7 Contaminated soil materials

7.1 Abstract

The sites of former process and handling facilities are often highly contaminated with explosives, mostly TNT, their precursors and degradation products. Soil materials from three ammunition plants and one storing facility have been tested with the collembola- and the enchytraeid-biotest and classified on the basis of these tests. Three soil materials from the ammunition plant “Werk Tanne” at Clausthal-Zellerfeld (Lower Saxony, Germany) CTNT1a with 1600 mg TNT/kg soil (dw), CTNT2a with 2500 mg TNT/kg soil (dw) and CTNT4a with 3100 mg TNT/kg soil (dw) were tested. One soil material, ETNTa with 4577 mg TNT/kg soil (dw), was taken from the open-burning site of the ammunition plant Elsnig near Torgau (Saxony, Germany). The storing facility was at Hambühren near Celle (Lower Saxony, Germany) and its soil material LTNT1a was originally contaminated with 350 mg TNT/kg soil (dw). The last soil material STNTa with 15 mg TNT/kg soil (dw) originated from the ammunition plant Stadtallendorf (Hesse, Germany). This soil material was not further investigated, as its toxicity in the reproduction test with both species could not be explained with its TNT-content. For a detailed comparison of the other soil materials the LC50(7d)- and EC50(28d)-values were compared. However, in case of the enchytraeid only for ETNT a LC50-value could be determined and thus the toxicity could not be compared. For the other tests the following order of toxicity can be given:

F. candida	LC50:	LTNT1a > ETNTa > CTNT1a > CTNT4a > CTNT2a
	EC50:	LTNT1a > ETNTa > CTNT1a > CTNT2a > CTNT4a
E. crypticus	EC50:	LTNT1a > ETNTa > CTNT1a > CTNT2a > CTNT4a

Thus LTNT1a was the most toxic soil material, although its TNT-contamination was the lowest, but it had the lowest content of organic matter. The second toxic soil material was ETNTa with the highest TNT-contamination and no clay content. The toxicity of the next toxic soil material CTNT1a could not the effect of TNT alone, but may result from the low pH in combination with a high content of heavy metals. The lower organic matter content of CTNT1a might explain that its toxicity was higher than in the other soil materials from Clausthal-Zellerfeld CTNT2a and CTNT4a with a higher TNT-contamination.

7.2 Theoretical background

Sites of former ammunition plants are often highly contaminated with explosives and their precursors in the manufacturing process. This contamination is partly the result of the bombardments and the improper dismantling by the allied forces after the war. Mostly,

however, the contamination can be attributed to the production of these plants under war conditions. Hence, the production was far more important than the protection of the workers´ health, often forced labourers, or the environment. Thus, the production waste was improperly discharged, although its toxicity was known. Especially the large amounts of waste water produced during the manufacturing process, called red water due to their colouring by nitroaromates. They were quite often just discharged in nearby rivers or seeped away in the soil.

Due to its being operated under war conditions, safety regulations for the manufacturing were not considered very important and accidents or even explosions were quite common on the plants, often with deadly outcome for some of the workers (BRAEDT et al, 1998). The sites were often located in forests due to camouflage and close to rivers or groundwater reservoirs to supply the plant with the huge amounts of water necessary for the synthesis of explosives. Unfortunately, these areas are today often used as drinking water reservoirs. Thus, the contamination of the former ammunition plants causes a particular threat to the environment (LEVSEN et al, 1993). Even today, more than 56 years after the end of World War II, the contamination at the sites is sill very high, as the environmental conditions are not favourable for the degradation of the explosives (BRADLEY & CHAPELLE, 1995).

7.2.1 “Werk Tanne” Clausthal-Zellerfeld

The ammunition plant “Werk Tanne” at Clausthal-Zellerfeld in Lower Saxony, Germany, was one of the biggest manufacturing sites in Europe during World War II. It was built on an area of 120 ha from 1934-1939 by the "Dynamit Nobel" and from 1939 onwards run by a subsidiary firm, the "Verwertchemie". In 1943/44 it produced 2800 t TNT per month (PREUß & HAAS, 1987). In addition a plant for the reprocessing of old explosives was built since 1942 (BRAEDT et al, 1998: 34).

In October 1944 the plant was partially destroyed by a bombing, which reduced its capacity (BRAEDT et al, 1998: 58 & 61). After World War II it was dismantled and blown up in parts by the British Army. However, in 1977 blastable TNT was found and in the following three years 150 t of explosives were removed (BRAEDT et al, 1998: 102), but even then huge areas remained contaminated with explosives or their precursors and degradation products with a maximum off 362 170 mg TNT/kg soil (dw) (BRAEDT et al, 1998: 111).

In the surrounding area provisions for drinking water had to be closed (OBERHOFER, 1991: 67; BRAEDT et al, 1998: 115; PREUß et al, 1988). This is particularly alarming as the region around Clausthal-Zellerfeld, the Harz, is an important drinking water reservoir for Lower Saxony.

