8 Remediation

8.1 Abstract

Five different soil materials were tested with the collembola- and the enchytraeid-biotest before and after the remediation. Overall, the remediation improved the habitat function of the soil materials in comparison to the soil materials before the remediation process, although the result was not always satisfying and further treatment is required.

After the remediation with the white rot fungus by "Wistrans" the soil material CTNT2aVA was still very toxic for the collembola and toxic for the enchytraeid. This is thought to be the effect of the high concentrations of the heavy metals Zn and Pb, but also of TNT-metabolites produced during the degradation process. They are usually further transformed, which might not have happened in this soil material as it was tested before a resting period. The soil material CTNT3b was neither toxic for the collembola nor for enchytraeid after the remediation with the anaerobic-aerobic windrow process of "Plambeck ContraCon" by indigenous microorganisms. The soil material CTNT4b remediated with indigenous microorganisms in a dynamic anaerobic-aerobic treatment by "U-Nord" was only classed as ecotoxicologically critical for the collembola and non-toxic for the enchytraeid. A negative effect of the remediation process itself was considered as likely. Hence, the process was altered yielding to the remediated soil material CTNT7b, which was non-toxic for either species.

After the remediation in a bioreactor with indigenous microorganisms and the amendment of 1% or 2% of molasses ENTb1% and ETNTb2% were still very toxic for both species. However, the habitat function of ETNTb2% had improved, as adults of both species survived the duration of the reproduction test and *E. crypticus* even showed a very low reproduction. The enchytraeid was also able to survive in the reproduction test with ETNTb1%.

A soil washing procedure resulted in a low contaminated sand fraction LTNTb and a highly contaminated fine grain fraction LTNTc. The latter, however, was not toxic for the enchytraeid and for the collembola, since the TNT-content had significantly decreased during the resting period. The sand fraction on the other hand proved to be very toxic for both species.

8.2 Theoretical background

Generally, three different processes are distinguished: in-site (in-situ) or ex-situ, which can be further separated in on-site and off-site. For in-site processes the earth remains on the side and is not moved, whereas it is excavated for all ex-situ processes and either treated on the site (on-site) or elsewhere (off-site). Obviously, the ex-situ processes are much more expensive due to

the movement of the earth, but they offer a better control of the remediation success and protection for the groundwater (BANK, 1994: 741-743).

Munitions contaminated soil materials have generally been remediated by incineration and soil washing, but biological methods are more and more favoured (DRZYZGA et al, 1999). All processes have to achieve the index value of 1 mg/kg soil (dw) for a sum of 11 nitroraromates usually found on TNT-contaminated sites. This index value is so low as to be reasonably acceptable and represents the detection limit for the single substances (SCHÄFER, 1992). However, if the site should be used as a residential area the recommended soil value for TNT is 0.13 mg TNT/kg soil (dw) due to its cancerogenity (SCHNEIDER et al, 1994 a,b).

8.2.1 Physico-chemical remediation

Incineration and soil washing are typical physico-chemical soil remediation processes applied for munitions contaminated soils. By the incineration treatment the contaminated soil material is burned at 800-1000°C (BANK, 1994: 758), but it should take place in special plants, which ensure a detoxification of the exhaust fumes (MARTINETZ, 1994: 238). Hence, the process itself is very expensive, not even considering the additional costs for excavation and transportation of the soil material, as incineration is a mere off-site process. The decontamination, however, is very successful, although the soil material is biologically dead and the texture is destroyed (BANK, 1994: 757-758).

Soil washing, too, is mostly applied off-site. The contaminated soil material is usually crushed and then washed. To ensure the passing from the soil material to the washing solutions either acids, bases, tensides as well as organic solvents or physical methods like kinetic energy are used. A flow chart of a soil washing process, which is used for munitions contaminated soil materials, is represented in Fig. 8.2-1.

The contaminants remain in the fine grain fraction, which has to be separated, dried and disposed. Although, a disposable fraction remains, this is only a percentage of the original contaminated soil material. Advantages are the relatively low costs compared to incineration, the rapidity of the process and the preservation of the soil texture (BANK, 1994: 751-756).

The applicability of the process depends on the characteristics of the toxicant as well as on the ones of the soil material. Good conditions offer soluble substances and sandy and gravely soil materials (MARTINETZ, 1994: 237-238).

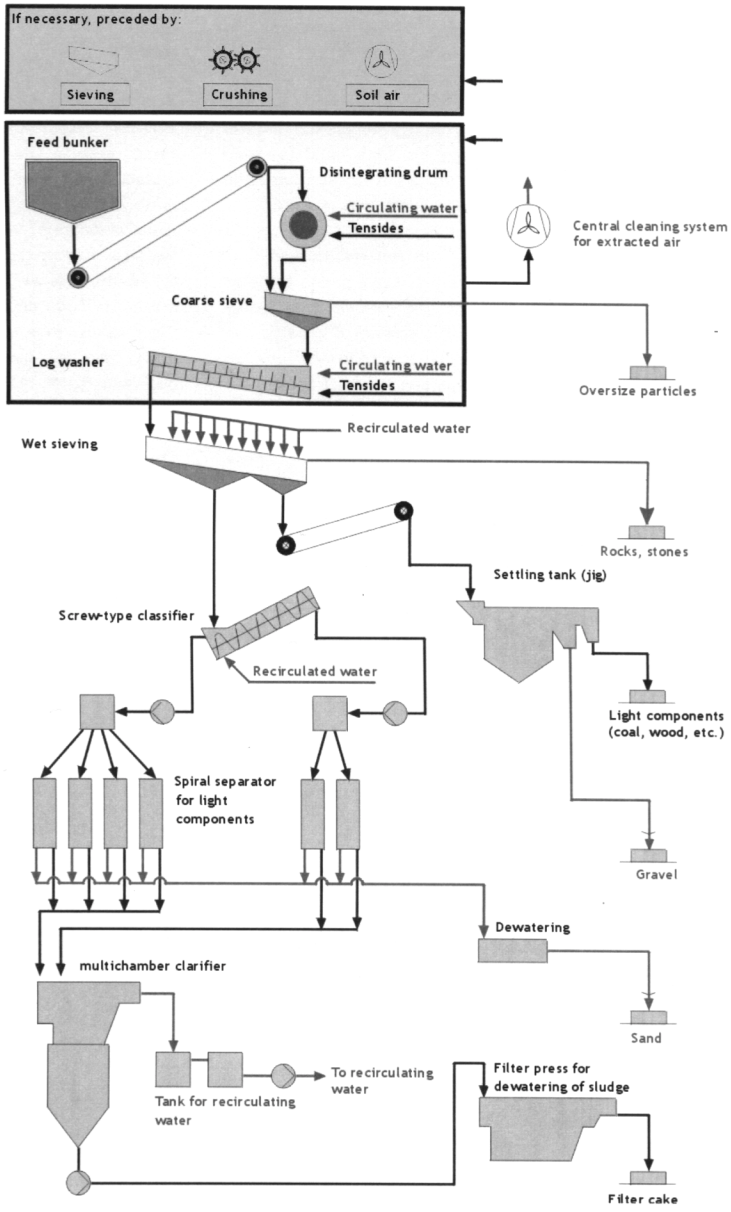

Fig. 8.2-1: Flow chart of a soil washing process by the AB Umwelttechnik GmbH (ABU, 2001).

8.2.2 Biological methods

Since physico-chemical methods, especially the incineration, are very expensive, scientific research has increasingly focused on biological methods, although they are so far not accepted by the authorities (BRUNS-NAGEL et al, 2000). Under investigation are the degradation by bacteria, fungi or by plants. These methods can be carried out either as in-situ or as ex-situ processes (RITTER & SCARBOROUGH, 1995), such as land farming, composting, bioreactors, soil-slurries or windrows.

The aim of any biological treatment is the complete degradation of an organic chemical to stable inorganic forms of carbon, hydrogen, nitrogen, phosphorus and others. This mineralisation

process entails several successive biological transformations (SCHWARZENBACH et al, 1993: 485). However, mineralisation is not always possible for all xenobiotics. On the one hand the microorganisms might not be capable of complete transformation. On the other hand dead-end products might be formed, which can interact with the soil matrix. However, if this binding is irreversible it renders the toxicant immobile and might thus offer an alternative to mineralisation (HAWARI et al, 2000).

