fridge. L/S 10 leachates were made in triplication. All tests done with the leachate and the results presented were averages of those triplicates.

The leaching of fine fraction with liquid to solid ratio 5 L/kg was performed according to Estonian standard method (EVS-EN 12457). The leaching was performed with end-over-end rotator. After 24 hours the samples were allowed to settle approximately 30 min and supernatant was decanted and stored at 4°C.

For chemical analysis the leachate was filtered through a GF/C Whatman filter (1.2 µm).

2.2 Physico-chemical analyses

The pH, electrical conductivity and redox potential were measured with the WTW pH/Cond340i, in the beginning of the L/S 10 leaching every hour as well as towards the end. Since the data is incomplete (no measurements were made during the night), only results from the beginning and the end of leaching was used. For the L/S 5 leachate measurements were taken only at the end of leaching.

Chemical oxygen demand (COD) and total organic carbon (TOC) for leachate L/S 10 were analyzed with Hach Lange cuvette tests and measured spectrofotometrically. Both filtered and unfiltered samples were tested for COD and TOC. Total carbon (TC), TOC, inorganic carbon (IC) and total nitrogen (TN) for filtered leachate L/S 5 were measured with Carbon/Nitrogen analyzer (TOC-V CPH, TNM-1, Shimadzu)

Metals (Cu, Zn, Pb) in the leachate was determined with the flame atomic absorption spectrometer (AAnalyst 400) and Cd with atomic absorption spectrophotometer (AA-6800, Shimadzu) equipped with graphite furnace. Operational conditions were according to the manufacturer’s recommendations. The leachate was filtered prior to testing with 0.45 µm filters (attached to 50 mL syringes) or centrifuged at 15000 g for 20 min.
2.3 Ecotoxicological tests

*Artemia salina tests* – The brine shrimp *Artemia salina* cysts were hatched 48 hours prior to testing in a 500 mL beaker which was set in a water bath at 25°C. Seawater was exchanged after 24 hours. The hatched artemia were collected into a petri dish for better collecting and placing into the test wells on multiwell plates and the leachates were added at different concentrations. The multiwell plates were then put into the water bath at 25°C and immobile (dead) animals were counted after 24 and 48 hours.

Two experiments were conducted, because the first test had too many deaths in the control wells to be considered acceptable. In the second test, the multiwell plates with artemia were wrapped in tinfoil and put under a lamp for a steady temperature range of approximately 25-28°C. To calculate the total number of animals, all of the artemia were killed at the end of the test with HCl (ratio 1:2 with distilled water) and counted afterwards.

*Rotifer test* – RotoxKit™ M, a kit which included everything needed to perform the tests on the rotifer *Brachionus plicatilis*, was used. Unfiltered samples were tested on the rotifers to evaluate toxicity and one sample was prepared for one multiwell plate. All the procedures were done according to the manual. To test different concentrations different amounts of samples were added to the wells and later corresponding amounts of diluent (10 ppt seawater) were added. 10 ppt seawater was used because of the similar (low) salinity of the Baltic Sea.

*MicroTox* – MicroTox Acute Toxicity Test was carried out with the test protocol named the „81% Screening Test“*. That was the test with the highest concentration available. The screening test was performed according to the guide in the Microtox Omni software, which is a standard method for the screening test. 24 samples were measured in 2 sets. The results were measured after 5 minutes and 15 minutes.

In order to evaluate the data from the three toxicity tests, the results must be classified. The toxicity data from tests were controlled with a classification system that calculates acute hazard classes (by the % of inhibition in tests) and weight scores for each class (Persoone *et al.* 2003). The data used for this classification was from the undiluted L/S 10 samples that were used for toxicity testing.
**Preliminary plant seed tests**- The first test was carried out to get a general idea about the effects of both the fine fraction and the cover material that was supposed to be used for the closed landfill in Kudjape. For the samples, a fine fraction sample from a random spot on the landfill, H2B1 specifically, and a sample from the soil/fine fraction/sludge compost mixture (3:1:1) that was supposed to become the cover material for the closed landfill, were tested. For the test, seeds from the lettuce *Lactuca sativa* „Grand Rapids” were used. The leachate L/S 10 was added to the lettuce seeds on filter paper in petri dishes. In total, 20 seeds per petri dish and 4 dishes per sample composed the set for the experiment. The results were compared to the control dishes with distilled water. Seed germination was observed for three days and root length were measured in the end.

