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TOXICITY EVALUATION OF SPENT DRILLING MUD AND DRILLING WASTE***

1. INTRODUCTION

Drilling waste belong to one of the types industrial waste, which is being deposited in large amount during drilling works into nature. Drilling waste mainly consist if spent drilling fluids and drilling cuttings [11]. Different addittives and pollutants make the spent drilling muds hazardous and dangerous wastes which cannot be discharged anywhere without treatment. Harmfulness of drilling fluids was considered and investigated since long time [3, 4, 6, 7, 13–15]. This knowledge is still limited. Most of investigations concerns marine ecosystems or marine species whereas drilling fluids generate problems in terrestrial ecosystems too. This problems concerns liquid brine phase, mineral suspended particulate phase (SPP), solid phase (SP) and all additions improving feature of drilling fluids. Both liquid and solid phase return to environment.

For many years, a method of reducing the dangers of drilling mud has been sought. Practical experience reveals that the most common solution lies in leaving out drilling mud in the area of waste-lands or disposing of dry, dehydrated mass in a utilization place as arranged for with an investor or the respective organ of local administration [12].

Both drilling mud and drained effluent have harmful environmental impacts on areas surrounding the storage/disposal sites, to a greater or lesser extent. Therefore, our aim in this study is to qualify the toxicity of seven kinds of drilling fluids to target plant, animal and soil types, both before and after treatment.

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2. MATERIALS AND METHODS

In our experiments, we used different types of spent drilling fluids and drilling waste from 12 wells. Spent drilling fluids and drilling waste were received from companies engaged in well drilling in Poland. These samples were from different depths, from 30 to 4,040 m deep. Before the experiments were performed, basic physical properties were determined. Each fluid was also analysed ex post for features important from a biological point of view. All bioassay results relate to drilling fluids, dry mass, excluding the liquid phase.

Water content of sample: determined in accordance with ASTM D2216 – 98 [1].

Density of the solid phase of soil: determined using pycnometry procedure ASTM D854 – 10 [2].

Bioassay testing: carried out on the spent drilling fluids, brine after filtration and solid phase (SP) after filtration on the press. Filtrate without spent drilling mud was papered on Baroid standard API low-pressure $\Delta P = 0.7$ MPa for 30 minutes. The general scheme of this procedure is shown in Figure 1.

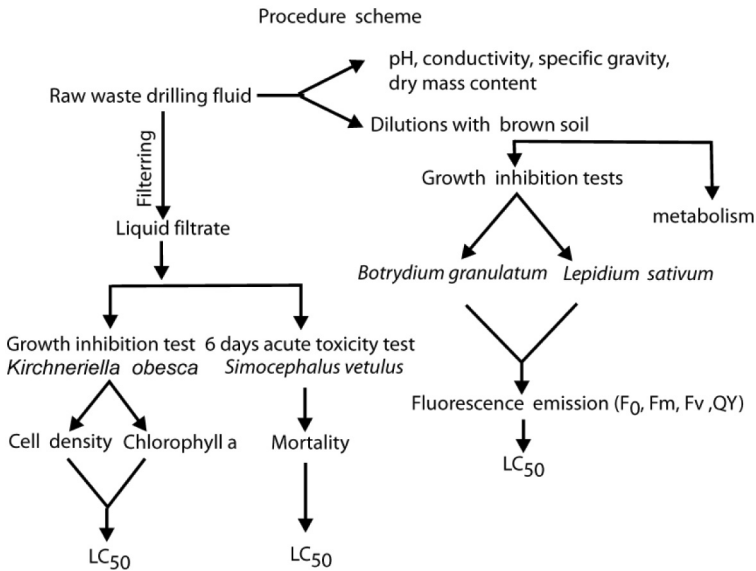


Fig. 1. Scheme of main procedures for testing harmfulness of drilling fluids

Three kinds of growth inhibition tests were carried out:

1. with *Kirchneriella obesa*,
2. with *Botrydium granulatum*,
3. with *Lepidium sativum*.

Additionally, one toxicity bioassay test was carried out:

4. Toxicity bioassay with *Simocephalus vetulus*.

Origins of strains

Kirchneriella obesa were isolated in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk.

The *Botrydium granulatum* strain, 805/3B, was isolated at the Scottish Marine Institute, Scotland. For cultivation of greens (*Kirchneriella* and *Botrydium granulatum*) Jaworski's medium (JM) was used.

Simocephalus vetulus was isolated from an oxbow near Kraków.

Lepidium sativum seeds were purchased from a garden centre.

