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Report on effect-based assessment of biodegradation and remediation success

Description of toxicity thresholds for acute and mechanism-specific effects as a measure of remediation success

D2.4

WP2: Oil biodegradation and bioremediation

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Executive summary

In the context of remediation studies, bioassays can function as effect-based tools to verify remediation conclusions based on chemical analysis data, as they provide insight into resulting toxicity and can thus also account for non-analysed contaminants. As part of the GRACE work package 2, bioassays were to be used as effect-based tools to determine the success of remediation by biodegradation in water samples. No accompanying investigations of biodegradation experiments could be conducted, due to low amounts of sample. However, in a different part of WP2, a polluted bay in Finland characterized by high anthropogenic pollution over the last decades was selected for a pilot study of electrokinetic treatment in respect to remediation of oil contaminated sediments. We investigated the electrokinetically treated sediments to complement the results from the chemical characterization (details in deliverable 2.3).

From the results of D2.3 no clear remediation success based on electrokinetic treatment was concluded. However, in one out of three experimental areas (area III) a degradation in petroleum hydrocarbons during the treatment period was observed. For the present report, 4 samples of 2 experimental areas (I and III) were selected to investigate whether the observed degradation of petroleum hydrocarbons in one area (III) also resulted in a lower toxicity towards biota. It is important to keep in mind that the present report does not compare the toxicity before and after the electrokinetic treatment but addresses the question on remediation success indirectly by comparing samples chemically indicating (area III) and not indicating (area I) a remediation success.

The results of three individual bioassays indicate a comparable trend that with reduced hydrocarbons including PAHs in experimental area III, a reduced toxicity is observed. In experimental area I, where no remediation success based on unchanged chemical profile was concluded, also effect-based toxicity assays did not detect reduced toxicity of treatment groups compared to control groups. However, a direct comparison of selected control sediments with electrokinetically treated sediments should be treated with caution as one out of three replicate sediment cores were chosen.

This was a proof-of-concept for complementing chemical analysis with effect-based measurements as an integrating parameter. To clearly evaluate the remediation success on toxicity level more data on additional sampling campaigns of the pilot study would be necessary. Furthermore, the implementation of additional ecotoxicological endpoints on higher biological organization levels would complete the big picture of electrokinetic treatment for remediation success in this scenario. Nonetheless, the selected *in-vitro* bioassays are time- and cost-efficient first screening tools to characterize toxic actions of extracts from electrokinetically treated sediment.

I Introduction

One part of the GRACE work package 2 addressing the oil biodegradation and bioremediation was to use bioassays as effect-based tools to determine the success of remediation by biodegradation in water samples. No accompanying investigations of biodegradation experiments could be conducted, due to low amounts of sample. However, we investigated instead electrokinetically treated sediments to complement the results from a chemical characterization. In this context, a polluted bay in Finland characterized by high anthropogenic pollution over the last decades with heavy metals, petroleum and polycyclic aromatic hydrocarbons (PAHs) was selected for a pilot study of electrokinetic treatment. A report on the chemical profile of elektrokinetically treated sediments during the long term experiment over more than 12 months can be found in deliverable 2.3 (Tunturi et al. 2018).

Several studies in the field of ecotoxicology have demonstrated that the combination of chemical and biological examination methods for environmental pollution risk assessment is important to understand the whole picture of toxicity and to identify the responsible chemical groups (Escher et al. 2013, Hecker and Hollert 2009). Also in the context of remediation studies, bioassays can function as effect-based tools to verify remediation conclusions based on chemical analysis data, as they provide insight into resulting toxicity on different biological organization levels and integrated also non-analysed contaminants. Hence, the aim of the present report was to complement the results of the chemical analysis reported in deliverable 2.3.

From the results of deliverable 2.3 it was concluded that a remediation success could not be unambiguously identified in the experimental areas. The results indicated an electrokinetic treatment remediation success for one out of three experimental areas. Hence, the concrete question of the present report was whether the observed degradation of petroleum hydrocarbons in one area also resulted in a low toxicity towards biota. No modification or even an increase in observed toxicity after electrokinetic treatment must not be excluded without proper investigation, as the treatment leads to a number of simultaneously occurring changes in the sediment. The mobilization of organic contaminants such as PAHs might occur, but also changes in soil moisture or dissolved oxygen content, strongly dependent on sediment characteristics, will be a result of electrokinetic treatment (Ortega-Calvo et al. 2013).

Based on this central question, four samples of one sampling campaign (for details see chapter II) were selected for mechanism-specific toxicity investigations. It is important to keep in mind that the present report does not compare the toxicity before and after the electrokinetic treatment but

addresses the question on remediation success indirectly by comparing samples chemically indicating and not indicating a remediation success.

The performed bioassays focus on cell viability (MTT assay), oxidative stress response (Nrf2-CALUX®) and dioxin-like activity (micro-EROD assay). All endpoints are *in-vitro* based methods using ecotoxicologically established cell lines. The advantages of *in-vitro* based methods are cost and time efficiency. Furthermore, they support the effort to reduce, refine and replace animal testing (3R principle). Several *in-vitro* bioassays focus on concrete toxicity pathways like receptor activation or inhibition, which helps to identify the underlying molecular mechanisms of toxicity. However, cellular responses will not always imply effects on higher biological organization levels like organs, whole organisms or even populations (Escher et al. 2013). When considering the limitations of the *in-vitro* methods for the interpretation of ecotoxicological effect data, cell based methods are useful tools in the characterization of toxic actions of environmental samples or chemicals.