**Seed germination tests** (with leachates L/S 5) - Seed germination and root elongation test was carried out with leachate L/S 5 with seeds of three different plants, the perennial ryegrass *Lolium perenne*, the lettuce *Lactuca sativa* and the timothy-grass *Phelum pratense*. Each leachate (8 samples in total, mixed by layers and sampling spots) was put onto 5 petri dishes with filter paper, and 20 seeds were added into each dish (100 seeds total for each sample). 4-5 mL of leachate was added to each dish and the petri dishes were stored in darkness in an incubator. The germination and root growth were measured in the end. For ryegrass the test was carried out for five days at 25°C, for the lettuce for three days at 25°C. For the timothy-grass the seeds required a three-day phase at 7°C followed by seven days at 25°C.

The germination rate was calculated by dividing the amount of germinated seeds from a sample with the amount of germinated seeds from the controls and multiplying it with 100, to get a percentage. The germination index was calculated using the germination rate, which was multiplied by the quotient of the mean root length from a sample and the mean root length of the control.

**Statistical analysis:** In order to determine if there was a difference in the effects of the eight L/S 5 leachates as well as between the plants themselves, root elongation data from the tested plant seeds were included in t-tests with a 5 % (0.05) significance level. Linear regression and correlation analysis to determine the correlation between physico-chemical measurement results and the results from toxicity tests with microorganisms was also carried out. All of the statistical analysis was done with Microsoft Excel 2013.
3. RESULTS

3.1 Physico-chemical characterization of leachate samples

The physicochemical parameters were measured at the beginning (0 h) as well as at the end (24 h) of the leaching process for the L/S 10 leaching and included pH, electrical conductivity and redox potential. The pH values at the beginning of the leaching ranged from 7.2 to 8.6 (average= 7.9; st.dev= 0.5). At the end of the leaching process the pH values ranged from 7.3 to 7.5 (average= 7.4; st.dev= 0.1). The figure as well as the standard deviations indicate that the pH values had a wider range at the beginning and were more even in the end of the leaching (Figure 3).

Electrical conductivity varied more (Figure 4) during the leaching process. At the beginning of the leaching the measurements ranged from 0.5 mS to 1.5 mS (average= 0.9; st.dev= 0.4). At the end of the leaching the conductivities were between 2.6 mS and 4.6 mS (average=3.2; st.dev= 0.6). Samples H2, H4, L2, L3 and L4 had a slightly lower conductivity than H1, H3.
and L1 at the beginning of the leaching. Sample H3 had the highest conductivity at the end of leaching.

Figure 4. Electrical conductivity of the L/S 10 samples at the beginning and end of the leaching. Error bars indicate standard deviations

The redox potential (Figure 5) had a wider range of values at the beginning of the leaching, like the pH and electrical conductivity. It ranged from 144 mV to 224 mV (average = 178; st.dev = 31). The redox potential values at the end of the leaching were between 203 mV and 220 mV (average = 211; st.dev = 6).

Figure 5. Redox potential of the L/S 10 samples at the beginning and end of the leaching. Error bars indicate standard deviations
The pH and electrical conductivity in the L/S 5 leachates were measured after leaching test. The pH values (Figure 6) for different samples varied only slightly, from 7.0 to 7.2 (average= 7.1; st.dev= 0.1). Electrical conductivities in the L/S 5 leachate samples were higher than in the L/S 10 leachate samples, ranging from 4.0 to 7.0 (average= 5.3; st.dev= 1.1). Similarly to the L/S 10 leachates, sample H3 had the highest conductivity.

The fine fraction sample H2B1 (L/S 10) prepared for the initial plant seed test had a pH of 7.7 and electrical conductivity 2.5 mS/cm. The sample from bio cover of landfill (fine fraction/soil/sludge mixture 3:1:1 v/v) had pH 7.7 and conductivity 2.3 mS/cm.