In the liquid phase tests, counting of algae was carried out electronically using a Coulter counter. One millilitre of the sample was diluted in 50 ml of physiological saline and counted. The background count of physiological saline was then subtracted from the sample. In small concentrations, negative values were sometimes obtained and assumed as zero during calculations. Algal tests of the liquid base of drilling fluids were carried out in 100 ml Erlenmeyer flasks.

Filtrates of drilling fluids were diluted using distilled water in proportion $1/2^n$: 1:2, 1:4, 1:8, 1:16, ..., 1:512. At each step, the two halves of the solution were obtained. One was further diluted while the second remained for the assay. This part was enriched by 0.1 ml of each compound of concentrated Jaworski's medium and was supplemented with redistilled water to reach a total volume of 100 ml. This whole volume of 100 ml had the same concentration as the normal medium. The flasks were inoculated with the same amount of *K. obesa* cells. Every three to four days, cell concentration was counted to determine the growth curve. Some concentrations were omitted according to the conclusions of the previous series. Finally, the following dilutions were tested: 0, 1/4, 1/16, 1/64, 1/256 and 1/512, and in the second series 0, 1/8, 1/16, 1/32, 1/128 and 1/256. The inoculated flasks were exposed on shakers under photosynthetically active radiation (PAR) of $47.3 \text{ mM s}^{-1} \text{ m}^{-2}$. The day/night proportion was 14:10. The shaking rhythm was 15 minutes every hour. After the final counting of algae concentration, the test flask contents were filtered through a 0.45 μm Sartorius membrane filter and the contents of chlorophyll *a* were determined with 90% acetone extract at 665, 645 and 630 nm, in accordance with UNESCO, 1966. The toxicity of the filtrate and drilling mud was calculated for both the final density of the culture, expressed in cells per millilitre, and the final concentration of chlorophyll *a* after the tests.

Each tested drilling fluid was diluted in the same proportion as in the *K. obesa* test, i.e., in the sequence $1/2^n$: 1:2, 1:4, 1:8, 1:16, ..., 1:512. This proportion concerns the dry mass of brown soil used in both dilution and the drilling fluids. Dilutions were prepared using a fluid drilling mud with a known content of dry mass. Sixty grams of prepared mixtures were poured onto Petri dishes and divided into two parts by rigid plastic foil. One half was sown with 0.68 g of *L. sativum* seeds, equivalent to about 600 seeds. The second half was sprayed with *Botrydium granulatum* culture. The basic density of this culture was

estimated by counting the cells/colonies in the membrane filter after filtering two millilitres of the basic culture. The dishes were exposed on a shelf under PAR $63.5 \text{ mM s}^{-1}\text{m}^{-2}$. The day/night proportion was 14:10.

After eight days, the mud surface with growing *L. sativum* and *B. granulatum* was photographed, as was the luminescence of chlorophyll *a*. The test with *L. sativum* ended here while the growth test of *B. granulatum* continued. These results were compared to the growth on the brown soil control. Plants were then cut off neatly at the mud surface, weighed as wet mass and then as dry mass. The uprooting caused an unreliably high dry mass, due to soil particles sticking to the roots. Washing the roots was ineffective. Cress seed gum has high viscosity [18] and absorbs large amounts of water. After 24 hours, the initial dry mass of seeds absorbed 650% of the water into gelatinous sheaths. These sheaths stuck to the soil particles, which were later difficult to remove and rendered the plant mass measurements inaccurate. The dry mass of plants, therefore, was not a reliable unit of substrate toxicity. It appeared that the better measure of this dependence would be photosystem efficiency. We thus decided to grow two measures of *L. sativum* as mutual controls. Analysis of photosystem efficiency and scores of growth were laid out as follows:

- 0 – lack of germination,
- 1 – germination without further growth sprouting,
- 2 – growth.

After the *L. sativum* test concluded, the second halves of the Petri dishes were continued, exposed in the same light as the *Botrydium*. Growth was found to be slower. After 19 days, dishes with algae on the soil surface were photographed in FluorCam FC-800-C, Photon Systems Instruments, and chlorophyll fluorescence was analysed to assess the state of the photosystem II (PS II). The same procedure was applied to the *L. sativum* test. The following parameters were measured:

- F_0 – Minimal fluorescence (arbitrary units). Indicates the fluorescence level when all antenna pigment complexes associated with the PS II are assumed to be open (i.e., dark-adapted).
- F_m – Maximal fluorescence (arbitrary units). Indicates the fluorescence level when a high-intensity flash is applied. All antenna sites are assumed to be closed.
- F_v – Variable fluorescence, calculated as $F_v = F_m - F_0$.
- QY – Maximum quantum efficiency, calculated as $F_v/F_m = (F_m - F_0)/F_m$.