In the following section background information on the bioassays of the present report is given.

Viability examination using MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a water soluble monotetrazoleum salt, which is used to detect cytotoxic events in cells. Hence, in this case the assay was selected as a marker for general cytotoxicity. The MTT assay is reported as a very sensitive method (Fotakis and Timbrell 2006). The underlying principle is based on the uptake of the yellow tetrazolium salt via the plasma membrane potential due to its net positive charge (Berridge et al. 2005), followed by the reduction to the unsoluble purple product formazan, which accumulates in crystals in viable cells (Gonzalez and Tarloff 2001). After lysing cells and formazan crystals, absorbance measurement quantifies the formazan content. High absorbance values correlate with high formazan contents and consequently with high cell viability.

Oxidative stress response using Nrf2-CALUX® assay

Human osteoblastic U2OS cells have been stably transfected with the transcription factor Nrf2, which is involved in the activation of genes containing anti-oxidant responsive elements contributing to the oxidative stress response (van der Linden et al. 2014). The activation of the Nrf2 can indirectly be a first indicator for genotoxic effects as reactive radicals can interact with cellular macromolecules possibly leading to DNA damage (Van der Oost et al. 2003).

The Nrf2 - chemical activated luciferase gene expression (CALUX) bioassays is based on a reporter-gene method using the induction of bioluminescence signals. The reporter-gene luciferase is expressed when the transcription factor Nrf2 is activated. With addition of the reporter-genes substrate luciferin the enzyme converts to substrate to oxyluciferin with light photon emission as a byproduct of the reaction. The luminescence signal correlates with the strength of luciferase expression, allowing a quantitative evaluation of oxidative stress response.

Dioxin-like activity using micro-EROD assay

The micro-EROD assay is an aryl hydrocarbon receptor (AhR)-based bioassay to determine cytochrome P450 activity (CYP1A), which are enzymes contributing to phase I of xenobiotic metabolism (Schiwy et al. 2015). Besides the detoxification function by converting toxic compounds into more hydrophilic compounds for further transformation steps until excretion, it has been shown that some non-toxic parent compounds can be converted to genotoxic metabolites (e.g. benzo(a)pyrene (Behnisch et al. 2001).

The EROD assay was selected for the present study as several oil-sediment containing compounds are well described for their CYP induction. In principle, xenobiotics bind the AhR in the cytosol of the non-transfected rat hepatoma cells. The formed complex, which is translocated into the nucleus, binds the dioxine-responsive elements leading to the expression a set of enzymes including CYP. With the addition of the substrate 7-ethoxyresorufin the ethoxyresorufin-O-deethylase (EROD, CYP enzyme) converts the substrate to the fluorescent product resorufin. Measured fluorescence is quantified by comparing the EROD activity with the concentration-response curve of the reference compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

II Sample background

Töölönlahti bay in Helsinki, close to the Gulf of Finland was selected as the study site for the pilot experiment using EKOGRID^{TM} method of electrokinetic treatment developed by Lamor Corporation Ab. The upper sediment layers are well known for the anthropogenic pollution accumulated during the last decades of industrial and municipal waste water discharge. Various organic and inorganic contaminants including crude oil relevant hydrophobic petroleum compounds such as polycyclic aromatic hydrocarbons have been determined. In total, 4 sampling campaigns from before treatment over during treatment and finally after treatment were conducted. More details about the study region as well as the EKOGRID^{TM} method are described in deliverable 2.3.

Selected samples of the present report for effect-based bioassays can be found in Table 1. All samples were collected during sampling campaign 3 (summer 2018). Two experimental areas (area I and III), each with one control and one treatment replicate (out of 3 replicates per treatment) were selected. Area I and III have been chosen based on differences in remediation success detected via chemical analysis with area I characterized by no and area III characterized by significant hydrocarbon reduction.

Table 1 Sediment samples of electrokinetic treatment pilot experiment in Töölönlahti bay (Helsinki, Finland) used in effect-based bioassays of this report. Roman numbers indicate experimental areas, followed by the number of sampling campaign on which control (C) or treatments (T) were collected in three replicates (A-C). Chemical analysis data were provided by WP2.

sample codes	sampling date	Petroleum hydrocarbons C10 – C40 [mg/kg dw]	Sum PAHs [mg/kg dw]
I3KC	4.06.2018	1900	9.3
I3TC	4.06.2018	2100	11
III3KC	27.08.2018	2600	13
III3TC	27.08.2018	780	8

III Material and methods

III.1 Sediment extraction

Pressurized liquid extraction (PLE) was used to extract the sediment samples using the SpeedExtractor® E-916 (BÜCHI GmbH, Flawil, Switzerland). Before extraction sediment samples were lyophilized at 0.2 bar for 72 h using the freeze drier Alpha 2-4 LD plus (Christ, Osterode am Harz, Germany) followed by storage at -20 °C until further usage. 10 g of freeze-dried sediment were filled in extraction cells in alternating layers of sample and silica sand. One extraction cell filled with silica sand only served as a process control (ProCo) and will be treated as an additional sample in the following steps of extract processing and bioassays. Operation conditions of one extraction cycle were characterized by 1 min preheating phase, followed by a 10 min static extraction phase at 100 bar and 100 °C, a 4 min discharge phase and finished with 2 min of nitrogen purge flush. As extraction solvents, HPLC-pure acetone and n-hexane (1:1 (v/v)) were used. 2 extraction cycles were performed in total.