Chemical oxygen demand (COD) was tested for both filtered and unfiltered samples to see the difference between them (Figure 7) in order to bring knowledge regarding the proportion of chemical constituents in the particulate and dissolved phase. Tests performed with the unfiltered samples showed erratic results, oxygen demand spanned in the range from 386 mg/L to 765 mg/L (average= 630; st.dev= 132 mg/L), showing higher values in samples H2, H3 and L3. For the filtered samples COD values were between 70 mg/L and 196 mg/L (average= 134; st.dev= 44). The higher values were among samples H1, H3 and L3.
Total organic carbon was also tested for both unfiltered and filtered samples to evaluate the proportion of organic carbon that was in soluble form. The results for unfiltered samples ranged from 225 mg/L to 412 mg/L (average = 303; st.dev = 58) and for the filtered samples from 45 mg/L to 104 mg/L (average = 73; st.dev = 19) (Figure 8).

The L/S 5 filtered leachate samples were tested for TC, COD and (TN) (Figure 9). TC results ranged from 66 mg/L to 132 mg/L (average = 93; st.dev = 24), samples 3 and 7 having the highest
values (132 mg/L). TN concentrations in leachates were between 31 mg/L and 288 mg/L (average= 151; st.dev= 91), having highest values 262 mg/L and 288 mg/L in samples H3 and L4, respectively (Figure 9). The lowest nitrogen content (31 mg/L) was in sample L1. COD ranged from 61 mg/L to 250 mg/L (average= 129; st.dev= 60).

![Figure 9. Total nitrogen, total carbon and chemical oxygen demand from L/S 5 leachate](image)

Metal concentrations in the L/S 10 samples (Figure 10) were the following: copper and lead concentrations were considerably lower than zinc concentrations, copper in the range of 16-45 µg/L, lead between 112 and 143 µg/L. Zinc concentrations in the samples were greater, between 311 µg/L and 801 µg/L (average= 529; st.dev= 15 µg/L), with the highest concentrations in samples H3 and L3. Cadmium concentrations (Figure 11) in the leachates were from 0.15 to 0.26 µg/L (average= 0.20; st.dev= 0.04 µg/L).
3.2 Ecotoxicological characterization of leachate samples

Toxicity tests with the brine shrimp *Artemia salina* yielded the following results: samples L2 and L3 showed no inhibition (mortality) in the undiluted leachate. The highest mortality rate after 24 h was in sample L1 (12 %) and sample L4 (10 %). Overall the 24 hour results were steadily low throughout all the samples. After 48 h the two samples with the highest mortality were sample L4 with 50 % and sample H3 had 48 % mortality (Table 1).
Table 1. Results of ecotoxicological tests with L/S 10 samples (undiluted) to *A. salina, B. plicatilis* and *V. fisheri*

<table>
<thead>
<tr>
<th>Sample</th>
<th>A. salina 24 h</th>
<th>A. salina 48 h</th>
<th>B. plicatilis 24 h</th>
<th>B. plicatilis 48 h</th>
<th>5 min.</th>
<th>15. min</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>2%</td>
<td>21%</td>
<td>31%</td>
<td>31%</td>
<td>11%</td>
<td>23%</td>
</tr>
<tr>
<td>H2</td>
<td>2%</td>
<td>21%</td>
<td>34%</td>
<td>37%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>H3</td>
<td>6%</td>
<td>48%</td>
<td>36%</td>
<td>37%</td>
<td>8%</td>
<td>12%</td>
</tr>
<tr>
<td>H4</td>
<td>8%</td>
<td>39%</td>
<td>4%</td>
<td>24%</td>
<td>19%</td>
<td>24%</td>
</tr>
<tr>
<td>L1</td>
<td>12%</td>
<td>31%</td>
<td>3%</td>
<td>13%</td>
<td>23%</td>
<td>27%</td>
</tr>
<tr>
<td>L2</td>
<td>0%</td>
<td>27%</td>
<td>16%</td>
<td>21%</td>
<td>20%</td>
<td>24%</td>
</tr>
<tr>
<td>L3</td>
<td>0%</td>
<td>33%</td>
<td>34%</td>
<td>46%</td>
<td>10%</td>
<td>17%</td>
</tr>
<tr>
<td>L4</td>
<td>10%</td>
<td>50%</td>
<td>41%</td>
<td>41%</td>
<td>7%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Red - Test results validity questionable due to mortality in control being over 10 %. In bold and underlined – highest mortality rates/highest light inhibition.

However, the different dilutions of samples showed variable results in mortality (Figure 12). Although the overall mortality did not exceed 20 %, the figure shows no dose-response relationship. The highest mortality actually presented itself in the 6.25 % dilution of sample L3 with a mortality rate of 15 %. At the other dilutions however there was no response from the brine shrimp at all. Mortality rates at different dilution levels after 48 hours varied as well and showed even less regularity (Figure 13).