8.2.2.1 Degradation by bacteria

Many bacteria of various families are able to transform TNT, however a lot of them stop their activity above 20-50 mg TNT/L. Only some are active at the saturation concentration of 130 mg TNT/L (MARTINETZ & RIPPEN, 1996: 764). The rate of degradation can be enhanced either by improving the conditions for the microorganisms already present on the site (microbial ecology approach) or by augmenting the number of microorganisms (the microbiological approach). For that specialised microorganisms can be introduced or cultured site-specific strains are added (RITTER & SCARBOROUGH, 1995). The latter is mostly preferred, as the microorganisms are already adapted to the site specific conditions (BANK, 1994: 750).

As already mentioned (see p. 72, chapter 6.2.2) the mayor pathway for TNT-degradation is the reduction of the nitro groups, since TNT is very resistant to electrophilic attack at the aromatic ring. The reduction of the nitro groups proceeds via the nitroso- (NO-) and hydroxylamino-derivates (OH-) as intermediates (PREUß & RIEGER, 1995: 72). As a consequence, TNT is converted in a stepwise process via the aminodinitrotoluenes (ADNT) and diaminonitrotoluenes (DANT) to triaminotoluene (TAT). Only the last step, the formation of TAT, is strictly anaerobic and the rate-limiting-step of the whole process (PREUß & RIEGER, 1995: 76).

So far TAT has to be considered as the endproduct, since no further degradation of TAT is known in soil. Thus, no mineralisation is achieved, only transformation to TAT. This substance, however, is irreversibly bound to the soil matrix, either to the clay minerals or to humic substances (DAUN et al, 1998). These bindings range from ionic interactions of the TAT monomer to very complex subsequent oxygen-dependent reactions and the formation of polymers (RIEGER & KNACKMUSS, 1995: 13). The irreversible binding of reduced TNT-metabolites to humic substances has also been demonstrated to take place in the biological treatment of TNT-contaminated soils (ACHTNICH et al, 1999; BRUNS-NAGEL et al, 2000; DAUN et al, 1998; DRZYZGA, et al, 1999; CATON et al, 1994; LENKE et al, 1998 and PENNINGTON et al, 1995). A study with radioactively labelled TAT proved that only 2% of the radioactivity was extractable after an anaerobic-aerobic degradation, whereas 98% was bound to the soil material of which 85% was bound to the humin fraction, 8% to humic acids and 7% to fulvic acids (ACHTNICH et al, 1999). Hence, the binding of TNT-derivates to humic substances, the

humification, is considered to be a satisfying end-point of remediation (BRUNS-NAGEL et al, 2000).

Since TNT is not used as a nutrition, this pathway functions only cometabolic with an additional carbon- and nitrogen-source (MARTINETZ & RIPPEN, 1996: 767-769). An overview of the pathway with the structural formula of the intermediates is given in Fig. 8.2-2.

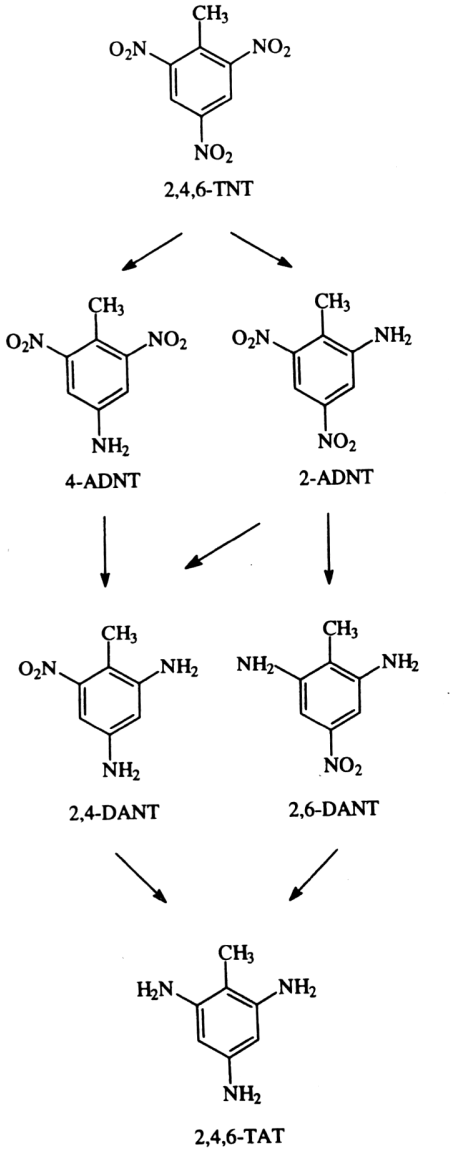

Fig. 8.2-2: Microbial reduction of TNT (MARTINETZ & RIPPEN, 1996: 768).

Under aerobic conditions many different intermediates and condensation products like azoxy compounds can be formed (see p. 73, Fig. 6.2-1), some of them even more toxic than their precursors (RIEGER & KNACKMUß, 1995: 8-9). The azoxy compounds are also formed during the anaerobic degradation, but can then be further reduced to TAT (RIEGER & KNACKMUSS, 1995).

Another possible transformation by *Pseudomonas putida* under aerobic conditions starts with a nucleophilic addition of a hydrid ion to TNT to form a hydrid-Meisenheimer-complex (VORBECK et al, 1994). This is followed by successive reductive splitting of the nitro groups into nitrite

groups and the formation of toluene, which can be completely degraded by other bacteria. A cloned *Pseudomonas* strain, in which the toluene transforming gene was introduced, was able to mineralise 50% of the provided TNT (RAMOS et al, 1995).

This process is very slow and competes with the reduction of the nitro groups. Thus, the latter is favoured as the faster pathway (MARTINETZ & RIPPEN, 1996: 776). By the addition of amino compounds, however, the formation of the hydrid-Meisenheimer-complex can be enhanced and thus it might become possible to bind TNT and initiate its further remediation yielding to a complete mineralisation of TNT (FANT et al, 2001).

Other transformation pathways of TNT are known, but so far they have no significance for the remediation. Hence, they are not discussed.

8.2.2.2 Degradation by fungi

Wood- and litter-decaying fungi were found capable of mineralising up to 42% of the originally present TNT (SCHEIBNER et al, 1997). Although, many are inhibited by high TNT-concentrations in solution, they can tolerate and mineralise much higher concentrations in soil. Thus, the white-rot fungus *Phanerochaete chrysosporium* was able to reduce an initial TNT-concentration from more than 10 000 to 3500 mg TNT/kg soil (dw) (STAHL & AUST, 1995), whereas it had stopped mineralisation at concentrations of 15 mg TNT/L in solutions (SPIKER et al, 1992).

The mineralisation becomes possible by an oxidative splitting of the aromatic ring due to the extra cellular enzymes oft the ligninolytic system (FRITSCHE et al, 1998). The complete pathway is so far not known (MICHELS & GOTTSCHALK, 1995), but involves an initial reduction of TNT to ADNT via hydroxlaminodinitrotoluene (OH-ADNT).

8.2.2.3 Degradation by plants

Plants are capable of directly or indirectly adsorbing, accumulating or metabolising various organic substances and have thus the potential to be used in the remediation of contaminated environments (BEST et al, 1999) – a process called phytoremediation. So far it has been proved that TNT can be taken up by plants and also be transported over short distances (Palazzo & Leggett, 1986, Folsom et al, 1988, Cataldo et al, 1989 all in GÖRGE et al, 1995). It accumulates particularly in the roots of plants and very few amounts can be traced in the leaves or the fruits (WERNER et al, 1998). In addition, the uptake is species specific and depends on the soil concentration (GÖRGE et al, 1995). However, very little of the initial TNT can be accounted for, as it might remains in the plants in an unextractable and/or in an undetectable form (PAVLOSTHATIS et al, 1998).

Phytoremediation offers the advantage of an in-situ process and it restricts soil movement, which might otherwise enhance the threat for the environment (PETERSON et al, 1996).

The innate biodegradative ability of plants is much lower than the one of bacteria. Thus, it was suggested to use transgenic plants expressing bacterial enzymes, which are known to degrade TNT. Hence, tobacco plants (*Nicotiana tabacum*) with a pentaerythritol tetranitrate (PETN)-reductase were able to survive higher TNT-concentrations than the wild-type plants (FRENCH et al, 1999). If the enzyme nitroreductase was transferred into tobacco plants, the transgenic plants were able to tolerate TNT-concentrations up to the aqueous solubility of TNT (HANNINK, et al, 2001).