Extracts were reduced close to dryness to eliminate solvents that could result in cytotoxicity using a rotatory evaporator. Extracts were re-dissolved in 1 mL DMSO, resulting in a concentration equivalent to 10 g dry sediment per mL solvent (10 g SEQ/ mL). Extracts were stored in darkness at -20 °C until investigation in bioassays.

III.2 Cell culture

III.2.1 Human osteosarcoma U2OS cells transfected with transcription factor Nrf2

The osteosarcoma cells U2OS purchased from BioDetection Systems BV (BDS, Amsterdam, The Netherlands) have been used to investigate the cell viability as well as the oxidative stress response.

Cells were cultured in a mixture of Dulbecco's modified Eagle's medium and F12 medium (1:1), which was supplemented with 7.5 % fetal calf serum (Biowest, France), non-essential amino acids and a penicillin-streptomycin solution as described in the SOP from BDS (b.v. 2017). Cells were cultivated at 37 °C with an atmosphere containing high humidity and 5 % CO_2. Periodically, cells were passaged when reaching 90% confluence.

For the MTT and the Nrf2-CALUX® assay an assay medium was prepared from Dulbecco's modified Eagle's medium and F12 medium (1:1) without the pH indicator phenol red, which was finally supplemented with 5 % FCS (charcoal stripped, Biowest, France), non-essential amino acids and penicillin-streptomycin as described in the SOP from BDS (b.v. 2017).

III.2.2 Rat hepatoma cells H4IIE

The non-transfected rat hepatoma cell line H4IIE is used in this study to determine the potential of oil sediment extracts to induce cytochrome-P450 activity. Cells were cultured in a Dulbecco's modified Eagle's medium (DMEM) without phenol red, which was supplemented with 10 % fetal calf serum (Biowest, France), GlutaMAX and HEPES buffer. Cells were cultivated at 37 °C with an atmosphere containing high humidity and 5 % CO_2. Periodically, cells were passaged when reaching 90% confluence.

III.3 Cell viability examination using MTT bioassay

The cell viability was investigated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay first described by Mossmann et al. (Mosmann 1983) with modifications regarding the selected cell type specific conditions (transfected U2OS cells).

Briefly, cells were seeded in a 96-well plate at a density of 1 x 10^5/mL in assay medium (see II.2.1). Afterwards, cells were incubated at 37 °C and 5 % CO_2. 24 h after seeding, cells were exposed to a dilutions series of the samples with a maximum solvent content of 0.1 %. The 1:2 dilution series was prepared beforehand in DMSO. Afterwards, the exposure medium was removed, cells were washed with PBS and yellow MTT salt (500 µg/mL) was added. After an incubation for 30 min at 37 °C the MTT solution was replaced by DMSO to dissolve the cell membranes and the formed formazan crystals on an incubation shaker for 15 min. The absorbance at 492 nm was measured using an Infinite M200 plate reader (Tecan Group AG, Männedorf, Schweiz). The intensity of absorption is proportional to the amount of viable cells. Cell viability was calculated by correcting the absorbance values first for the response of the blank values. A control absorbance of untreated cells was defined as 100 % cell viability while all sample dilutions were calculated relative to this viability.

For cell exposure in all mechanism-specific bioassays only sample concentrations resulting in at least 80 % cell viability were used.

III.4 Oxidative stress response using Nrf2-CALUX® assay

The Nrf2-CALUX® assay was performed according to the SOP provided by BioDection Systems (BDS) Amsterdam as described briefly in van der Linden et al. (2014).

Seeding and incubation conditions were described in the previous chapter (cell viability assay procedure). In the Nrf2-CALUX® assay cells were exposed to a dilution series of sample extracts

and the standard reference compound curcumin ($1*10^{-8}$ - $1*10^{-4}$ M). To avoid non-specific cytotoxic effects, the highest test concentrations (ranging from 0.63 to 10 mg SEQ/ mL, for details see results MTT assay) for each extract were determined in the MTT assay. The maximum solvent content was 0.1 % for sample extracts and 1 % for the curcumin reference, respectively.

After 24 h of exposure the medium was removed, cells were lysed and luciferase activity was measured with the application of luciferin substrate mixture in a luminescence reader (Glomax 96-microplate reader, Promega, Madison, USA). The intensity of the luminescence signal correlated with the activation of the transcription factor Nrf2.