![Figure 12. Effect of L/S 10 samples on mortality of *Artemia salina* after 24 hours](image-url)
The RotoxKit M™ test with the rotifer *Brachionus plicatilis* showed results that were more even throughout the samples. After 24 hours, samples H4, L1 and L2 had the lowest mortality rate in the undiluted leachate with mortality between 3% and 16%. Their dose-response curves however move downward at the end of the scale, so their highest effect is actually exhibited at the 50% dilutions (Figure 14). The highest mortality rate in the undiluted leachates after 24 hours was in sample L4, with 41%. The 48 hour results show slightly higher mortality rates, but the dose-response curves followed the shapes of curves from the 24 hour results, with slight deviations in samples H4, L2 and L4 (Figure 15).
The MicroTox *Vibrio fisheri* screening test results showed a maximum of 23.5 % inhibition of luminescence after 5 minutes and 27.4 % after 15 minutes, both results from sample L1 (Figure 16). The least inhibition was exhibited by sample H2, with 2.5 % after 5 minutes and 2.4 % after 15 minutes. Sample 1 showed the largest increase in-between measurements, doubled from 11.4 % to 22.8 %. The other samples had only an increase of ~4-5 %.
According to the toxicity classification system (Persoone et al., 2014) for wastes discharged into the aquatic environment, almost all of the samples (besides H4) belong to the toxicity class 2 – slight acute toxicity (Table 2). The upper table shows a classification based on the results of the 24 hour/5 minute results, the lower table results based on the 48 hour/15 minute results (A. salina was not included in the second classification due to invalid test results). H1, H4 and L2 have a higher weight score percentage in the second table, nonetheless all samples remained in class 2.

Table 2. Classification of toxicity test results according to the toxicity classification system for wastes discharged into the aquatic environment (Persoone et al. 2003)

<table>
<thead>
<tr>
<th>Sample</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H4</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity class</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Weight score (%)</td>
<td>33%</td>
<td>33%</td>
<td>33%</td>
<td>0%</td>
<td>33%</td>
<td>33%</td>
<td>33%</td>
<td>33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H4</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity class</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Weight score (%)</td>
<td>100%</td>
<td>50%</td>
<td>50%</td>
<td>100%</td>
<td>50%</td>
<td>100%</td>
<td>50%</td>
<td>50%</td>
</tr>
</tbody>
</table>

The preliminary tests with Lactuca sativa seeds were performed with the L/S 10 leachate of fine fraction H2B1 and biocover. The average root lengths were 4.4 cm and 4.1 cm in leachates of H2B1 and the biocover, respectively. The control had an average root length of 1.6 cm. In H2B1 leachate the germination rate was 85 % and germination index 226 %. For the leachate of biocover sample the germination rate was 92 % and germination index 229 %. In the control, 90 % of the seeds germinated.

Further the effect of leachates of fine fraction on seed germination was tested with L/S 5 samples. The average root length of Lolium perenne in the leachates ranged from 2.4 cm (H3) to 3.1 cm (H2) (Table 3). The average in control test with distilled water was 1.9 cm. For Lactuca sativa, average root lengths were between 2.2 cm (H3) and 2.8 cm (H1), the control
having an average of 1.2 cm. *Phelum pratense* germinated seeds had average root lengths between 1.7 cm (L1) and 2.5 cm (H2), the control’s average being 1.8 cm.

Table 3. Average root lengths of *Lolium perenne*, *Lactuca sativa* and *Phelum pratense* in L/S 5 leachate

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>L. perenne</em></th>
<th><em>L. sativa</em></th>
<th><em>P. pratense</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>H1</td>
<td>2.8</td>
<td>2.8</td>
<td>1.8</td>
</tr>
<tr>
<td>H2</td>
<td>3.1</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>H3</td>
<td>2.4</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>H4</td>
<td>2.9</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>L1</td>
<td>2.8</td>
<td>2.7</td>
<td>1.7</td>
</tr>
<tr>
<td>L2</td>
<td>2.7</td>
<td>2.6</td>
<td>1.9</td>
</tr>
<tr>
<td>L3</td>
<td>2.5</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>L4</td>
<td>2.7</td>
<td>2.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The germination rates, i.e. amount of seeds germinated in a sample compared to the amount of seeds germinated in the control of the perennial ryegrass (*Lolium perenne*) were all above the control, except for sample H3 with a germination rate of 93 % (Figure 17), being the only sample resulting slightly lower germination of seeds than the control group. Sample H4 had the highest germination rate with 121 %.