8.3 Materials and methods

Some of the munitions contaminated soil materials prescribed in the previous chapter were part of a remediation project. They were tested before and after the remediation to evaluate the success of the remediation with a mortality and reproduction test with *F. candida* and *E. crypticus* (see p. 6, chapter 3.3.1.1). If the toxicity was very high the LC50- and EC50-values were determined in a dilution with Lufa 2.2. In general, Lufa 2.2 was used as a control soil material as the provided reference soil materials were not suitable for at least one of the test organisms.

The soil materials were not only contaminated with TNT, but also with heavy metals or PAK. These additional contaminations are listed, if their concentrations are higher than the interventional values for this soil type in the German soil protection law (BBodenSchG, 1999). A complete list of the contaminants is given in Appendix C. The analysis of the remediated soil materials was performed in the same way and by the same institutions as already described for the contaminated soil materials (see p. 95, chapter 7.3.2).

The contaminated soil materials are indicated by the suffix (–a) after the abbreviation of the site, and remediated ones by (–b) or (–c). An uncontaminated reference soil material is indicated by (0) after the short form of the site and the same suffixes as for the other soil materials.

For the soil material from Elsnig molasses was used as an additional carbon source. Thus this substance was tested for its toxicity on the test organisms. Not very much remediated soil material was available, so only reproduction tests with 15 g soil (fw) instead of 30 g soil (fw) were performed for each species. The lesser amount of soil material, however, did not cause a significant effect on the reproduction.

Choice tests were not performed, since it cannot be excluded that the animals chose one side due to differences in the soil material itself and not in regard to the contamination.

8.3.1 “Werk Tanne” at Clausthal-Zellerfeld: CTNT2, CTNT3, CTNT4

The ammuniton plant „Werk Tanne“ at Clausthal-Zellerfeld (Lower Saxony, Germany) was remediated in the course of a scientific project of the German Ministry of Education and

Research (BMBF) for the evaluation of biological remediation methods in the years 1996-1999, grant number 1491032. Three different remediation strategies were tested in a field study: anaerobic-aerobic composting (company "Umweltschutz-Nord"), dynamic anaerobic-aerobic windrow-process (company "Plambeck ContraCon") and remediation with the white rot fungus (company "Wistrans"). These projects were coordinated by the "Industrieanlagen-Betriebsgesellschaft" (IABG), Berlin. To ensure the comparability, soil materials from various contaminated spots were mixed and riddled to remove the coarse part > 60 mm. All three processes were started with 70 t of this soil mixture (KARUTZ et al, 2000).

CTNT2a was remediated with the white rot fungus in an anaerobic windrow without intensive soil treatment. In the middle of the treatment the soil material was mixed and the substrate of fungi was renewed (MICHELS, 1998). After 24 weeks the soil material was transferred to an open area for resting (KARUTZ, et al, 2000). The remediated soil material CTNT2aVA was tested before this resting period (VA is the short form for "vor Auslagerung" meaning before resting).

CTNT3a was remediated by a dynamic anaerobic-aerobic windrow process in a real two step mechanism yielding to CTNT3b. During the anaerobic phase the TNT was reduced by the aid of easily usable substrates without intensive soil treatment. The immobilisation took place in the successive aerobic phase under intensive soil treatment. The whole treatment lasted 12 weeks (DOHNALEK-DROSTE & ZIESNER, 1998). The unremediated soil material CTNT3a was not available for testing.

With the anaerobic-aerobic composting the contaminated soil material CTNT4a and an uncontaminated reference soil material CTNT04a, also known as CTNT02a, were treated, yielding to the remediated soil materials CTNT4b and CTNT04b. At the beginning of the treatment easily usable substrates were added and the soil material was turned over at least every second day, later only once a week. To optimise the process the amount of added substrate was varied (MICHELS, 1998). CTNT7b was the product of such an altered treatment of CTNT7a, a soil material equalling CTNT4a.

In Table 8.3-1 the properties of the soil materials from “Werk Tanne” before and after the remediation are given, as well as their contaminants. All those contaminants are listed which were above the intervention value for their soil type according to the German soil protection law (BBodenSchG, 1999). A complete list of the contaminants is given in Appendix C.

Table 8.3-1: Soil properties and contaminations above the German intervention values for the contaminated and the remediated soil materials from the ammuntion plant Werk Tanne at Clausthal-Zellerfeld (Lower Saxony, Germany)

	CTNT 2a	**CTNT 2aVA**	**CTNT 3a**	**CTNT 3b**	**CTNT 4a**	**CTNT 4b**	**CTNT 7b**	**CTNT 04a**	**CTNT 04b**
clay%	16	0	14	40	16	24	16	24	25
silt%	42	65	46	46	45	32	28	58	47
sand%	43	35	40	13	39	45	56	19	29
soil type[1)]	Slu	Us	Slu	Lt3	Slu	Ls3	Sl4	Lu	Ls2
pH	6.9	7.2	6.8	7.7	6.7	8.0	7.5	4.1	7.6
C_{org}%	5.4	10.4	4.7	8.8	5.6	7.7	7.2	6.3	9.4
contamination [mg/kg soil (dw)]									
TNT	2500		2100	n.d.	3100	n.d.	n.d.		n.d.
Cd	4.9	4.0	5.7	3.4	4.6	4.0	2.7		2.3
Cu	79	99	82	77	84	83	72		49
Ni	28		29		27		24		
Pb	990	1382	1510	1866	772	772	748	490	431
Zn	940	857	1270	664	747	747	594	202	375
PAK (EPA)	13.4		13.9	36.7	32	28	29.7	13.4	
remediation	white rot fungus in anaerobic windrow process		dynamic anaerobic-aerobic windrow process		anaerobic-aerobic composting				
remediator	Wistrans		Plambeck ContraCon		U-Nord				

1) Short forms of the soil types according to the German system (AG Boden, 1998); the full names are listed in abbreviations

n.d. not detected

As the threshold levels given by the soil protection law do not exclude a toxic effect of the heavy metals on the test organisms, the EC50(28d)-values were calculated for each soil material on the basis of the reference value formula used in the Dutch List of Soil Quality Reference (VEGTER, 1995), as already described for the contaminated soil materials (see p. 97, chapter 7.3.3). The calculation and the results are given in Appendix D.2.

8.3.2 Elsnig near Torgau: ETNT

The contaminated soil material ETNTa from the ammunitions plant at Elsnig near Torgau (Saxony, Germany) was taken from the open-burning site. It was remediated microbiologically in bioreactors by the "C-P-D Umweltschutz Oelzschau" in a research project of the German Ministry for Education and Research, grant No 14509503. As an additional carbon source 1% or 2% molasses were added as well as 0.15% NH_4Cl and 0.02% K_2HPO_4 as nutritious salts, yielding to the remediated soil materials ETNTb1% and ETNTb2%. The properties and the contaminations of the three soil materials are given in Table 8.3-2. Only those contaminants which were above the interventional values of the German soil protection law for the soil type are listed, the others are given in Appendix C.

Table 8.3-2: Soil properties and contaminations above the German intervention values for the contaminated and remediated soil materials from the open-burning site in Elsnig near Torgau (Saxony, Germany)

	ETNTa	ETNTb1%	ETNTb2%
clay%	0	n.t.	n.t.
silt%	65	n.t.	n.t.
sand%	35	n.t.	n.t.
pH	7.1	n.t.	n.t.
soil type[1)]	Su		
C_{org}%	3.0%	n.t.	n.t.
contamination [mg/kg soil (dw)]			
TNT	4577	787	41
Hexyl	296	144	48
Hexogen	482	729	168
Octogen	56	92	23
remediation		1% molasses	2% molasses
remediator	C-P-D Umweltschutz Oelzschau		

1) Short forms of the soil types according to the German system (AG Boden, 1998); the full names are listed in abbreviations

n. t. not tested

8.3.3 Hambühren: LTNT

The TNT-contaminated soil material LTNTa was taken from the storage facility at Hambühren near Celle (Lower Saxony, Germany). The site was contaminated across an area of about 10 000 m^2 with a maximum of 20 000 mg TNT/kg soil (dw) at the hot spot (SCHÄFER & BACKSEN, 1998). Hence, remediation became necessary, when the erection of residential area was planned on the site.