As the Curcumin calibration series does not have maximum response, it is not possible to use the typical relative induction evaluation (see ERα-CALUX®). Instead, the induction factors (IF) were calculated to quantify the response of a sample as recommended by BDS. IF was calculated for each dilution step of the curcumin standard and the samples by normalizing each luminescence value to the luminescence of the background of the standard. IF values were used to establish a dose-response fit (4 parameters non-linear regression with variable slope) using the GraphPad Prism 6. Cytotoxic concentrations of the reference compound leading to IF values of 0 were excluded for the dose-response fit. Within this fit the concentration of curcumin and the sample that results in an IF=1.5 was calculated because the curcumin standard gives a non-cytotoxic stable response in this range and does. At an IF of 1.5 the specific activity of each sample was calculated by dividing the sample concentration by the standard concentration and finally expressed in µg Curcumin/mgSEQ of sample.

III.5 Dioxin-like activity using micro-EROD assay

Measurement of EROD activity was conducted according to Schiwy et al (2015). H4IIE cells were seeded at a density of 200,000/mL in a 96 well plate. Afterwards, cells were incubated for 2 h followed by the exposure with TCDD standard dilution series (0.15 -6 pg/mL) and sample dilutions in quadruplicates. A 1:2 sample dilution series were prepared beforehand in 1% DMSO medium. Resulting maximum solvent concentration in the assay was 0.5 %. After an incubation period of 72 h, the exposure medium was removed and 100 µL of the substrate 7-ethoxyresorufin (8 µM) solution supplemented with dicoumarol (10 µM) were added to each well. During an incubation for 30 min at 37 °C the substrate ETX is reduced to the fluorescent product resorufin. To stop the reaction, 75 µL of methanol was added to each well.

Substrate deethylation was determined by measuring the formed resorufin at 540 nm excitation and 590 nm emission wave length. EROD activity was expressed in pmol resorufin/mg/min.

Whole protein of each well was measured in the same plate using a Bicinchoninic Acid kit (Sigma Aldrich GmbH, Germany) according to the manufacturer's instructions and quantified with a dilution series of bovine serum albumin (BSA) as an external standard (7.81 - 500 µg/mL). Briefly, 100 µL of BCA kit solution were added to each well followed by an incubation at 48°C for 22 min. Absorbance at 562 nm was then measured using a multiplate reader (Infinite M200, Tecan Group AG, Männedorf, Schweiz).

IV Results of bioassays for the investigation of remediation success

1. Cell viability examination using MTT bioassay

All sediment extracts were tested in the MTT bioassay for viability examination in the concentration range of 0.04 to 10 mg SEQ/mL (0.1% solvent). Cell viability of treatment concentrations was normalized to an untreated negative control always testes aside the samples.

In general, the exposure to each extract led to a concentration related increase in cytotoxicity with increasing sample concentration (**Figure 1**). The process control of extraction, which consists of inert silica sand, led to normal cell viability compared to the untreated control (**Figure 1** E). Hence, cytotoxicity of the samples based on the extraction procedure can be excluded.

Cell exposure to the untreated control group sediment extracts of experimental area I (**Figure 1** A) resulted in a lower cytotoxicity compared to the corresponding electrokinetically treated group of experimental area I **Figure 1** B). While for I3 KC (control) only the highest test concentration led to cell viability below the critical viability of 80 %, I3 TC induced cytotoxicity even in the 4th highest test concentrations.

For sediment extracts of experimental area III the exact opposite was observed: The untreated sediment extract (**Figure 1** C) induced a higher cytotoxicity compared to the electrokinetically treated extract (**Figure 1** D) with the highest 4 concentrations and only the highest concentration below the critical cell viability limit, respectively.

In respect to a reported reduction in hydrocarbon contamination of experimental area III compared to area I, the present experiments on viability observed the same trend of reduced cytotoxicity in experimental area III after electrokinetic treatment. However, this interpretation requires substantial precaution as the selected control group cannot be directly compared to the selected treatment group. Both replicates were chosen out of 3 sediment cores in the sampling field.

Based on the viability examination the maximum exposure concentrations for U2OS cells in the Nrf2-CALUX® assay were determined as 5 mg SEQ/mL (I3 KC, III3 TC) and 0.625 mg SEQ/mL (I3 TC, III3 KC). Process control was investigated from the highest concentration of 10 mg SEQ/mL.