7.2.2 Elsnig near Torgau

The former ammunition plant Elsnig was built in 1934-35 on an area of 6-8 km^2. It was run by the "Wetfälisch-Anhaltinische Sprengstoff AG" (WASAG) and by the army itself (HILDEBRAND, 1992). The monthly capacity was 2600 t TNT, 125 t Hexogen and 200 t Hexyl. In addition 4050 t of explosives per month were filled at the filling station (PREUß & HAAS, 1987) and at an open burning site the solid production wastes were blasted twice a week, contaminating the air and the soil.

Although twelve explosions during the manufacturing are reported between 1939-45 (HILDEBRAND, 1992), the plant remained nearly undamaged until it was dismantled and blasted by the Soviets. However, the soil and the groundwater were contaminated with tons of explosives as the result of these accidents. This is very concerning as the site is in the area of the drinking water reservoir for the Halle/Leipzig area (OBERHOFER, 1991: 71-74).

7.2.3 Hambühren

This soil material was taken from a storage facility for TNT at Hambühren close to Celle (Lower Saxony, Germany). It was remediated in Lägersdorf, which led to the abbreviation LTNT. The site had a maximum TNT-concentration of 20 000 mg TNT/kg soil (dw) (SCHÄFER & BACKSEN, 1998). For the remediation it was excavated, transported and riddled and only the fraction < 20 mm (LTNT1a) was remediated. The TNT-content of the soil material was given with 1700 mg TNT/kg soil (dw), but in a different institute working in the same scientific project 350 mg TNT/kg soil (dw) were determined (PFEIFFER, et al 2001). Hence, the TNT seems to be distributed very heterogeneously in this soil material and the higher TNT-concentration is likely to be the result of a small piece of crystalline TNT and thus the lower TNT-content is more probable.

7.2.4 Stadtallendorf

Stadtallendorf was the largest ammunition plant in Europe during World War II. It was run by the "Verwertchemie" and had a capacity of 5000 t TNT per month. At a filling station 5420 t TNT per month were turned over and delaboration was also carried out on this site (PREUß & HAAS, 1987). From 1938-1945 overall 125 000 t TNT have been produced on an area of about 4 km^2. During the manufacturing and the later dismantling of the plant the area was extensively contaminated with TNT and its precursors, but TNT is with 38% the most common contaminant (GÖRGE et al, 1995).

7.3 Materials and methods

Soil materials from four different contaminated sites were tested. As three soil materials were taken from different areas at the same site, altogether six soil materials were used. The toxicity of the pure soil material was determined in mortality and reproduction tests with *F. candida* and *E. crypticus* (see p. 6, chapter 3.3.1.1). If the toxicity was very high, the LC50(7d)- and EC50(28d)-values were evaluated in a dilution with Lufa 2.2. 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.

The soil material from Elsnig ETNTa was taken from an open-burning site and is thus also contaminated with Hexyl, Hexogen and Octogen apart from TNT. To exclude the possibility of a synergetic effect, the toxicity of each of these explosives was tested in combination with TNT for both test species in the mortality and reproduction test. As concentrations the evaluated LC50- or LC10 and EC50-values were used. From the non-toxic explosives like Hexogen, Octogen or Hexyl in the mortality test with the collembola 2000 mg/kg soil (dw) were applied.

7.3.1 Properties of the soil materials

The soil materials had different properties, which might influence their toxicity. In Table 7.3-1 an overview is given. The soil material from Stadtallendorf STNTa was a mixed sample from two fairly similar areas at the site. Thus, for the soil properties and the contamination the mean of both samples was taken.

7.3.2 Analysis of contaminated soil samples

If field soil materials are used in biotests they have to be analysed for certain contaminants. Most of the tested soil materials were analysed in the joint project "Biological soil remediation" sponsored by the German Ministry of Education and Research (BMBF) and coordinated by the TU Berlin, Institut für Ökologie, Fachbereich Abfallbelastung der Landschaft, grant number 141032. Approximately 100 soil samples were taken from each site, passed through a sieve (Ø 2 cm) and homogenised with a mechanical sample mixer (Riffelteiler from Retsch). A batch of these mixed samples was analysed for heavy metals by atomoic adsorption sectrometer (AAS) after destruction with aqua regia. The nitroaromates were analysed by high-pressure liquid chromatography (HPLC) and a sum paramer for the polycyclic hydrocarbons (PAK) was determined according to the guidelines of the Environmental Protection Agency (EPA) by gas chromatography (GC). The detection limits for heavy metals were in a range of 0.004-0.02 mg/kg soil (dw), for nitroaromates the detection limit was between 0.002-0.020 mg/kg soil (dw) and for PAK between 0.05-0.1 mg/kg soil (dw).