For the remediation about 2500 t soil material and building rubble were excavated and transported to the stationary soil washing plant of the "AB Umwelttechnik GmbH" (ABU) at Lägersdorf (Schleswig-Holstein, Germany) (see p. 115, Fig. 8.2-1). There, the soil material was first riddled and the fraction > 20 mm was searched for crystalline TNT, of which 300 kg were removed, before it was treated thermally.

The fraction < 20 mm was washed with water and additional sorbents, which remained in the light fraction. The wash water was continually treated with activated charcoal. After the soil washing remained a lightly contaminated sand fraction, a highly contaminated light fraction and the filtration residue from the wash water. The contamination of the sand fraction was in general with < 20 mg TNT/kg soil (dw) below the threshold values for the usage as building material (SCHÄFER & BACKSEN, 1998). The light fraction was supposed for further thermal treatment, whereas the filtration residue with more than 10 000 mg TNT/kg soil (dw) was supposed for the use in the cement production.

Overall 86.4% of the soil material could be reused. The remaining 13.6%, the gravel, the light fraction and the remainder from the riddling, had to be treated thermally (SCHÄFER & BACKSEN, 1998).

The lightly contaminated sand fraction LTNT1b and the highly contaminated light fraction LTNT1c were tested for their habitat function with the biotests. The light fraction of the TNT-washing process contained the mineral fraction < 63 mm as well as the organic matter up to 2 mm. The properties as well as all the contaminants above the intervention values for the soil type in the German soil protection law are given in Table 8.3-3, the other contaminants are listed in Appendix C.

Table 8.3-3: Soil properties and contaminations above the German intervention values for the contaminated and remediated soil materials from the washing process at Lägersdorf (Schleswig-Holstein, Germany)

	LTNT1a	**LTNT1b sand fraction**	**LTNT1c light fraction**
clay%	1	0.5	13
silt%	3	1.5	0.5
sand%	96	98	29
soil type[1)]	Ss	Ss	Uls
pH	7.4	7.4	7.8
C_{org}%	1.2	0.1	18
contamination [mg/kg soil (dw)]			
TNT	350	80	(570) 30
Pb	36	24	121
Zn	75	22	111
PAK(EPA)	2.7	0.8	55
remediation	soil washing		
remediator	ABU		

1) Short forms of the soil types according to the German system (AG Boden, 1998); the full names are listed in abbreviations

8.4 Results

8.4.1 „Werk Tanne“ at Clausthal-Zellerfeld

8.4.1.1 CTNT2

The remediated soil material CTNT2aVA showed no mortality, but a significant reduction of the reproduction in both biotests. In contrast, the original contaminated soil material CTNT2a with a TNT-content of 2500 mg/kg soil (dw) caused a 100% mortality and reduced the reproduction of *F. candida* completely. For the enchytraeid no mortality was observed not even in the pure soil material, but the reproduction rate was reduced to 12.4 % ± 1.9 in two different tests.

Table 8.4-1: Mortality and reproduction rate for *F. candida* and *E. crypticus* in CTNT2a and the remediated soil material CTNT2aVA in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest		Enchytraeid-biotest	
soil material	**% mortality**	**% reproduction rate**	**% mortality**	**% reproduction rate**
CTNT2a	100 ±0.0*	0.0 ± 0.0*	2.0 ± 4.0	12.4 ± 1.9*
CTNT2aVA	24.0 ± 10.2	0.0± 0.0*	7.5 ± 8.3	2.0 ± 1.7*

* significant difference to control with Lufa 2.2

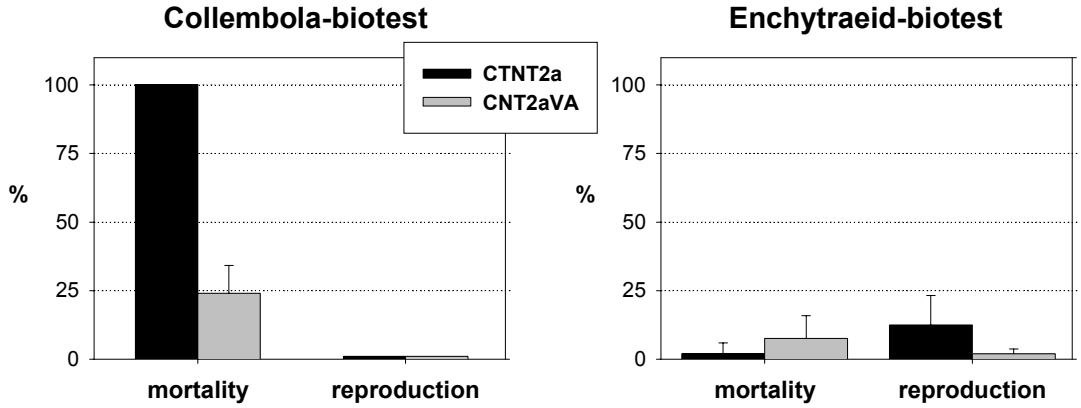

Fig. 8.4-1: Mortality and reproduction rates of *F. candida* and *E. crypticus* in the contaminated soil material CTNT2a and the remediated soil material CTNT2aVA from the ammunition plant "Werk Tanne" at Clausthal-Zellerfeld (Lower Saxony, Germany). Bars: mean ± SD.

8.4.1.2 CTNT3

The contaminated soil material CTNT3a was not available for testing, but the previously described soil material CTNT2a can be used for a comparison. CTNT2a was the same soil type as CTNT3a and contained only slightly less TNT as well as a bit more Pb and Zn (Appendix C). Thus, it was used as a basis for the evaluation of the remediation success in CTNT3b. The remediated soil material CTNT3b, however, showed no mortality and no significant reduction of the reproduction in either of the two biotests.

Table 8.4-2: Mortality and reproduction rate for *F. candida* and *E. crypticus* in CTNT2a and the remediated soil material CTNT 3b in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest		Enchytraeid-biotest	
soil material	**% mortality**	**% reproduction rate**	**% mortality**	**% reproduction rate**
CTNT2a	100 ± 0.0*	0.0 ± 0.0*	2.0 ± 4.0	12.4 ± 1.9*
CTNT3b	12.0 ± 9.8	107.2 ± 43.5	14.0 ± 8.0	82.8 ± 15.3

* significant difference to control with Lufa 2.2

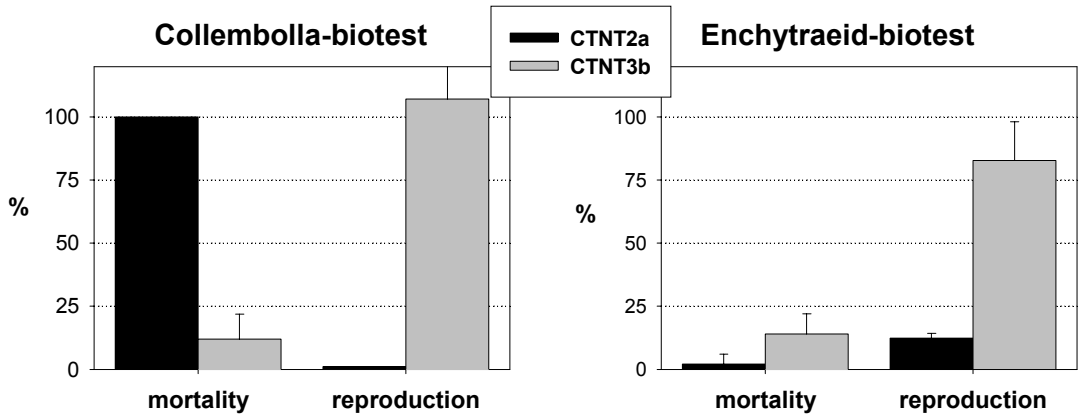

Fig. 8.4-2: Mortality and reproduction rates of *F. candida* and *E. crypticus* in the contaminated soil material CTNT2a and the remediated soil material CTNT3b from the ammunition plant "Werk Tanne" at Clausthal-Zellerfeld (Lower Saxony, Germany). Bars: mean ± SD.