The seeds from the lettuce (*Lactuca sativa*) had lower germination rates, samples H3 and L2 being the only one above control (101 % for sample H3 and 108 % for sample L2) and sample H4 having identical germination rates with control. The lowest rate was present in sample L4 with 77 %. The rest of the samples had germination rates between 93 % and 98 %.

Timothy-grass (*Phelum pratense*) had germination rates below the control in all eight samples. Sample L3 had the lowest rate with 88 % and sample H4 having the highest rate with 98 %. The control’s germination (seeds that germinated out of 100) for *L. perenne* was 76 %, for *L. sativa* the germination percentage was 84 % and for *P. pratense* the germination percentage was 97 %.
Figure 17. The germination rates of *Lolium perenne*, *Lactuca sativa* and *Phelum pratense* in the L/S 5 leachate

The germination indexes (relation between a sample’s seed germination and root length compared to the controls) of *Phelum pratense*, *Lactuca sativa* and *Lolium perenne* in the L/S 5 samples are displayed in Figure 18. *L. perenne* seeds had the highest germination indexes in sample H2 with 188 % and sample H4 with 186 %. The lowest index was in sample 3 with 116 %.

Figure 18. The germination indexes of *Lolium perenne*, *Lactuca sativa* and *Phelum pratense* in the L/S 5 leachate
3.3 Statistical analysis

The statistically significant correlations were investigated using Correlation and T.DIST in Microsoft Excel 2013. The results for the correlation between physico-chemical analysis results and toxicity test results with L/S 10 leachate (Appendix 1) show that there was a statistically significant (p<0.05) correlation between B. plicatilis test results and COD, TOC, electrical conductivity and copper concentration measurements.

COD measurements (Figure 19) showed a correlation with both B. plicatilis and V. fisheri results for up to 28% in the linear regression graph. A. salina results correlated with COD measurements for 15%, but the correlation was not statistically significant.

![Figure 19. COD linear regression with ecotoxicological tests](image)

TOC measurements (Figure 20) also correlated better with B. plicatilis and V. fisheri test results, while the correlation with A. salina results was low and showed no statistical significance.
Electrical conductivity measurements (Figure 21), to which only *B. plicatilis* and *V. fisheri* 24 h results showed statistically significant correlation, on the linear regression graph shows that 21-24 % of the results from those two tests is explained with the linear regression model.
Copper concentrations correlated best with *B. plicatilis* 24 hour results (Figure 22) and showed no correlation with *A. salina* test results.

![Cu linear regression with ecotoxicological tests](image)

**Figure 22. Cu linear regression with ecotoxicological tests**

*V. fisheri* test results correlated with the same physico-chemical measurements as *B. plicatilis*, except copper concentrations. However, the statistically significant correlations *V. fisheri* had were all negative, showing that, with the growing concentrations, the inhibitive effect was decreasing.

Although there was some correlation between the other physico-chemical analysis results and toxicity test results, they were not statistically significant (p>0.05).

Correlations between the physico-chemical analysis results from the L/S 5 leachate and average seed length, germination rate and index were also tested (Appendix 2). *L. sativa* root length data showed significant correlation with total carbon, dissolved organic carbon and chemical oxygen demand measurements. *L. sativa* seed germination rate data showed significant correlation with inorganic carbon data and its germination index correlated with total nitrogen data.
4. DISCUSSION

Since municipal landfills are essentially different from each other, from a compositional, climatic, hydrological, environmental, volumatic etc. standpoint, the statement about results from these tests being more of an informative, scientific and site-specific nature should be made. A lot of factors shape the physicochemical characteristics of a landfill, like the location (rural or urban), the technological level in consumer goods (different types of materials used for packaging), organic waste content etc. Therefore the results and conclusions about the ecotoxicity of fine fraction from Kudjape landfill cannot be used for decision making in other landfills. However, the data along with descriptions of methods can be used as guidelines when planning to use fine fraction as a part of the cover for a mined landfill.