For the analysis of ETNT the C-P-D Umweltschutz Oelzschau GmbH Berlin (formerly FZB Biotechnik GmbH Berlin) was in charge in a different research project of the German Ministry for Education and Research, grant number 14509503.

STNT was analysed by the Biologische Bundesanstalt at Berlin (BBA) in another research project of the German Ministry of Education and Research, grant number 14508581.

The soil materials were not only contaminated with TNT and its degradation products, but also with heavy metals or polycyclic hydrocarbons (PAK). In Table 7.3-1 these additional contaminations are listed if their concentrations would render a further investigation necessary. As threshold levels the concentrations given by the German soil protection law for the intervention values of each soil type were used (BBodenSchG, 1999). A complete list of the contaminants is given in Appendix C.

Table 7.3-1: Soil Parameters and contaminations above the German intervention values for the contaminated soils materials from the four sites

	Clausthal-Zellerfeld			**Elsnig**	**Hambüh-ren**	**Stadtal-lendorf**
	CTNT1a	**CTNT2a**	**CTNT4a**	**ETNTa**	**LTNT1a**	**STNTa**
clay %	18	16	16	0	1	0
silt %	55	42	45	29	3	34
sand %	27	43	39	71	96	66
soil type[1)]	Lu	Slu	Slu	Su3	Ss	Su3
pH	4.5	6.9	6.7	7.1	7.4	6.8
C_{org}%	2.9	5.4	5.6	3.0%	1.2	0.7
contamination [mg/kg soil (dw)]						
TNT	1600	2500	3100	4577	350	14.5
Hexyl	n.t.	n.t.	n.t.	296	n.t.	n.t.
Octogen	n.t.	n.t.	n.t.	482	n.t.	n.t.
Hexogen	n.t.	n.t.	n.t.	56	n.t.	n.t.
Cd		4.9	4.6		[< 2]	
Cu	102	78.5	83	120	36	
Ni		28	27			
Pb	254	990	772	200		
Zn	875	940	747	430	75	
PAK (EPA)	18	13.4	32	22	2.7	n.t

1) short forms for the soil type according to the German system (AG Boden, 1998); the full version is listed in abbreviations

n.t. not tested

[ ] detection limit above interventional value

7.3.3 Evaluation of the toxicity of heavy metals in the contaminated soil materials

Unfortunately, the threshold levels do not exclude a toxic effect of the heavy metals on the test organisms in some of the soil materials. Thus, the EC50(28d)-values were calculated for each soil material by applying a formula given in the Dutch List of Soil Quality Reference Values for

the calculation of reference values in a standardised soil material with 25% clay and 10% organic matter.

Table 7.3-2: Reference values and the soil reference value formulas for some metals (VEGTER, 1995)

metal	reference value in standard soil material	soil reference value formula
Cd	0.8	(Cd) = 0.4 + 0.007 x (clay +3 OM)
Cu	36	(Cu) = 15 + 0.6 x (clay + OM)
Ni	35	(Ni) = 10 + clay
Pb	85	(Pb) = 50 + clay + OM
Zn	140	(Zn) = 50 + 1.5 x (2 clay + OM)

() concentrations in mg/kg on a dry matter basis
clay: weight percentage (dry matter) of clay fraction in soi
OM: weight percentage of organic matter on a dry matter basis of the soil

From these reference values a standardised EC50-value was obtained with the following formula (derived from SMIT & VAN GESTEL, 1998):

$$EC50_{st} = \frac{\text{EC50 x reference value}}{\text{soil reference value formula}}$$

The standardised EC50-values ($EC50_{st}$) were determined either from the EC50(28d)-values found in Lufa 2.2 or from the literature or both. By reversing the formula for the normalisation of the EC50-values, the EC50-values for each soil material could be calculated and compared with the concentration of the metal in the soil material. The calculation and the results are given in Appendix D.2.

Table 7.3-3: Calculated EC50-values for some metals in the tested soil materials

soil material	EC50 in mg/kg soil (dw)							
	Collembola-biotest				Enchytraeid-biotest			
	Cd	**Cu**	**Pb**	**Zn**	**Cd**	**Cu**	**Pb**	**Zn**
CTNT1a	127.3	613.6	2462.5	489.4	5.0	395.0	369.2	475.2
CTNT2a	153.6	676.0	2627.2	508.4	11.9	435.2	393.9	493.6
CTNT4a	144.2	647.4	2551.7	493.6	9.6	416.8	382.6	479.3
ETNTa	102.6	385.7	1861.0	253.5	5.2	248.3	279.0	246.1
LTNT1a	90.9	358.9	1790.3	246.3	2.1	231.0	268.4	239.1
STNTa	85.8	335.1	1727.6	370.2	1.2	215.7	259.0	259.0

7.4 Results

7.4.1 „Werk Tanne“ Clausthal-Zellerfeld

7.4.1.1 CTNT1a

The toxicity for the soil material CTNT1a with 1600 mg TNT/kg soil (dw) was assessed with mortality and reproduction tests. It caused 100% mortality and no reproduction for *F. candida,* but was less toxic to *E. crypticus*. For this test organism the mortality increased to 28.0% and the reproduction rate in the pure soil material was significantly reduced to 10.4%.