Simocephalus vetulus tests were carried out in 100 ml Erlenmeyer wide-neck flasks. Dilutions were the same as in the algal tests. Filtrate of drilling fluids was diluted with natural filtered pond water, instead of the artificial medium used in the algae tests.

Each flask was inoculated with 10 neonates. Every day, the animals were observed and they were counted every two days. To avoid the mistake of jumped animals during counting, samples were poured into a special chamber of 10×10 cm, internally divided into a matrix of 2×2 cm sub-chambers, before counting. Animals were exposed for six days.

For calculation of LC₅₀ and EC₅₀, the modified source code of the program *Spearman*, obtained courtesy of the EPA, was used. This program is an implementation of the Spearman–Karber method [8, 9]. Due to the method's requirements, mortality was calculated always in relation to the initial 10 individuals, irrespective of the increase in numbers in some concentrations. For growth inhibition tests, the ToxCalc program was used.

3. RESULTS

The specific gravity of dry drilling mud ranged between 4.2 and 1.1 g cm⁻³. The raw drilling fluids demonstrated specific gravity of between 1.10 and 2.20 g cm⁻², as shown in Table 1. The content of dry mass ranged between 37.38 and 83.23%.

Table 1

List of sample codes and key to drilling fluid types and content of solid compounds and specific gravity of dry drilling and raw drilling mud

Sample No.	Sample code	Type of drilling mud	Specific gravity of spent drilling fluids [g/cm ³]	Content of dry mass [%]
1	L- 2K	Saline barite	2.20	37.38
2	K-1/P-Cl	Chloride-polymer	1.23	69.74
3	W- 2	Polymer	1.26	68.25
4	P-2/Dow	Non-clay ultradrill	1.10	82.80
5	KRAM-1/K	Potassium	1.51	55.86
6	W-1/Cl-P	Chloride-polymer inhibited	1.28	68.87
7	P-19K	Potassium	1.20	74.21
8	P-1/K-P	Potassium polymer	1.15	79.81
9	L-1/B	Bentonite	1.17	83.23
10	K-1/B	Bentonite	1.28	74.44
11*	S-4	Potassium polymer	1.24	45.90
12*	S-5K	KCl polymer	1.26	65.60
13*	S-7	Clay less with blockers	1.25	66.10

* After press

From a biological point of view, pH and electrolytic conductivity are important parameters in the general estimation of life conditions. Solutions prepared for bioassays had similarly weak alkaline reactions of above a pH of eight in all dilutions.

Generally such a pH is easily tolerable by algae and plants. Sample No. 12 (S-5K) changes properties during dilution with distilled water and after enrichment by media compounds.

The filtrate of each drilling mud was highly saline, with an electrolytic conductivity usually greater than 100 mS cm^{-1} . This creates very high osmotic pressure, which kills freshwater organisms. The conductivities of natural and moderately polluted inland waters are in the range $0.1\text{--}0.7 \text{ mS cm}^{-1}$, whereas those of oceanic waters are in the range $64\text{--}55 \text{ mS cm}^{-1}$.

For *Simocephalus vetulus*, a harmless concentration of the liquid phase requires dilution of more than the LC_{50} value, usually more than 1:128 (0.0078).

Dilutions up to 1:8 (0,125) kill *S. vetulus* within between 30 seconds and one hour. Greater dilutions are less toxic and tolerable despite relatively high concentration of salt. The three tested drilling fluids – sample No. 6 (W-1/CI-P), 9 (L-1/B) and 12 (S-5K) have relatively low toxicity for *S. vetulus*. All remaining samples demonstrate LC_{50} of between 0.0036 and 0.0078.

Growth of *Kirchneriella obesa* in the liquid phase following filtration of the drilling mud was tested in dilutions according to the scheme described in the Figure 1. Growth curves demonstrate typical sigmoid shapes according to cell numbers, as seen in Figure 2. The content of chlorophyll *a* varies from 0 to approximately $10 \mu\text{g/ml}$ of extract, as shown in Figure 3.

Kirchneriella seems to be tolerant in respect to both pH and salinity. This species is capable of slowly growing on salty samples, such as that in the filtrate of sample number three (W-2) in dilution 1:16, where electrolytic conductivity was $7250 \mu\text{S cm}^{-1}$. This value, transformed by the coefficient 0.56, gives us a total dissolved solids (TDS) value of 4640 mg dm^{-3} . This falls under the category of brackish water, which covers TDS values of between 1000 and $10.000 \text{ mg dm}^{-3}$.