Results from the physico-chemical analyses in both the L/S 5 and L/S 10 leachate of fine fraction as well as from the preliminary test showed different results, as it would be because of the different amount of fine fraction used to make the leachate. The pH measured in all samples was the only parameter to be more similar. The pH measurements of L/S 10 leachate at the beginning and ending of leaching also showed that during the process changes were minimal.

Electrical conductivity in the L/S 10 leachate was lower in the beginning of leaching, as expected, the hourly measurements in the beginning indicated a quite steady rise in conductivity as more salts or ions from salts from the fine fraction dissolved into the water in time. Conductivity in the L/S 5 leachate was expectedly higher than L/S 10 leachate due to more solid material being used in leaching.

Redox potential, which was only measured in the L/S 10 leachate showed more even results between samples at the end of leaching and together with the other physicochemical parameters, like organic carbon concentrations, chemical oxygen demand etc. serve as a reference or support in helping to identify the possible cause of ecotoxicological effects. Metals, specifically copper and lead, are also in the role of possible explanatory elements in case of some samples showing higher toxicity than others.
Low mortality rates in the *A. salina* toxicity tests quite clearly showed that the L/S 10 leachates were not very toxic. With a maximal 24 h mortality rate of 12 % in sample L1, it is quite evident. However, due to the dose-response curves showing no correlation between dilutions and mortality, and the fact that after 48 hours mortality increased randomly among dilutions (even controls, which caused the 48 hour data to be invalid), it is possible to make a few deductions. First, the cause for the 48 hour data to become invalid could be due to low quality or too weak specimen of *A. salina*. That would explain the absence of correlation between dilutions. Also, because the 24 h mortality data showed only a small range of mortality, the specific cause for them could not be attributed to the physico-chemical parameters.

The RotoxKit™ toxicity tests with the rotifer *Brachionus plicatilis* yielded much more reliable results until the end of the test as well as showed better correlation between concentrations of leachate and mortality rate. The differences between results from 24 hour data and 48 hour data were apparent, though some samples had almost no change in mortality. The higher mortality rates compared to the results from brine shrimp *A. salina* are likely due to the rotifer’s higher sensitivity (Tsarpali *et al.*, 2012; Elnabris, 2014). The RotoxKit test is also the main reason that all the samples got a Class 2 (slight acute toxicity) rating from the toxicity classification system. Still, the results of mortality were so low that not even LC50 (concentration causing 50 % of mortality among test organism) was achieved in the tests, indicating a low toxicity.

The data from *Vibrio fisheri* 81.9 % screening test also did not reach the 50 % inhibition, in this case, of luminescence. The screening test is originally meant to be a pre-test to determine how toxic the samples could be and what kind of dilutions are needed for samples in tests with other organisms. The results with the relatively high standard deviations, indicate high variability of inhibition among triplicates.

Since data of different kinds of tests can often give different results, a general evaluation of the results would give a better opportunity to make logical conclusions. The toxicity classification system developed by many research groups from different countries under coordination of Ghent University (Persoone *et al.*, 2003) was used. Since in all three toxicity test carried out in this work the 50 % effect was not reached, the “Toxicity classification system for wastes discharged into the aquatic environment” was well suited.
Combining all results from three ecotoxicity tests, the Class 2 – slight acute toxicity was calculated for almost all of the samples (except sample H4 in the first table, which was class 1 – no acute toxicity). The quite low weight scores after the 24 hour/5 minute data (1 of 3 tests had significantly higher levels of effect, >20%) suggest that the quantitative importance of the toxicity in those classes is low. Three samples had weight scores of 100 % in the 48 hour/15 minute data and the rest was 50 %, which only reinforces their affiliation with class 2, remaining slightly acutely toxic.

Seed germination and root elongation test data should give additional information about the ecotoxicity of fine fraction. Since the results from the preliminary test showed the low effect of the standard, L/S 10 leachate, the stronger, L/S 5 leachate was prepared to see if the fine fraction leachate could have a stronger effect.

Seed germination rates in the tests showed no inhibitive effect on L. perenne, for L. sativa and P. pratense the rates were slightly below control, indicating only a slight inhibition. Since the germination rates alone do not suffice in determining the effect of the L/S 5 leachate, calculating the germination index is a better way of getting results. The comparison of the two however can show certain aspects of growth. For example, the germination rate for sample H1 in L. perenne was 117 %, in L. sativa 98 % and P. pratense 92 %. All relative to the germination in the controls, so useful information here would be that L. perenne germinated slightly better than L. sativa, which in turn germinated better than P. pratense in H1 leachate. Their respective germination indexes were 176 %, 231 % and 90 %, meaning that L. perenne and L. sativa had higher root lengths compared to control. P. pratense however had a germination index lower than the germination rate, meaning that its mean root lengths were either equal or lower than that in control.