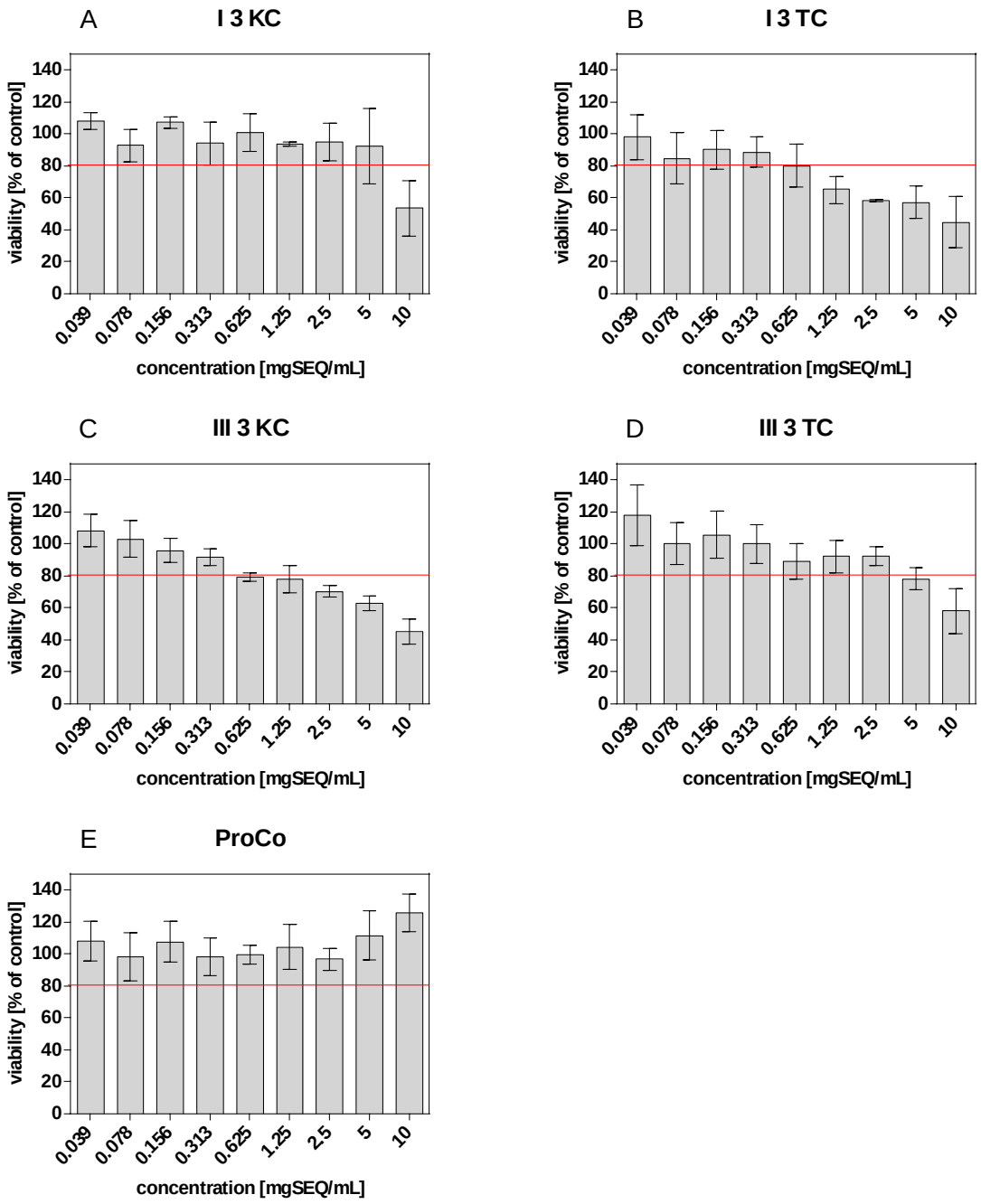

Figure 1 Relative viability of U2OS cells exposed to oil sediment extracts after electrokinetic treatment in the MTT bioassay. Panels A and B show the untreated control (A) and treated sediment (B) samples of experimental area I, while panel C and D show the corresponding treatment groups of experimental area III. Cytotoxic effects of sample concentrations were normalized to the negative control (100 % viability). Bars represent mean values of 3 independent replicates. Error bars denote the standard deviation and the red dotted line illustrates the critical threshold for normal cell viability (80 %).

2. Oxidative stress response using Nrf2-CALUX® assay

The extraction process control (ProCo) did not activate the transcription factor Nrf2 (see **Figure** 2, Panel E) with induction factors (IF) corresponding to the background level of untreated control (IF = 1). Hence, potential oxidative stress responses are based on sampled compounds only and effects from the extraction procedure can be excluded.

In general, all tested sediment extracts activated the transcription factor Nrf2 in a concentration-response relationship (see **Figure 1** A-D). The maximum induction factors calculated for the second highest test concentration of the reference compound Curcumin varied between 8 and 20, which is the common response variability of this bioassay, as oxidative stress induction is biologically close to cytotoxicity. For all experiments, the highest test concentration of the reference compound ($1* 10^{-4}$ M) had to be excluded based on cytotoxic effects.

All tested sediment extracts of Töölönlahti bay induced oxidative stress above the IF threshold of 1.5 in their highest test concentrations varying from 1.63 to 2.8 (see **Table 2**). However, based on viability examination experiments (see chapter IV.1) the highest non-cytotoxic test concentrations in the Nrf2-CALUX® bioassay varied for each sample. The calculation of the specific activity accounts for different sample dilutions and allows the comparison of each sample.

As can be seen in Figure 3 the treated sediment core of experimental area I (I3 TC) showed high variations between the 4 individual experiments with no activation of the Nrf2 up to a specific activity of 44 µg Curcumin/mg SEQ. However, the results indicate a trend of increased oxidative stress potential of electrokinetically treated sediment extracts compared to untreated control. This trend was not found to be statistically significant.

In contrast, results for experimental area III show a clear difference in oxidative stress induction potential. The exposure to the electrokinetically treated sample resulted in a lower specific activity compared to untreated sample.

Thus, the results of increased hydrocarbon degradation in experimental area III (D2.3) is supported by a reduced toxicity observed in this report. Furthermore, the present study also did not detect a reduced but rather increased oxidative stress potential for the electrokinetically treated samples of experimental area I, where no reduction of petroleum hydrocarbons and the PAH concentration was found.