The calculated values of LC_{50} demonstrate LC_{50} dilutions between 0.6 and 0.019. The liquid phase of the 11 (S-4) sample was 31.6 times less toxic than the 9 (L-1/B) sample, as shown in Table 2.

Samples with algae (*Botrydium granulatum*) growing on waste drilling fluid/soil mixtures were analysed only for PS II efficiency. Efficiency values of algal PS II were relatively high at all dilutions (F_v/F_m approximately 0.5) and similar for all tested drilling mud. The most efficient was grown on brown soil 0.754. This quantum efficiency (QY) parameter was independent of the concentration of the drilling fluid in the soil, shown in Table 3. This means that, if the growth is possible, the PS II is efficient. Injury to life processes does not affect PS II.

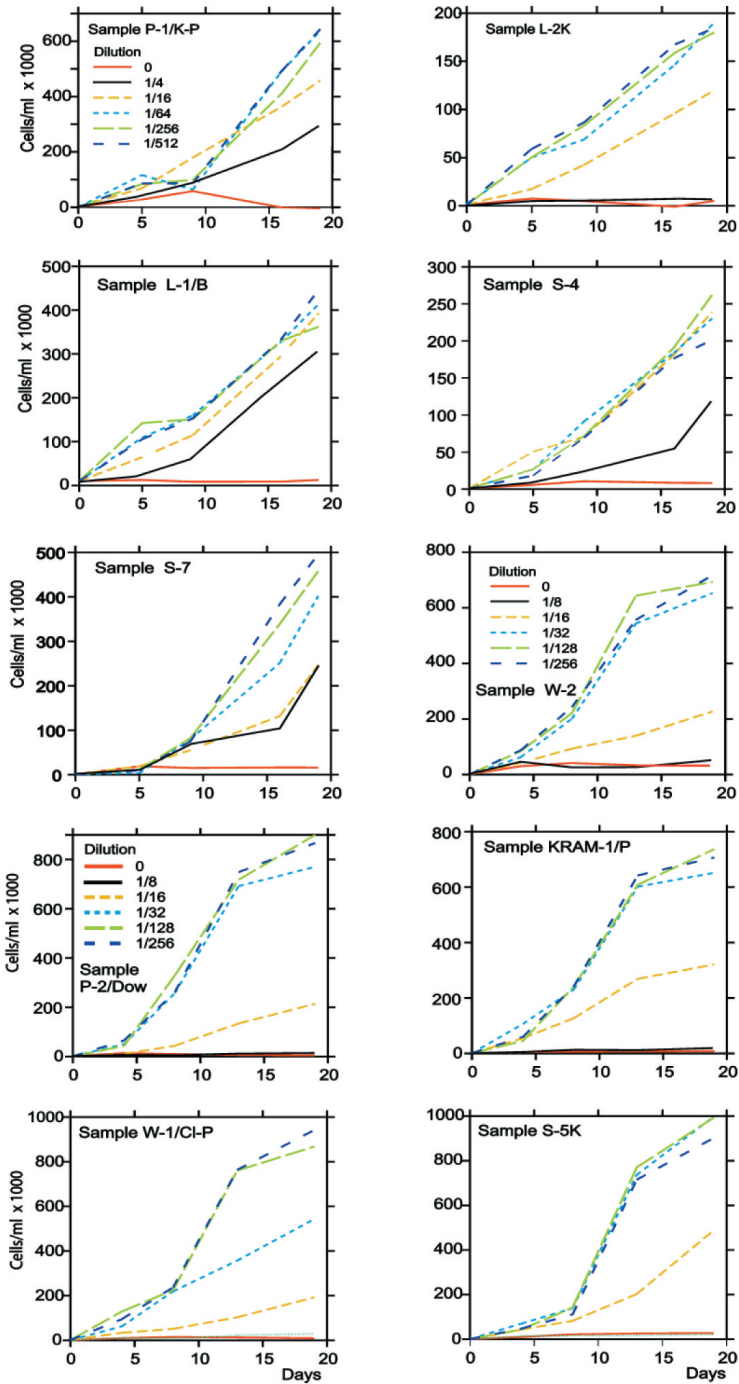


Fig. 2. Growth of *K. obesa* in the liquid phase

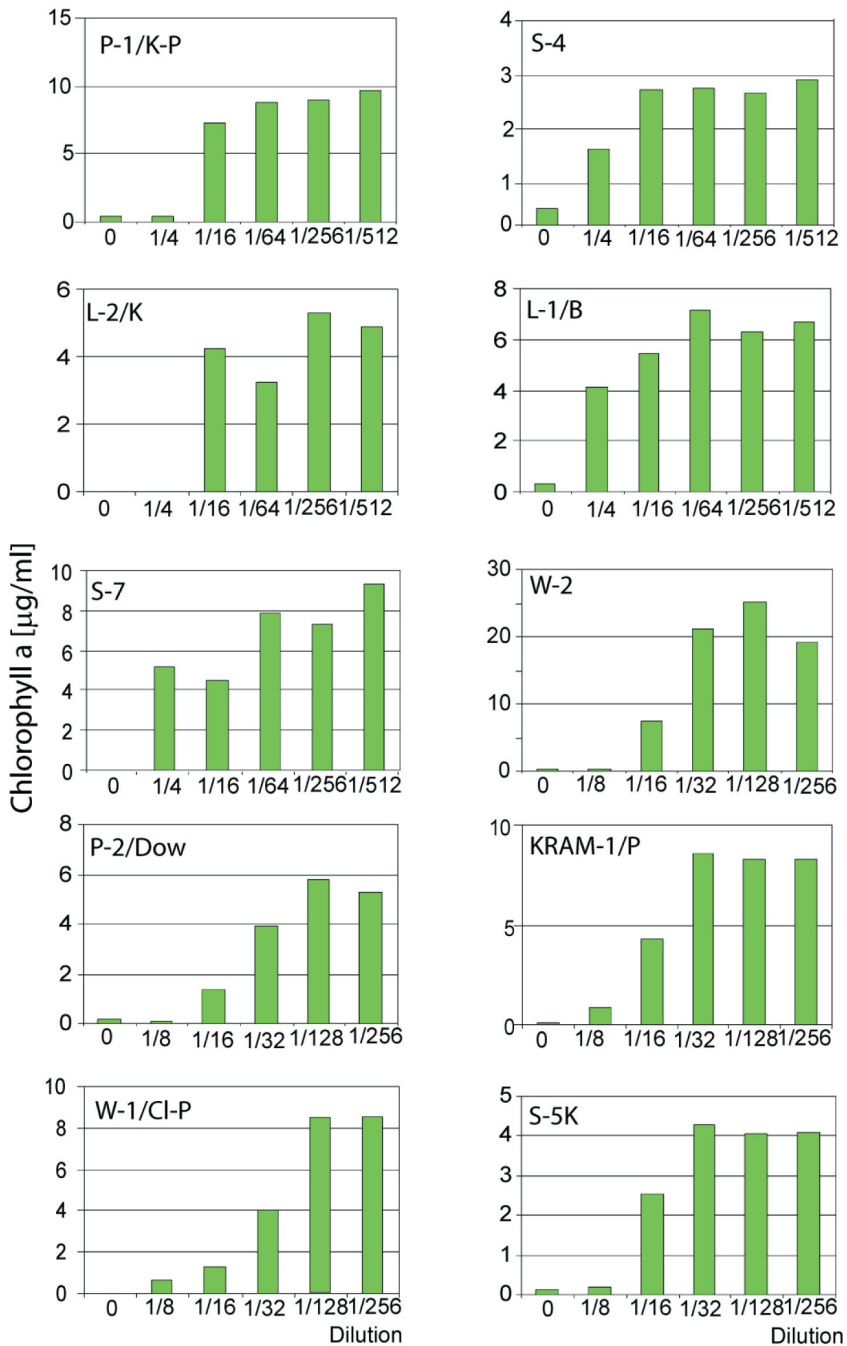


Fig. 3. Final concentrations [$\mu\text{g/ml}$] of chlorophyll *a* in the acetone extract. Volume of extract = 10 ml

Table 2

LC₅₀ estimation for drilling mud toxicity of *Simocephalus vetulus* and *Kirchneriella obesa* after six days

Sample No.	LC50 <i>S. vetulus</i>	LC50 <i>K. obesa</i>
1	0.0078	0.11
3	0.0073	0.053
4	0.0078	0.047
5	0.0036	0.061
6	0.0118	0.029
8	0.0078	0.094
9	0.125	0.019
11	0.0078	0.6
12	0.0313	0.073
13	0.0042	0.21

Table 3

Quantum efficiency (QY) of *Botrydium granulatum* photosynthetic system and values of minimal fluorescence (F_0), for *Botrydium granulatum* (B) and *L. sativum* (L) growing on diluted drilling mud/brown soil mixtures