Considering that, a slight inhibitive effect was exhibited from the L/S 5 samples towards P. pratense from both germination rate and index, meaning its seeds germinated less and roots grew shorter than in control. This fact would go well with the results from the toxicity tests, implying a minor toxic effect towards the fine fraction leachate. However, the indexes for the other two plant species showed a rather different result, exhibiting a clear stimulating effect toward root growth. As additional data to be taken into account, the indexes and germination rates from the preliminary test with L/S 10 leachate on L. sativa were similar to the L/S 5 tests, but since the sample used in the preliminary test was not mixed, any conclusions drawn from
that similarity would not be scientifically sufficient. The results of the seed germination tests indicated that from three tested plant species *P. pratense* was the most sensitive.

The statistical analysis for determining correlation between the physico-chemical data and results of toxicity tests revealed that there were no clear correlations. The only statement that can be made from correlation analysis in this research is that the most likely physico-chemical parameter to be affecting the inhibition in the toxicity tests is COD. COD is a measure of organic compounds in water. Therefore it is safe to say that the most affecting factor towards the tested organisms was the content of organic compounds in the leachate of fine fraction. Correlation does not imply causation, especially with a limited amount of analyses and not accounting for all the possible factors. The no statistically significant correlation between the concentrations of metals and results of toxicity tests can be explained with the low concentration of studied metals in the leachate.

For the plant seeds, also organic compounds were statistically more affecting, but the effect was only present for *L. sativa* and it was stimulating, not inhibiting. Nitrogen concentration also negatively correlated with the germination index of *L. sativa*, indicating the stimulating effect.

To conclude the discussion, the following general statements can be made:

1. Physicochemical characteristics between the L/S 5 and L/S 10 leachate had greater differences in electrical conductivity and TOC.
2. The ecotoxic effect of L/S 10 leachate from fine fraction in the acute toxicity tests with aquatic organisms was low and can be classified as slightly acutely toxic.
3. Seed germination/root elongation tests showed stimulating effects of the L/S 5 leachate on *L. perenne* and *L. sativa* and only low inhibitive effects were observed on *P. pratense*.
4. The most affecting leachate parameter for ecotoxicological test organisms were COD measuring content of organic compounds.
5. Organic compounds and nitrogen in leachate were affecting the germination of *L. sativa.* Statistically significant correlations between physico-chemical parameters and data from tests with the other two plants were not observed.
SUMMARY

Leachate from fine fraction with particle size below 10 mm, excavated from the Kudjape closed landfill from Saaremaa, Estonia, was tested for ecotoxicity. Physico-chemical analyses, ecotoxicological tests with different types of organisms and plant seed germination and root elongation tests were carried out to characterize the leachate.

Acute toxicity tests with the three microorganisms: the brine shrimp Artemia salina, the rotifer Brachionus plicatilis and the bioluminescent bacteria Vibrio fisheri showed low levels of toxicity in the L/S 10 leachate. However, some results were high enough as not to be considered non-existent.

Mortality rates of A. salina in the 24 hour results from L/S 10 leachate were the lowest of the three tests with highest mortality of 12 %. The 48 hour data had to be discarded because of invalidity due to overly high mortality rates in controls.

Mortality rates of B. plicatilis in the RotoxKit™ tests with L/S 10 leachate were higher in all of the samples mortality rates remained well below the LC50. The 24 hour and 48 hour results varied slightly, as did mortality rates between the different L/S 10 samples.

Inhibition of light emission caused by leachate to V. fisheri in the MicroTox 81.9 % screening test was also low, with the maximum inhibition effect of 27 %. Results varied between samples, like in the other two tests, and inhibitive effects were higher in the 15 minute measurements than the 5 minute measurements.

Since the data collected from the three ecotoxicological tests varied and a position on a scale for evaluation should be reached, the results were included in the “Toxicity classification system for wastes discharged into the aquatic environment”, which resulted in a general categorization of the L/S 10 leachate from the fine fraction to be Class II – slight acute toxicity. One sample was even determined to be in Class I – no acute toxicity.