8.4.1.3 CTNT4

The remediated soil material CTNT4b had no effect on the mortality either of the collembola or the enchytraeid. The reproduction rate, however, was reduced to 63.9% ± 16.0 for *F. candida* and to 57.6% ± 15.0 for *E. crypticus*. For the collembola the reduction was significant in a t-Test. The originally contaminated soil material CTNT4a with 3100 mg TNT/kg soil (dw) caused 100% mortality and complete reduction of the reproduction in the collembola-biotest. For the enchytraeid no significant mortality was observed in the pure soil material, but a reduction of the reproduction rate to 10.6% ± 12.5.

An uncontaminated reference soil material, CTNT04b, treated with the same remediation process, did not affect the mortality of both species. However, the reproduction of both was significantly reduced: completely for *F. candida* and to 5.9% ± 3.3 for *E. crypticus*. Before the remediation the reference soil material CTNT04a did not affect the mortality of either species. The reproduction rate of *F. candida* was significantly increased to 125.7%± 9.2 and for the enchytraeid it was significantly decreased to 22.3% ± 2.6.

After an alteration of the treatment the soil material CTNT7a equalling CTNT4a was remediated yielding to the remediated soil material CTNT7b. This soil material had no significant effect on the mortality or reproduction of either species.

Table 8.4-3: Mortality and reproduction rate for *F. candida* and *E. crypticus* in CTNT4a, the remediated soil materials CTNT4b, CTNT7b and the uncontaminated soil materials CTNT04a and CTNT04b before and after the remediation in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest		Enchytraeid-biotest	
soil material	**% mortality**	**% reproduction rate**	**% mortality**	**% reproduction rate**
CTNT4a	100 ± 0.0*	0.0 ± 0.0*	12.0 ± 11.7	10.6 ± 12.5*
CTNT4b	8.0 ± 4.0	63.9 ± 16.0*	2.0 ± 4.0	57.6 ± 15.0
CTNT7b	22.0 ± 7.5	79.1 ± 37.9	10.0 ± 7.1	82.0 ± 14.2
CTNT04a	0.0 ± 0.0	125.7 ± 9.2*	0.0 ± 0.0	22.3 ± 2.6*
CTNT04b	8.0 ± 7.5*	0.0 ± 0.0*	4.0 ± 8.0	5.9 ± 3.3*

* significant difference to control with Lufa 2.2

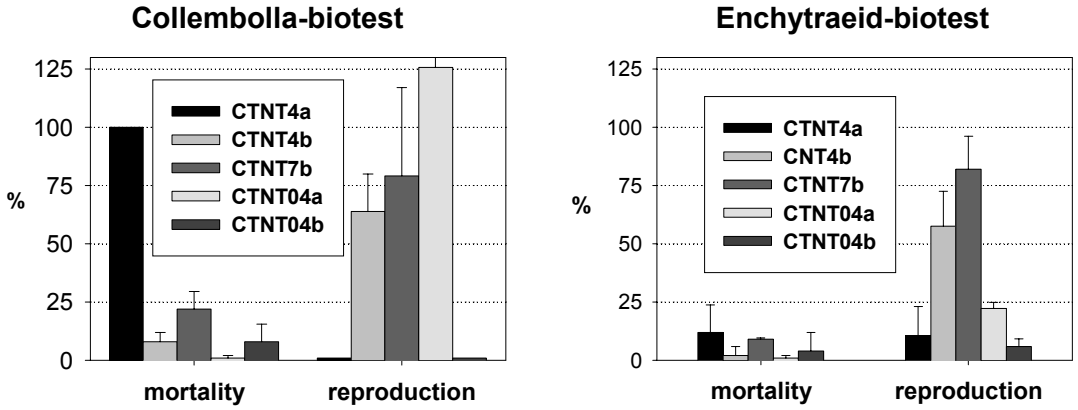

Fig. 8.4-3: Mortality and reproduction rates of *F. candida* and *E. crypticus* in the contaminated soil material CTNT4a and the remediated soil materials CTNT4b, CTNT7b as well as in the uncontaminated soil material CTNT04a before and after the remediation (CTNT04b) from the munition plant "Werk Tanne" (Lower Saxony, Germany). Bars: mean ± SD.

8.4.2 Elsnig near Torgau

For the remediation process molasses as an additional carbon source for the microorganisms as well as 0.15% NH_4Cl and 0.02% K_2HPO_4 as nutritious salts were added. One batch of soil material was remediated with 1% molasses yielding to ETNTb1% another with 2% molasses resulting in ETNTb2%. Only reproduction tests were performed as not enough soil material was available for mortality tests. In the collembola-biotest no reproduction was observed, but in ETNTb2% adults survived. For *E. crypticus* a reproduction rate of 13.6% ± 10.4 was evaluated in ETNTb2%. In ETNTb1% adult animals survived, although the reproduction was completely reduced.

The original contaminated soil material ETNTa from the open burning site at Elsnig was strongly contaminated with ammunition like compounds (ALC) (5765.0 mg ALC/kg soil (dw)), but TNT contributed the major part with 4577.0 mg TNT/kg soil (dw). In the pure soil material no animals survived and the reproduction was completely reduced for both test species.

Table 8.4-4: Reproduction rates for *F. candida* and *E. crypticus* in the ETNTa and the remediated soil materials ETNTb1% and ETNTb2% in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest	Enchytraeid-biotest
soil material	**% reproduction rate**	**% reproduction rate**
ETNTa	0.0 ± 0.0*	0.0 ± 0.0*
ETNTb1%	0.0 ± 0.0*	0.0 ± 0.0*
ETNTb2%	0.0 ± 0.0*	13.6 ± 10.4*

* significant difference to control with Lufa 2.2

As an effect of the added substances on the test species cannot be excluded they, too, were tested with mortality and reproduction tests in the standard soil material Lufa 2.2.

Table 8.4-5: Mortality and reproduction rate for *F. candida* and *E. crypticus* with the additives of the remediation process for ETNTa

	Collembola-biotest		Enchytraeid-biotest	
additives and soil treatment	**% mortality**	**% reproduction rate**	**% mortality**	**% reproduction rate**
1% molasses; 0.15% NH_4Cl, 0.02% K_2HPO_4	1.7%	14.3%*	1.7%	57.5%*
1% molasses	4.0%	37.5%*	0.0%	100.4%
0.15% NH_4Cl; 0.02% K_2HPO_4	6.0%	77.1%	4.0%	82.6%
1 month ageing: 1% molasses; 0.15% NH_4Cl; 0.02% K_2HPO_4	100%*	0%*	100%*	0%*

* significant difference to control without any additives

In the collembola-biotest the reproduction was significantly reduced if molasses and the nutritious salts were added together, but also by molasses alone. The mortality was not affected, neither was the reduction in the reproduction significant if the nutritious salts were added without molasses. However, after the soil material containing molasses and the nutritious salts had been aged for one month, it was 100% lethal and the reproduction was completely reduced.

In the enchytraeid-biotest the reproduction was only significantly reduced by molasses together with the nutritious salts, but not by either of them separately, nor was the mortality affected. After the ageing process, however, the mortality was 100% and the reproduction was completely reduced.

8.4.3 Hambühren

After the soil washing process two soil fractions remained: a low contaminated sand fraction LTNT1b with 80 mg TNT/kg soil (dw) and a highly contaminated fine grain fraction LTNT1c with

570 mg TNT/kg soil (dw). After the biotests the TNT-content of LTNT1c was analysed again and only 30 mg TNT/kg soil (dw) were detected. In the collembola-biotest this soil material did not effect the mortality, but significantly reduced the reproduction rate to 58.9% ±18.6. For the enchytraeid the soil material had no effect either on the mortality (0.0% ± 0.0) or on the reproduction rate (94.1% ± 18.0). The low contaminated soil material LTNT1b, however, significantly increased the mortality of *F. candida* to 82% ± 24.0 and reduced the reproduction completely. In the enchytraeid-biotest the soil material had no effect on the mortality (0% ± 0.0), but reduced the reproduction completely.

The original soil material LTNT1a was contaminated with 350 mg TNT/kg soil (dw). It reduced the mortality of *F. candida* completely and no reproduction could be detected. For the enchytraeid the mortality increased insignificantly to 18% and the reproduction rate was reduced significantly to 25.5% ± 10.4 in the pure soil material.