Dilution	Parameter	Drilling fluid number								
		1	2	3	4	5	6	11	12	13
1:0	QY	0	0	0	0	0	0	0	0	0
	F_0 (B)	0	0	0	0	0	0	0	0	0
	F_0 (L)	0	0	0	0	0	0	0	0	0
1:4	QY	0	0	0	NA	NA	0	NA	NA	NA
	F_0 (B)	0	0	0	NA	NA	0	NA	NA	NA
	F_0 (L)	0	0	0	NA	NA	0	NA	NA	NA
1:8	QY	NA	NA	NA	0	0	NA	0.66	0.46	0.51
	F_0 (B)	NA	NA	NA	0	0	NA	420.5	314.3	630.5
	F_0 (L)	NA	NA	NA	0	0	NA	1154	765.5	400
1:16	QY	0.70	0.71	0.7	0.57	0.67	0.67	0.54	0.65	0.66
	F_0 (B)	57.6	182.8	367.4	799.7	640.4	468.8	304.9	539.4	421
	F_0 (L)	0	0	0	561.2	861.8	12.25	497.6	368.77	1 354

Table 3 cont.

Dilution	Parameter	Drilling fluid number								
		1	2	3	4	5	6	11	12	13
1:32	QY	NA	NA	NA	0.62	0.57	NA	0.64	0.65	0.53
	F_0 (B)	NA	NA	NA	558.5	460.8	NA	331.4	235.7	290.7
	F_0 (L)	NA	NA	NA	655.8	527.5	NA	1163	809.19	914.1
1:64	QY	0.7	0.7	0.70	NA	NA	0.63	NA	NA	NA
	F_0 (B)	199.4	172.5	176.5	NA	NA	262.4	NA	NA	NA
	F_0 (L)	9.29	8.395	9.64	NA	NA	9.11	NA	NA	NA
1:128	QY	NA	NA	NA	0.61	0.60	NA	0.65	0.67	0.65
	F_0 (B)	NA	NA	NA	558.5	460.8	NA	331.4	235.7	290.7
	F_0 (L)	NA	NA	NA	746.4	487.4	NA	1101	1593.4	1417
1:25	QY	0.7	0.69	0.69	0.59	0.67	0.6	0.65	0.60	0.47
	F_0 (B)	181.6	626.8	206.8	670.8	336.1	474	426.8	440	594.9
	F_0 (L)	9	8.6	9.89	1144	1181	9.11	767	1181.2	1006
1:51	QY	0.65	0.66	0.7	NA	NA	0.4	NA	NA	NA
	F_0 (B)	88.8	286.9	189.6	NA	NA	480.5	NA	NA	NA
	F_0 (L)	9.34	8.435	8.37	NA	NA	8.59	NA	NA	NA

NA – not available (skipped concentration). 0 – lack of growth

Somewhat different were minimal fluorescences (F_0). Table 3 shows that samples one, two, three and six (L-2K, K-1/P-Cl, W-2, W-1/Cl-P) had lower F_0 than the remaining samples, though the difference between these two sample groups was not large. For *B. granulatum*, F_0 varies in the range between 200 and 500, whereas for *L. sativum* the range is between, approximately, 10 and 1000. With regard to LC_{50} , *B. granulatum* varies between 0.094 and 0.56, as shown in Table 4, while *L. sativum* returns lower values, in the range 0.039 to 0.29. Algae were able to grow on higher concentrations of waste drilling fluids in the case of *B. granulatum* as compared to *L. sativum*, as we can see from Figure 4.

After eight days of growth, the plantation of *Lepidium sativum*, was analysed for PS II efficiency. F_0 was taken to be when all antenna pigment complexes associated with the PS II were dark-adapted. This variable seemed to depend on the kind of drilling fluids used. Even small additions to the soil caused lowering values of F_0 . This applied to samples one, two, three and six (mud L-2K, K-1/P-Cl, W-2, W-1/Cl-P). Samples 4, 5, 11, 12 and 13 (P-2/Dow, KRAM-1/K, S-4, S-5K, S-7) had relatively high values of F_0 , as shown in Table 3.