The data obtained from the physico-chemical analyses showed differences between L/S 10 and L/S 5 leachate. The correlation analysis results revealed that, from all the physico-chemical
parameters measured, the most likely to affect the inhibition in the toxicity tests was chemical oxygen demand.

Seed germination and root elongation tests with the L/S 5 leachate were carried out with lettuce *Lactuca sativa*, perennial ryegrass *Lolium perenne* and timothy grass *Phelum pratense*. Germination rates were highest for *L. perenne* (93–121 %) and lowest for *P. pratense*, which showed germination rates slightly under 100 % in all samples. Germination indexes, which took germination and root lengths into account, were highest for *L. sativa* and lowest for *P. pratense*. The high germination indexes also indicated a stimulating effect of leachates on the root growth of *L. sativa* and *L. perenne*.

Results from the ecotoxicological tests and seed germination tests lead to the conclusion that the leachates made from fine fraction excavated and sorted out from Kudjape landfill pose no ecotoxic hazard.
Kudjape prügilast kaevandatud peenfraktsiooni ökotoksilisuse määramine

KOKKUVÕTE


Ökotoksikoloogilised katsed viidi läbi soolavähikesega (Artemia salina), keriloomaga (Brachionus plicatilis) ning bioluminestseeruva bakteriga (Vibrio fisheri) leovee peal, mis valmistatud 1:10 suhtega (kuivainet : vedelikuühiku kohta).

A. salina katsetes vaadeldi leovee mõju loomade suremusele 24 ning 48 tunni möödudes. Tulemustest sai arvesse võtta ainult 24 tunni tulemusi, sest 48 tunni möödumisel oli suremus kontrollkatsetes ületanud lubatud piiri (10% suremus). Suurim suremuse määr 24 tunni möödumisel katsete proovides oli 12 %.

B. plicatilis testid sooritati RotoxKitM™ komplektidega. Leovee katsetes selle organismiga oli suremus mõnevõrra suurem, kuid mitte üheski katse proovis ei ületanud see 50 %. Suremust kontrolliti samuti 24 ning 48 tunni möödudes, mille käigus selgus, et suremus oli vähesel määral pikema aja möödudes suurenenud. Esines ka erinevusi suremuse osas erinevates proovides ning veidi suurenev pikema aja möödudes (5 minuti ja 15 minuti mõõtmistulemuste põhjal). Suurim valguse eritamise inhibeerimise määr oli 27 %.

V. fisheri valguse inhibeeriv mõju MicroTox-i katsetes oli samuti erinev erinevates proovides ning veidi suurenev pikema aja möödudes (5 minuti ja 15 minuti mõõtmistulemuste põhjal). Suurim valguse eritamise inhibeerimise määr oli 27 %.

Kõigi kolme katse andmeid kasutati ühise klassifikatsiooni süsteemis, mille kohaselt kuulusid kõik katsete proovid Toksiliisuse hindamise süsteemis teise klassi, mis näitab minimaalset toksilisust.
Taimede seemnete isanevuse ning juurte pikkuste katsed viidi läbi kangema leoveega (1:5) lehtsalati (Lactuca sativa), raiheina (Lolium perenne) ning põldtimuti (Phelum pratense) peal. Idanevuse määrad, mis näitavad seemnest idanevust võrreldes kontrollkatsega, olid kõige kõrgemad proovides raiheinaga (93-121 %). Madalaimad määrad olid põldtimutil, mille määrad jäid kõikides proovides veidi alla 100 %. Idanevuse indeksid, mis lisaks seemnete idanevusele võtab arvesse ka juurte pikkused, andsid aga järgmised tulemused: kõrgeimad indeksid ilmnesid lehtsalatil ning madalaimad põldtimutil, mis jääid taas veidi alla kontrollile.

Füüsikalis-keemiliste analüüside tulemusi ökotoksikoloogiliste katsete tulemustega korrelatsioonanalüüsi tehes selgus, et kõige rohkem korreleerub mikroorganismide suremuse/valguse inhibeerimisega keemiline hapnikutarve ning orgaaniline süsinik.

Kõiki tulemusi arvesse võttes võib kokkuvõtvalt öelda, et Kudjape prügilast kaevandatud peenfraktsiooni ökotoksilisus on madal.
REFERENCES


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APPENDIXES