Table 8.4-6: Mortality and reproduction rate for *F. candida* and *E. crypticus* in LTNT1a and in the fractions of the washing process LTNT1b and LTNT1c in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest		Enchytraeid-biotest	
soil material	**% mortality**	**% reproduction rate**	**% mortality**	**% reproduction rate**
LTNT1a	100 ± 0.0*	0.0 ± 0.0*	18.0 ± 18.3	25.5 ± 10.4*
LTNT1b	82.0 ± 24.0*	0.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0*
LTNT1c	14.0 ± 4.9*	58.9 ± 18.6*	0.0 ± 0.0	94.1 ± 18.0

* significant difference to control with Lufa 2.2

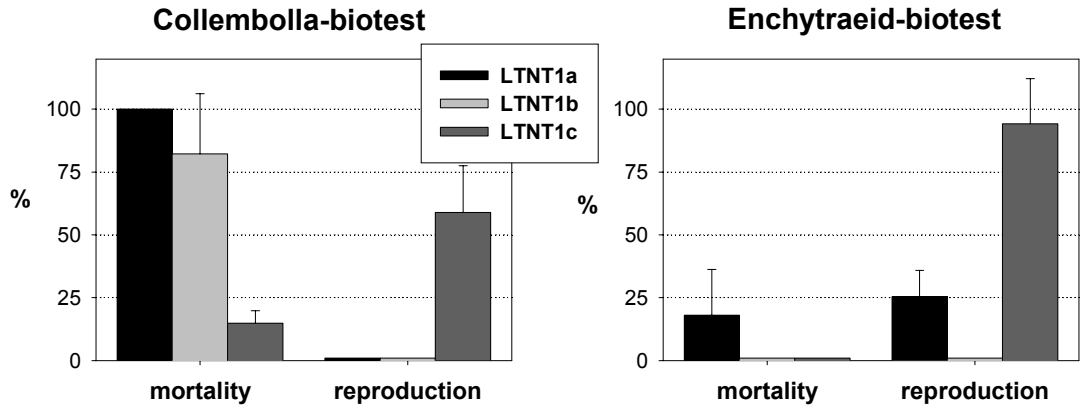

Fig. 8.4-4: Mortality and reproduction rates of the original soil material LTNTa from Hambühren (Lower Saxony, Germany) and the two soil fractions LTNT1b and LTNT1c after the washing process in the stationary soil washing plant of the ABU at Lägersdorf. Bars: mean ± SD.

To determine the toxicity of the sand fraction the 50%-effect-values were evaluated in a dilution with Lufa 2.2. For the collembola the LC50(7d) was 78.1 and the EC50(28d)-value was 68.0 mg TNT/kg soil (dw). For the enchytraeid an EC50(28d)-value of 70.9 mg TNT/kg soil (dw) was evaluated.

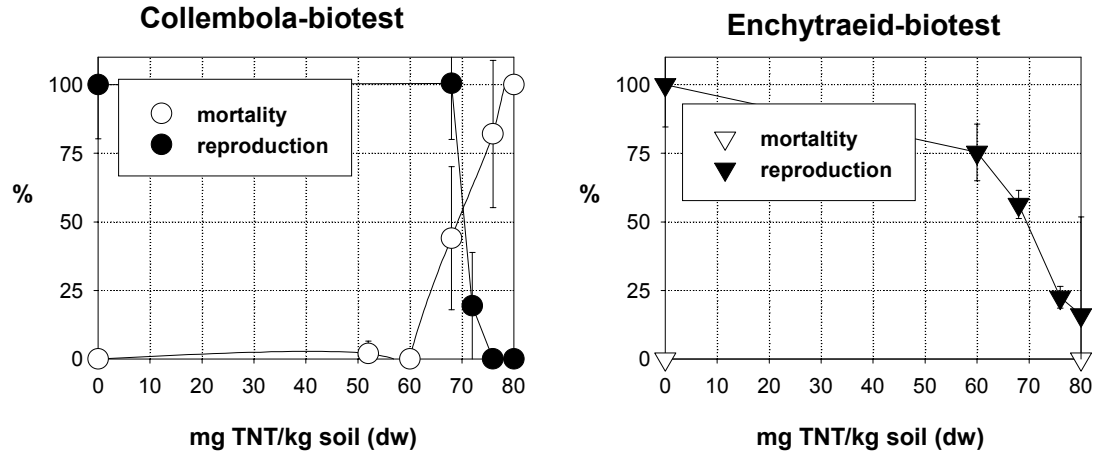

Fig. 8.4-5: Concentration-effect relationship with LTNT1b for *F. candida* and *E. crypticus*. Symbols: mean ± SD.

8.4.4 Summary of results

For contaminated soils special criteria have been established to evaluate the toxicity (see p. 105, Table 7.4-10) (ACHAZI et al, 2001). They can also be applied for the remediated soil materials, but then the more subtle differentiation between very toxic and toxic becomes useful.

Table 8.4-7: Toxicity of the contaminated and remediated soil materials according to the guideline established for contaminated soils

soil material	mg TNT/kg soil (dw)	Collembola-biotest		Enchytraeid-biotest	
		mortality	reproduction	mortality	reproduction
CTNT2a	2500	**++**	**++**	**--**	**+**
CTNT2aVA	n.d.	**--**	**++**	**--**	**+**
CTNT3a	2100	n.t.	n.t.	n.t.	n.t.
CTNT3b	n.d.	**--**	**--**	**--**	**--**
CTNT4a	3100	**++**	**++**	**--**	**+**
CTNT4b	n.d.	**--**	**(+)**	**--**	**--**
CTNT7b	n.d.	**--**	**--**	**--**	**--**
CTNT04a	0.049	**--**	**--**	**--**	**+**
CTNT04b	n.d.	**(+)**	**++**	**--**	**+**
ETNTa	4577	**++**	**++**	**++**	**++**
ETNTb1%	786.9	n.t.	**++**	n.t.	**++**
ETNTb2%	41.1	n.t.	**++**	n.t.	**+**
LTNT1a	350	**++**	**++**	**--**	**+**
LTNT1b	80	**+**	**++**	**--**	**++**
LTNT1c	30	**(+)**	**(+)**	**--**	**--**

\+ non-toxic
(+) ecotoxicologiclly critical
\+ toxic
++ very toxic
n.d. not detected
n.t. not tested

8.5 Discussion

As a result of the remediation process the TNT-concentration decreased in some soil materials even below the detection limit and the habitat function of the soil materials generally improved. However, the improvement was not complete for all soil materials (see p. 129, Table 8.4-7), hence, a more closer look is necessary.

8.5.1 Evaluation of the remediation processes

8.5.1.1 „Werk Tanne“ at Clausthal-Zellerfeld

CTNT2a was remediated by "Wistrans" with the white rot fungus in a windrow. After the remediation, but before the resting period the soil material CTNT2aVA was tested. It caused no significant mortality for both test organisms, which is an improvement for the collembola in comparison to the unremediated soil material. However, the reproduction of the collembola remained completely reduced, although adult animals survived. The reproduction of the enchytraeid on the other hand was significantly reduced, but not completely. Thus, the remediated soil material has to be classed as very toxic for *F. candida* and toxic for *E. crypticus*.

This toxicity cannot be attributed to TNT, as the soil material contained no detectable concentration of TNT. However, the remediated soil material still contained concentrations of the heavy metals Cd, Cu, Pb and Zn above the intervention values for this soil type according to the German soil protection law (BbodenSchG, 1999). In the case of Zn the concentration was even 3.5 times above the calculated threshold level for *F. candida* (see Appendix D.2). For the enchytraeid the concentration of Pb was 4.0 times and the concentration of Zn 2.6 times higher than the calculated threshold levels (see Appendix D.2). In addition, metabolites produced during the remediation process might also contribute to the toxicity of the soil material. Usually, such products are further transformed in the following resting phase. As no soil material after the resting was available, no final statement of the remediation success can be given.

Although the unremediated soil material CTNT3a was not available, the remediation success could be judged on the basis of the toxicity of the similar soil material CTNT2a. Especially since the remediated soil material CTNT3b was not lethal and the reproduction for both species was not significantly different from the control in Lufa 2.2. Therefore CTNT3b has to be classed as non-toxic for the collembola and for the enchytraeid according to the guideline for soils. Thus the remediation of the "Plambeck ContraCon" with an anaerobic-aerobic windrow process can be considered as successful for TNT. However, the contents of the heavy metals Cd, Cu, Pb and Zn were still above the interventional values for this soil type according to the German soil protection law (BbodenSchG, 1999).