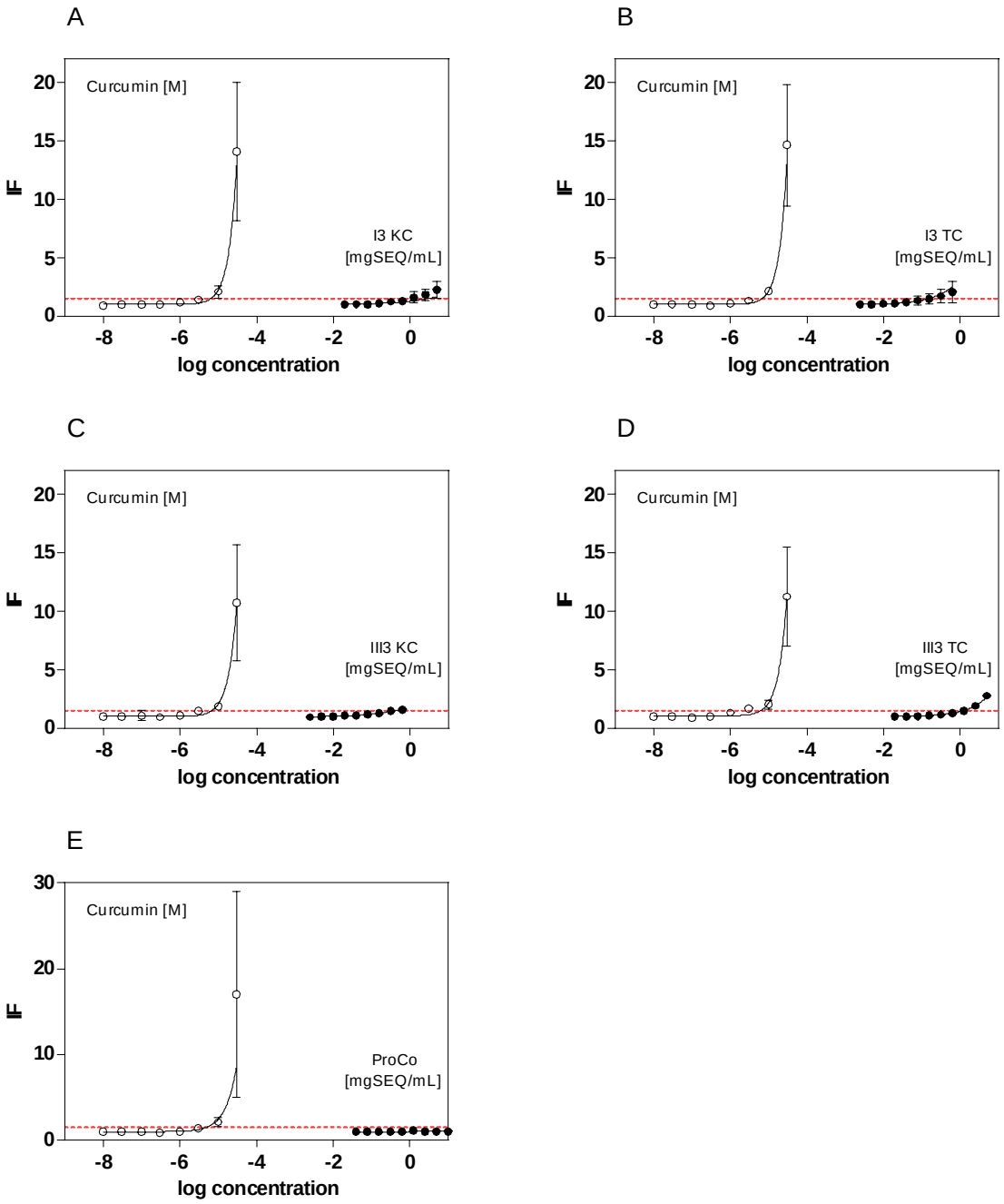

Figure 2 Oxidative stress induction by sediment extracts of electrokinetic treatment experiments in the Nrf2-CALUX® assay. Based on luminescence data induction factors (IF) were calculated as relative values to the background of the Curcumin calibration series. Dots and error bars represent the mean IF of 4 individual experiments with standard deviation. Filled circles show the IFs of sediment extracts, while empty circles show the IFs of the reference compound. In panel A and B the oxidative stress induced by untreated (A) and electrokinetically treated (B) sediment extracts of experimental area I are plotted. Panels C and D show respective data of experimental area III. An extraction process control (E) was included. Red dotted line indicates the oxidative stress threshold of IF=1.5, which is used for further calculations.