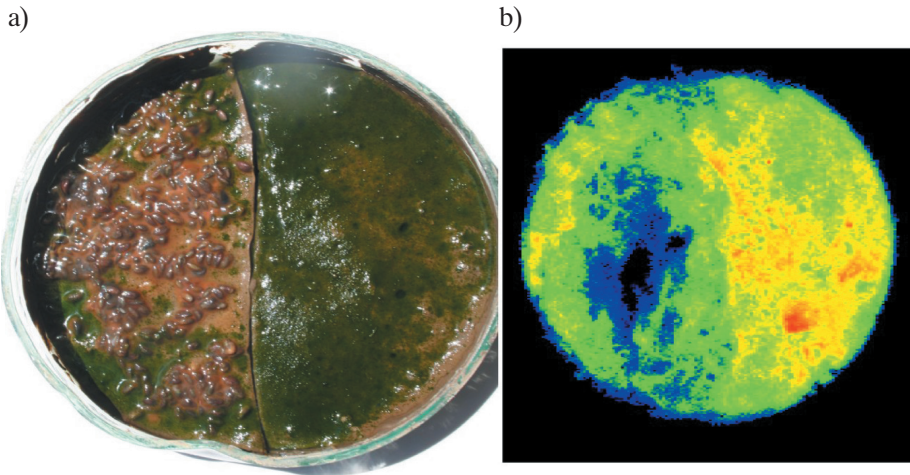


Fig. 4. Growth of *Botrydium granulatum* on brown soil mixed with sample 11 at the dilution 1:8. Species on the right side of the Petri dishes (a) are able to grow whereas *L. sativum* is unable to germinate. *B. granulatum* is more tolerable than seeds of *L. sativum*. On the right (b) we see the F_0 view of the same sample

These values of the efficiency coefficient F_0 were used in the calculation of LC_{50} . The LC_{50} of the analysed samples varied between approximately 0.04 and 0.25 in the category of sample dilution, as seen in Table 4.

Table 4

LC_{50} values for *L. sativum* and *B. granulatum* according to the QY parameter.
Growth on solid substrate of drilling fluids/brown soil mixtures.
Unit of toxicity – dilution of drilling fluid

Sample No.	<i>L. sativum</i>	<i>Botrydium granulatum</i>
1	0.039	0.156
3	0.093	>1/16
5	0.094	0.094
6	0.039	0.156
8	0.094	0.094
9	0.093	>1/16
10	0.039	0.156
11	0.25	0.56
12	0.25	0.56
13	0.25	0.56



All partial measurement of PS II necessary for QY calculation were taken from image analysis similarly to the *B. granulatum* test. Usually, the dilution 1:64 was sufficient for plant growth.

Due to the excessively high toxicity obtained in preliminary bioassay tests, drilling muds were modified in the next series of experiments using procedure rinsing salt out in a proportion of 1:1. These procedures, in theory, should lower the concentration of harmful compounds. Five samples were tested with *L. sativum*, after washing out part of the soluble compound. This treatment was effective only for bentonite drilling fluid from the L-1/B borehole. Remaining samples were still toxic despite the dilution, as shown in Table 5.

These same samples were additionally tested with *B. granulatum* and provided the same results. W-2, L-2K, KRAM-1K and W-1/CI-P muds were still toxic, while L-1/B permitted plant growth. Analysis of PS II showed only vestigial activity, as outlined in Table 5.

Table 5

Growth of *L. sativum* and of *B. granulatum* on washed 1:1 drilling fluids
0 – lack of growth, 1 – germination, 2 – moderate growth, 3 – very good growth

Species		Sample No.					
		Control Soil	9	3	1	5	6
<i>L. sativum</i>	Score of growth	3	3	0	0	0	0
	View of the test						
<i>B. granulatum</i>	Score of growth	3	3	0	0	0	0
	PS II activity	$F_v/F_m = 0.754$	$F_v/F_m = 0.507$	Vestigial activity of PS II	Vestigial activity of PS II	Vestigial activity of PS II	Vestigial activity of PS II Contaminated by <i>Penicillium sp</i>
	View of the test						

4. DISCUSSION

Drilling fluids are complex chemical products containing many potentially harmful constituents. As a result, application of drilling fluids, and thereby exposure to certain components of those drilling fluids, is considered a health risk for personnel [10] and the environment.

In the environment, even natural and chemically-neutral suspensions arising from soil erosion during high flows is capable of exterminating populations of filtrators like water fleas. This mineral zero, calorically worthless food exterminated cladocerans within one week [16, 17]. This same mechanism may concern bentonite suspensions in specific environments. For example, mineral suspension in rivers is, for this reason, limited to 25 mg dm^{-3} by the European Union [5].