The original contaminated soil material CTNT4a from the ammunition plant "Werk Tanne" at Clausthal-Zellerfeld contained 3100 mg TNT/kg soil (dw). It was classed as very toxic to *F. candida* and toxic to the enchytraeid on the basis of the reproduction test. After the remediation with an anaerobic-aerobic compost no TNT could be detected in the remediated soil material CTNT4b. The habitat function of the soil material had also improved. Especially for the collembola the improvement is striking: the mortality decreased from 100% in the contaminated soil material to 8% ± 4.0 in the remediated one (see p. 129, Table 8.4-3). The reproduction, too, improved from 0% ± 0.0 to 63.9% ±16.0. This was still significantly lower than in the corresponding control, but on the basis of the guideline for soils outlined earlier this soil material is considered to be only ecotoxicologically critical, although the concentration of Zn was slightly above the calculated threshold value for this soil material (see Appendix D.2).

In the enchytraeid-test the mortality was neither strongly affected by the contaminated nor by the remediated soil material. The reproduction, however, raised from 10.6% ± 12.5 before to 57.6% ± 15.0 after the remediation with no significant difference to the corresponding control for the latter. Hence, the remediated soil material CTNT4b was non-toxic for the enchytraeid, although the concentrations of Pb and Zn were slightly above the calculated threshold values for this soil material (see Appendix D.2).

The uncontaminated control soil material CTNT04b, which was treated with the same remediation process was more toxic than the contaminated soil material after the remediation. In the collembola-biotest the mortality was 8.0% ± 7.5 and the soil material had to be classed as excotoxicologically critical. This was proven by a completely reduced reproduction for the collembola and a reproduction rate of only 5.9% ± 3.3 for the enchytraeid. According to the guideline for soils, this control soil material has to be classed as very toxic for the collembola and toxic for the enchytraeid.

In contrast, the reproduction of the collembola was even significantly increased in CTNT04a, the uncontaminated reference soil material before the remediation, whereas it was significantly decreased to 22.3% ± 2.6 in the enchytraeid-biotest. For *E. crypticus* the soil material has thus to be classed as toxic, which might be a result of the low pH of 4.1. After the remediation the pH-value of 7.6 cannot affect the reproduction of the enchytraeid anymore, hence, the reduction of the reproduction for both test species has to be attributed to the remediation process.

An altering of the treatment yielded to the remediated soil material CTNT7b. In this soil material no significant mortality occured and the reproduction for both species had significantly increased. According to the guideline for soils CTNT7b had to be classed as non-toxic for the collembola and for the enchytraeid, although the Zn-content was with 594.0 mg Zn/kg soil (dw) above the standardised EC50-value of 496.9 mg Zn/kg soil (dw) for *E. crypticus* (see Appendix D.2).

Hence, the remediation process of "U-Nord" with an anaerobic-aerobic compost can be considered as successful for TNT. The content of the heavy metals, however, has to be judged carefully, as in CTNT4b the contents of Cd, Cu, Pb and Zn and in CTNT7b also of nickel (Ni) were above the interventional values for these soil types according to the German soil protection law (BbodenSchG, 1999).

8.5.1.2 Elsnig

From the remediated soil materials from Elsnig near Torgau not much soil material was available, as the remediation is still in progress. Thus only reproduction tests were possible. In the soil material with 1% molasses, ETNTb1%, the reproduction of the collembola was completely reduced and no surviving adults were found at the end of the test. The TNT-content of this soil material was with 787 mg TNT/kg soil (dw) still quite high and even above the 50%-values of the original contaminated soil material ETNTa with a LC50(7d) of 179.3 and an EC50(28d) of 123.5 mg TNT/kg soil (dw).

In the soil material ETNTb2% with 2% molasses the reproduction was completely reduced, but adults had survived even after four weeks. This indicates an enormous improvement of the soil material, as the original contaminated soil material had to be diluted down to 8% before any adults could survive for a week in the mortality test. Thus it can be assumed that this remediated soil material would not be lethal in a regular mortality test. However, the low TNT-content of ETNTb2% with 41 mg TNT/kg soil (dw) does not justify the still apparent toxicity, as it was much lower than the 50%-values of the contaminated soil material ETNTa with 123.5 mg TNT/kg soil (dw). The higher toxicity might be caused by the additional carbon source molasses, which was added for the remediation process. It was found to reduce the reproduction significantly (see p. 127, Table 8.4-5). After one month of ageing the standard soil material with molasses and the nutritious salts became even more toxic, because then it was 100% lethal and did reduce the reproduction completely. The increased toxicity might be the result of a fermentation process, as the soil material smelt fermented and the jars had not been aerated during the ageing at room temperature. During the fermentation process toxic compounds might have been produced.

The enchytraeid, too, was not able to reproduce in ETNTb1%, but adult animals had survived at the end of the test indicating a reduced mortality. This result corresponds with the 50%-values determined for the original contaminated soil material ETNTa, as the TNT-content of ETNTb1% was with 787 mg TNT/kg soil (dw) only slightly higher than the LC50(7d) of 695.6 and much higher than the EC50(28d) of 315.9 mg TNT/kg soil (dw).

In ETNTb2% the enchytraeid was even able to reproduce with a reproduction rate of 13.6% ± 10.4 (see p. 127, Table 8.4-4). This might not seem to be a major improvement, but

compared to the original soil material, the difference is huge, as the contaminated soil material had to be diluted to 15% before reproduction became possible. However, the TNT-content of ETNTb2% was with 41 mg TNT/kg soil (dw) much lower than the 50%-values determined in the original contaminated soil material ETNTa with 315.9 mg TNT/kg soil (dw). Thus, the remediated soil material ETNTb2% was more toxic than the original contaminated soil material. This might again be the effect of molasses and the nutritious salts, since they significantly reduced the reproduction of *E. crypticus* if added together (see p. 127, Table 8.4-5). For the enchytraeid, too, the soil material with the additives became even more toxic after one month of ageing, as it was then 100% lethal and did reduce the reproduction completely. This might again be the effect of the fermentation process as well as of the additives.

For the remediated soil materials, but especially for ETNTb2%, with its relatively low TNT-content, it can thus not be excluded that the additives do affect the test organisms. In addition, metabolites produced during the degradation process might enhance the toxicity. Hence, a period of resting might improve the habitat function of ETNTb2% for the test organisms.

The concentrations of Hexyl, Hexogen (RDX) and Octogen (HMX) on the other hand should not affect the test organisms, since they were below the concentrations found to be effective for both test species (see p. 101, Table 7.4-5) and also below the concentrations for synergetic effects (see p. 101, chapter 7.4.2). However, their concentrations and the concentration of TNT were still too high, to consider the remediation as completed, but an improvement has already been achieved in comparison to the original contaminated soil material. On the basis of the present results a completely satisfying result seems feasible.

8.5.1.3 Hambühren

The contaminated soil material from Hambühren LTNT1a was highly toxic for the collembola in both tests and toxic for the enchytraeid in the reproduction test, but non-toxic according to mortality. The soil washing of the contaminated soil material LTNT1a with 350 mg TNT/kg soil (dw) led to two contaminated soil fractions: a low contaminated sand fraction and a highly contaminated light fraction.

During a period of resting before the start of the biotest, the TNT-content decreased in the light fraction from 570 to 30 mg TNT/kg soil (dw). This reduction in the TNT-content can be attributed to sorption to the high content of organic carbon (see p. 123, Table 8.3-3) as well as to bacterial degradation indicated by the increase of ADNTs found in this soil fraction (see Appendix C). After the resting the soil material was only slightly toxic in the collembola-test (see p. 128, Table 8.4-6), as the mortality and the reproduction had improved (mortality 14% ± 4.9; reproduction 58.9% ± 18.6) but both were still significantly different from the control. For *E. crypticus* on the other hand, the soil material was non-toxic.

In comparison to the toxicity of the original soil material the remediation was successful for both test species. In particular, for *F. candida*, as the mortality of the original soil material was 100% and the reproduction was completely reduced. For the enchytraeid the better habitat function could only be observed for the reproduction, which increased from 25.5% ± 10.4 to 94.1% ± 18.0, as the mortality was not even significantly affected by the original contaminated soil material. This improvement, however, has to be judged carefully as the TNT might be only disguised by the high content of organic carbon.