Table 2 Calculated induction factors (IF) of elektrokinetically treated sediment extracts in the Nrf2-CALUX® assay. IFs of untreated and treated extracts of experimental area I and III were calculated by dividing the luminescence values of each sample dilution by the background of the curcumin calibration. The table contains mean IF with standard deviation (SD) calculated for the highest test concentration of 4 independent experiments. Extraction process control (ProCo) did not induce IFs elevated to the negative control.

treatment	highest test concentration [mg SEQ/ mL]	IF_{mean}	SD
I3 KC	5	2.29	0.75
I3 TC	0.625	2.09	0.89
III3 KC	0.625	1.63	0.10
III3 TC	5	2.8	0.22

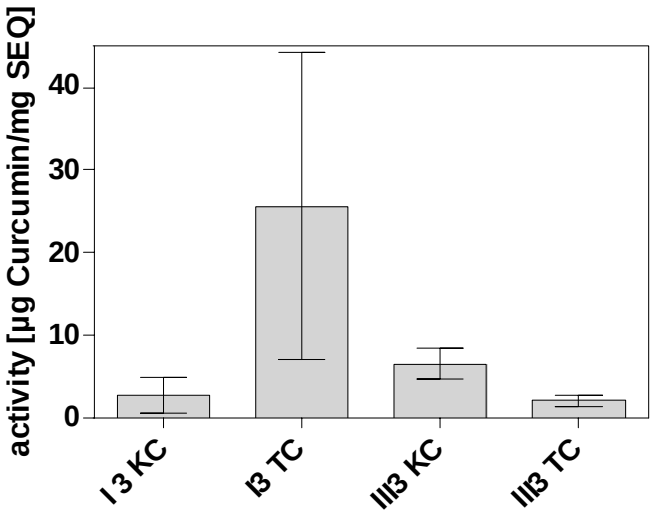

Figure 3 calculated specific activity of electrokinetically treated sediments in the Nrf2-CALUX® assay. Bars and error represent the mean and standard deviation of 4 independent experiments. Specific activity was calculated based on interpolated sample concentrations and curcumin concentrations leading to an induction factor (IF) of 1.5 compared to the reference background.

3. Dioxin-like activity using micro-EROD assay

In general, only 1 EROD assay with the sediment extracts was available for the present report for time reasons. However, even with this first experiment, trends of CYP activity in rat hepatoma cells could be observed and will be communicated.

Also in this bioassay, the extraction process control (ProCo) did not induce the CYP enzyme (see **Figure 4**, Panel C). Hence, potential EROD induction is based on sampled compounds only and effects from the extraction procedure can be ruled out.

In the tested concentration range, all sediment extracts from experimental area I and III induced cytotoxicity in the hepatoma cells as can be seen by decreasing EROD activity with increasing concentration (**Figure 4**, Panels A and B). Hence, no EC or TEQ values could be calculated based on the present results. To identify a dose response related EROD induction extracts have to be diluted further.

However, the lowest 2 exposure concentrations of all 4 samples seem to be non-cytotoxic to the cells. The EROD activity in this range (20,000 – 25,000 pmol mg^{-1} min^{-1}) was quite high and comparable to the upper range of the TCDD standard indicating high amounts of potential AhR-activating compounds.