Conductivity converted to TDS indicates that is in the range 154,000 to 70,500 mg dm^{-3} , depending on the type of drilling fluid. This is a very high mineralization. Mainly, these compounds are NaCl or KCl. To some degree, conductivity is an indicator of the amount of NaCl and KCl added to some drilling fluids. This study indicates that raw salty drilling mud will be more harmful, due to salinity, in land and freshwater ecosystems than in marine ecosystems. The simplest way to decrease this harmfulness is to dilute the drilling fluid and remove the salty water. Therefore, we tested only 1:1 dilutions of some drilling muds. This dilution was sufficient only for bentonite mud (L-1/B, as outlined in Tab. 5). For the remaining samples, washing the salt out of the mud in a proportion 1:1 was insufficient. This is easily explained by reference to the fact that salt concentration decreased to between 77,000 and 35,250 mg dm^{-3} after washing, while our tests show that *L. sativum* is capable of growing (as LC50) in soil-fluid mixtures containing only as much as 31,000 mg dm^{-3} of salt, for example in clayless with blockers fluid (sample 13 – S-7) and in lower concentrations of 12,314 to 6396 mg dm^{-3} (such as ultradrill 4 – P-2/Dow and saline-barytic sample one, L-2K). More tolerant of salinity is the alga *Botrydium granulatum*, which can grow on soil containing 69,440 mg dm^{-3} of TDS, for example in clayless with blockers fluid (sample 13 – S-7) and in 24,024 mg dm^{-3} concentrations (such as saline-barytic sample one, L-2K). More sensitive to salinity is the alga *Kirchneriella obesa*, which is capable of growing on concentrations of 16,940 mg dm^{-3} (such as saline-barytic sample one, L-2K). Salinity cannot explain the greater toxicity of drilling fluids like ultradrill – P-2/Dow (which displays a TDS as salinity equivalent of 12,314 mg dm^{-3} in sample five, KRAM-1/K) or chloride-polymer inhibited fluids (having LC50 at concentrations of 2071 mg dm^{-3} of TDS in sample six, W-1/Cl-P). This greater toxicity must be assigned to the other components. However salinity remains an important contributor to toxicity.

The harmfulness of drilling fluids can result from low or high pH. Tested samples had generally weakly alkaline pH, except for sample three (W-2), which had pH near 10. Octuple dilution diminished this pH to 7.6, which is environmentally acceptable.

The technology of image analysis used in plant physiology to analyse the quality of PS II is very useful for analysis of untypical experimental sets, like soil algae, when a binary response is satisfactory. This technology allows for the ongoing observation of progress of the experiment, whereas other techniques like chlorophyll extraction are destructive and definitively end the experiment. This technique allows us to estimate the efficiency (QY) of the photosynthetic system of growing algae or plants, but does not allow for the estimation of whether this growth is relatively fast or slow. If growth is possible it means that PS II is efficient and any damage on the biochemical level is to be found elsewhere. When growth of *B. granulatum* was possible in the soil mixture, the quantum efficiency was found to be quite good – usually 0.6 to 0.7.

A somewhat different situation was found in the test with *L. sativum*. Here the parameter F_0 seemed to depend on the kind of drilling fluids used. It is probable that this difference is due to the specific features in the biology of this species. Alga has short rhizoids and this rhizoidal 0.5 mm layer can be cleaned of harmful compounds by spraying water. *L. sativum* normally has a deep root system and spraying the culture with water does not remove harmful substances from the soil. The plant therefore remains under the influence of these substances. This may explain the difference in the validity of the F_0 and QY parameters for rooted plants and algae. Regardless of the reason why *Botrydium* tolerates higher concentrations of drilling fluids, it can be regarded as a pioneering species.

The liquid phase was sparsely tested with algae. Usually these growth inhibition tests are carried out with a marine diatom *Skeletonema costatum*, recommended by OSPAR. However, a culture of unicellular algae in the liquid phase of the drilling fluid posed technical problems. Due to precipitation of some compounds in some filtrates, the cells counted by the particle counter may have been unreliable as the electronic counter is unable to distinguish algal cells from the precipitated particles. Use of the chlorophyll concentration, instead, was determined to be the best solution to this problem.

Some liquid phases had a very narrow range of concentration (between the concentration that would block growth and that that would enable it) while others displayed a broad range of such concentrations.

K. obesa had, in some types of drilling fluids, a similar sensitivity to that of *B. granulatum*, such as in saline–barytic, whereas KCl-polymer filtrate was three times more toxic to it. Furthermore, the toxic effect was eight times greater in the same type of drilling fluids taken from different wellbores, like samples L-2K and S-5K.

The results of experiments on the toxicity of drilling fluids are sometimes difficult to compare. Differences between studies have primarily resulted from the variety of recipes of drilling fluid components used by different investigators and inconsistent units like dry mass, wet mass, dose per kg, dose per ha, etc.

5. CONCLUSIONS

This investigation shows that:

1. Both for terrestrial and water ecosystems, the first order problem in toxicity mitigation is very high salt concentration.
2. For mitigation of the salinity problem, dilution of drilling fluids with natural soil should be recommended. In moderate climates, excess salinity will be slowly leached by rains.
3. All of the tested drilling fluids damaged the PS II of the test plants.
4. The more sensitive organisms were cladocerans (here *S. vetulus*), freshwater planktonic alga *K. obesa* and *B. granulatum*, which can be considered as a pioneer alga and comparable in sensitivity to *L. sativum*.

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