The opposite was the case for the low contaminated sand fraction LTNT1b. This soil material reduced the reproduction of both test organisms completely and the mortality of the collembola was with 82.0% ± 24.0 significantly higher than in the corresponding control. Thus the soil material has to be classed as very toxic for both test organisms in spite of its low TNT-content of 80 mg TNT/kg soil (dw).

LTNT1b was even more toxic for both test species than the original soil material LTNTa as a comparison of the 50% effect values shows, although its original TNT-content was much lower than the one in LTNT1a with 350 TNT/kg soil (dw). For LTNT1a the LC50(7d)-value for the collembola was 145.5 mg TNT/kg soil (dw) in a dilution with Lufa 2.2 and above 350 mg TNT/kg soil (dw) for the enchytraeid. The EC50(28d)-values were 90.0 and 272.5 mg TNT/kg soil (dw), respectively.

The high toxicity of the sand fraction LTNT1b has to be attributed to its even lower organic carbon content with 0.1% and the low clay content with 0.5%. Hence, the possible sorption sides of TNT had been reduced and nearly all the TNT of the sand fraction was bioavailable and could be easily mobilised, as had been shown in water eluates from this soil material (PFEIFFER et al, 2001). Hence, it is not surprising that the toxicity of the sand fraction rapidly decreased in dilution with Lufa 2.2. Thus the LC50(7d)-value of 78.1 mg TNT/kg soil (dw) for the collembola corresponds to 98% of the contaminated soil material and the EC50(28d) of 68.0 mg TNT/kg soil (dw) to 85%. For the enchytraeid the EC50(28d) of 70.9 mg TNT/kg soil (dw) corresponds to 87% of the contaminated soil material.

Although the sand fraction still contained some TNT and remained toxic, its use as building material should be possible. The content of heavy metals, too, was not too high for this purpose. Whether the light fraction LTNT1c has to be incinerated or if a biological treatment might be more suitable should be reconsidered on the basis of these experiments and the TNT-content of other light fractions after the washing process should be tested after a period of resting, too. For an evaluation of the cost-effectiveness of the soil washing, it has to be considered that still 13.6% of the original soil material have to be treated thermally.

8.5.2 Comparison with literature

The effect of composting of munitions contaminated soils has already been assessed with biotests. GRIEST et al (1993) found that the mutagenity and the toxicity were reduced in the organic solvent extracts after composting as was the concentration of the explosives.

In a different study on the effect of composting, however, the mutagenity increased, whereas the toxicity for bacteria as well as the acute toxicity for the earthworm *E. fetida* decreased (JARVIS et al, 1998).

In a microcosm study it was evident that the soil material from a compost with contaminated soils had an effect on plants in comparison to the soil material from a control compost with uncontaminated soil. However, since the toxicity was low it was considered to be environmentally acceptable. For the earthworms and isopods tested in this microcosm study the risk was definitely reduced (GUNDERSON et al, 1997).

In the project “biological processes for soil remediation – ecotoxicological test batteries”, which was sponsored by the German ministry of Education and Research from 1996-1999, grant number 1491032, various remediation processes have been evaluated. In Table 8.5-1 an overview of the different tests with TNT-contaminated soil materials from this project before and after the remediation is given. The soil materials have already been described, as the evaluation of these soil materials with the collembola- and enchytraeid-biotests were performed as a part of this project during the course of this thesis.

For all used biotets the contaminated soil materials were toxic according to the criteria for toxicity given below the table. The evaluation of the remediated soil materials and the control soil material CTNT04a, however, differed. Only CTNT3b was classified consistently as non-toxic, but it was evaluated with only one other test, the respirative activation quotient (Q_R).

For the dicotyledon turnip (*B. rapa*), the remediated soil material CTNT4b was still toxic, but this was attributed to the high pH-value (RÖMKE & KALSCH, 2001).

The toxic effect of Q_R for the remediated soil materials CTNT4b and LTNT1c on the other hand was attributed to the high content of mineral nitrogen found in this soil materials, which was reduced in CTNT3b and CTNT7b due to its longer resting period (WILKE & WINKEL, 2001).

In case of the ciliate *C. inflata* the acute test was slightly more sensitive than the chronic growth test. However, the higher toxicity of the remediated soil materials CTNT4b, LTNT1b and LTNT1c in the acute test was considered to be the result of the substances added before the remediation. The higher toxicity of the control soil material CTNT04b on the other hand correlated with a higher sodium content (PAULI et al, 2001).

For the toxicity of CTNT4b to the earthworm no explanation was offered (HUND-RINKE, 2001), whereas the toxicity of the unremediated reference soil material CTNT04a to the enchytraeid might be the result of the low pH of this soil material.

The differences between the Ames- and the Umu-test were discussed, as in general a potential risk is given, if just one of the genotoxicity tests is positive (PFEIFFER et al, 2001).

Table 8.5-1: Evaluation of the tested contaminated and remediated soil materials with other biotests: results with the collembola- and enchytraeid-biotest are given as a comparison and indicated by shading

		CTNT3a	CTNT3b	CTNT4a	CTNT4b	CTNT7b	CTNT04a	CTNT04b	LTNT1a	LTNT1b	LTNT1c
phyto-toxicity	*B. rapa*[1)]	n.t.	n.t.	+	+	n.t.	?	--	n.t.	n.t.	n.t.
	A. sativa[2)]	n.t.	n.t.	+	?	n.t.	--	--	n.t.	n.t.	n.t.
	S. subspicatus[3)]	n.t.	n.t.	+	n.t.	n.t.	n.t.	n.t.	+	+	--
micro-biology	Q_R[4)]	+	--	+	+	--	+	--	+	+	n.t.
	nitrification[5)]	+	n.t.	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	C. inflata acute[6)]	n.t.	n.t.	+	+	n.t.	--	+	+	+	+
	C. inflata chronic[7)]	n.t.	n.t.	+	--	n.t.	--	--	+	--	--
geno-toxicity	*Ames-test*[8)]	n.t.	n.t.	+	--	n.t.	n.t.	n.t.	+	--	--
	Umu-test[9)]	n.t.	n.t.	+	+	n.t.	n.t.	n.t.	+	+	--
water risk	*V. fischeri*[10)]	n.t.	n.t.	+	--	n.t.	n.t.	n.t.	+	+	--
	D. magna[11)]	n.t.	n.t.	+	--	n.t.	n.t.	n.t.	+	+	--
terrestrial inver-tebrates	*E. fetida*[12)]	n.t.	n.t.	+	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	F. candida[13)]	n.t.	--	+	--	--	--	+	+	+	--
	E. crypticus[13)]	n.t.	--	+	--	--	+	+	+	+	--

-- non-toxic
\+ toxic
n.t. not tested

1) Length, fresh and dry weight of shoot, number of blossoms or buds, number of leaves, number of siliquas, fresh and dry weight of siliquas different from control (RÖMBKE & KALSCH, 2001).
2) Length, fresh and dry weight of shoot, number of blossoms or buds, number of leaves different from control (RÖMBKE & KALSCH, 2001).
3) $G_A > 4$ (G_A dilution in which the inhibition of growth is < 20%) (PFEIFFER et al, 2001).
4) $Q_R > 0.3$ (WILKE & WINKEL, 2001).
5) Nitrification of soil mixture with Lufa 2.2 as control soil is more than 10% lower than the calculated value (WILKE et al, 2000).
6) Inhibition of hatching of encysted cells > 30%.
7) Inhibtion of growth > 30%.
8) $G_{EA} > 3$ with *S typhimurium* TA98 or TA100 (G_{EA} dilution in which difference in induction < 80 (TA 100) or < 20 (TA 98)) (EISENTRÄGER et al, 2001).
9) $G_{EU} > 1.5$ *S typhimurium* TA1535 or pSK1002 (G_{EU} dilution in which induction > 1.5) (EISENTRÄGER et al, 2001).
10) $G_L > 8$ (G_L dilution in which inhibition of fluorescence is < 20%) ; G_L 3-8 evaluation with test for inhibition of growth: $G_{LW} > 4$ ($G_{LW:}$ dilution in which the inhibition of growth is < 20%) (EISENTRÄGER et al, 2001).
11) $G_D > 4$ (G_D dilution in which 9 out of 10 daphnia are still mobile) (PFEIFFER et al, 2001).
12) Reproduction rate < 50% (HUND-RINKE, 2001).
13) Reproduction rate < 50% (see p. 129, Table 8.4-7).