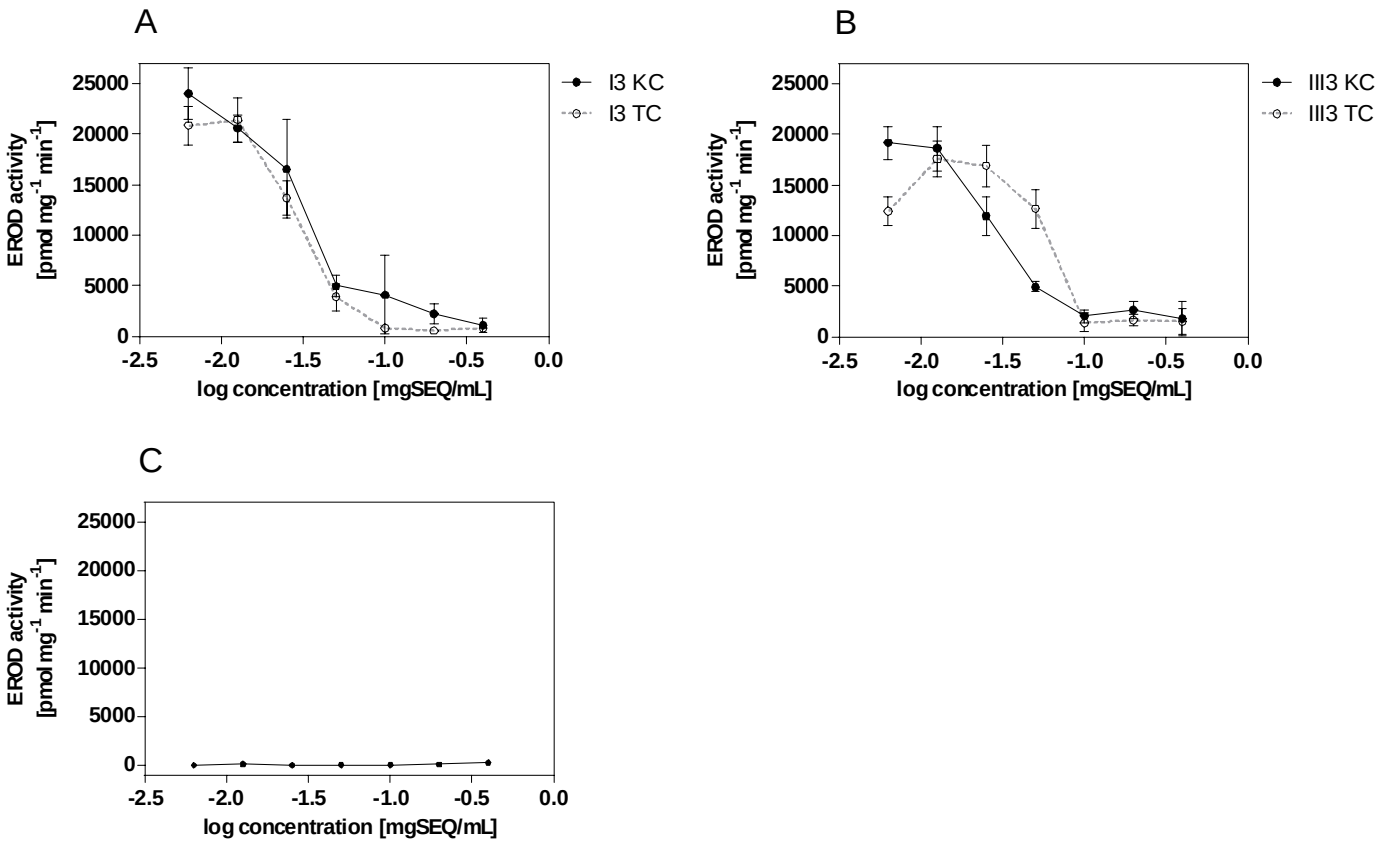

Figure 4 CYP1A activity in H4IIE rat hepatoma cells exposed to electrokinetically treated sediments in the µEROD assay. Based on fluorescence data of 7-ethoxyresorufn deethylation and measured protein content the CYP activity of the samples and the TCDD standard were calculated. Dots and error bars represent the mean EROD activity of 4 technical replicates with standard deviation. In panel A the EROD activity induced by untreated (A) and electrokinetically treated (B) sediment extracts of experimental area I are plotted. Panels B and C show respective data of experimental area III and the included extraction process control.

Comparing the elektrokinetically treated and untreated sediment extracts of experimental area I, no difference in the cell inhibition or EROD induction potential was observed as both curves show an identical shape. In contrast to this, the treatment of control and elektrokinetically treated sediment extracts of experimental area III resulted in deviating curve progressions. Especially in the lowest and simultaneously non-cytotoxic concentration the EROD activity of elektrokinetically treated sediment was below the EROD activity of untreated sediments. Hence, as already described in the other bioassays, the results carefully indicate a remediation success in the experimental area III. However, it has to be emphasized again that the EROD assay has to be repeated in a lower concentration range to identify a clear CYP activation potential in the sediment samples.

V Assessment of remediation success

The integration of electrokinetic treatment methods in remediation of contaminated soils and sediments is a current topic of research. Different studies have demonstrated that in respect to organic contamination with PAHs, the mobilization of the polluted fraction entrapped within the soil nanopores and/or strongly sorbed to black carbon, could have been enhanced due to electrokinetic treatment (Ortega-Calvo et al. 2013). However, the effectiveness of enhanced mobilization followed by enhanced biodegradation seems to be dependent on unique scenarios influenced by physical-chemical characteristics of sediments, the microbial community and the contaminants.
The aim of the present study was to complement the results of a long-term electrokinetic remediation experiment using chemical analysis of organic contamination by effect-based methods on toxicity.

The results of three individual bioassays indicate a comparable trend that with reduced petroleum hydrocarbons and PAHs in experimental area III, a reduced toxicity is observed. In experimental area I, where no remediation success based on unchanged chemical profile was concluded, also effect-based toxicity assays did not detect reduced toxicity of treatment groups compared to control groups. From the present results it can be concluded that in the contaminated sediments of Töölönlahti bay organic contaminants like petroleum hydrocarbons and PAHs are the main driver of toxicity as the reduced concentration of those chemical groups correlated with a reduced toxicity.

However, as already indicated in the introduction, a direct comparison of selected control sediments with electrokinetically treated sediments should be treated with caution as one out of three replicate sediment cores were chosen. Furthermore, the present report addresses the verification of chemical analysis data on remediation success indirectly by investigating samples of one sampling campaign that differ in their chemical profile leading to different levels of remediation success. To clearly evaluate the remediation success on toxicity level more data on different

sampling campaigns would be necessary. Based on the current chemical profiles that were established during the whole experiment no clear conclusion about the remediation success was drawn because of high variations between the sediment test plots, but promising trends were observed.

VI Conclusion and outlook

In general, limited data on the potential soil or sediment remediation success by electrokinetic treatment are available. However, electrokinetic treatment as well as combined approaches using additional treatment methods like surfactant application or rhizoremediation are promising tools in the field of bioremediation.

Even though the pilot experiment of electrokinetic treatment did not allow clear conclusions of remediation success, the finding that all three bioassays gave lower effects for the treated samples that also showed hydrocarbon reduction is an indication that the electrokinetic treatment actually was able to reduce toxicity.

For further investigations not only more data on different sampling campaigns would be necessary to identify the big picture of remediation success but also more ecotoxicological endpoints including higher biological organization levels would complete the toxicity characterization. The investigation of teratogenic and acute toxic effects on the early life stages of the zebrafish would be a useful endpoint to include a whole organismic vertebrate endpoint. The embryonic and early larval development up to 120 hours post fertilization (hpf) is not considered as an animal experiment, and hence no animal test authorization is required. Furthermore, not only sediment extracts but also whole sediments could be tested in an established sediment contact assay.

VII References

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