Table 7.4-1: Mortality and reproduction rate with CTNT1 for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

Collembola-biotest		Enchytraeid-biotest	
% mortality	% reproduction rate	% mortality	% reproduction rate
100 ± 0.0*	0.0 ± 0.0*	28.0 ± 9.8*	10.4 ± 11.6*

* significant difference to control with Lufa 2.2

To determine the LC50(7d)- and EC50(28d)-values the pure soil material was diluted with the usual standard Lufa 2.2. For the collembola-biotest a LC50(7d) of 601.1 mg TNT/kg soil (dw) and an EC50(28d) of 136.6 mg TNT/kg soil (dw) were determined. In the enchytraeid-biotest it was not possible to evaluate a LC50(7d) and the EC50(7d) was 1042.4 mg TNT/kg soil (dw). For the enchytraeid the zinc-concentration at the EC50-concentration of TNT was 568.8 mg Zn/kg soil (dw) and thus above the calculated threshold for zinc (Zn) with 475.2 mg Zn/kg soil (dw) in this soil material (see Appendix D.2).

A control soil material CTNT0 from the site was available, but even though it was hardly contaminated (Appendix C) it caused a high mortality and no reproduction in the enchytraeid-biotest, probably due to its low pH of 3.8. Hence, Lufa 2.2 was chosen for the dilution.

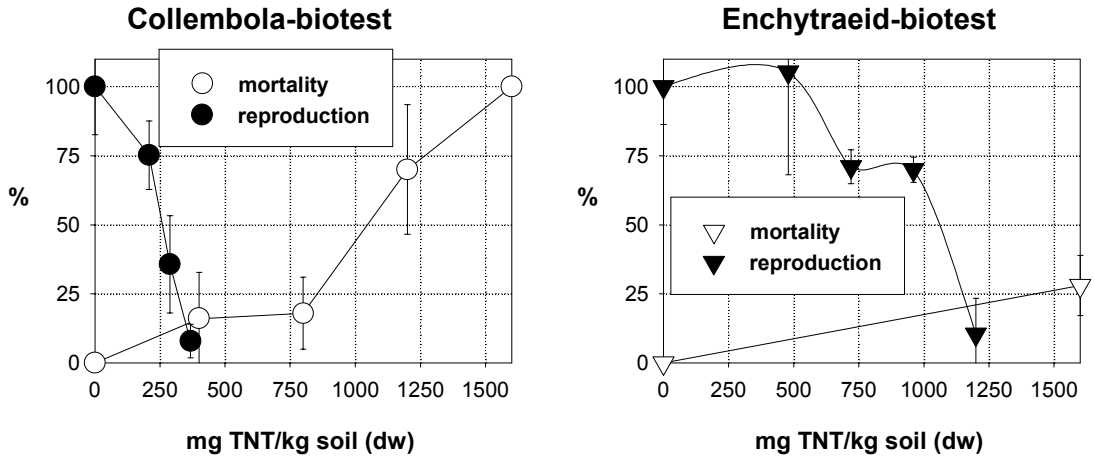

Fig. 7.4-1: Concentration-effect relationship with CTNT1a in dilution with the standard soil material Lufa 2.2 for *F. candida* and *E. crypticus.* Symbols: mean ± SD.

7.4.1.2 CTNT2a

The contaminated soil material CTNT2a with a TNT-content of 2500 mg/kg soil (dw) was evaluated with mortality and reproduction tests. It 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 17.6% ± 6.5 in two different tests.

Table 7.4-2: Mortality and reproduction rate with CTNT2a for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

Collembola-biotest		**Enchytraeid-biotest**	
% mortality	% reproduction rate	**% mortality**	**% reproduction rate**
100 ± 0.0*	0.0 ± 0.0*	2.0 ± 4.0	12.4 ± 1.9*

* significant difference to control with Lufa 2.2

In a dilution of the pure soil material with Lufa 2.2 the LC50(7d) for *F. candida* was determined at 888.2 mg TNT/kg soil (dw) and the EC50(28d) at 186.1 mg TNT/kg soil (dw). For the enchytraeid an EC50(28d)-value of 1780.3 mg TNT/kg soil (dw) was evaluated.

A reference soil material CTNT02a was available for this soil material, which had no negative effect for the collembola nor did it cause any mortality for the enchytraeid, but reduced its reproduction rate to 22.3% ± 2.6. Hence, the reference soil material was not used for dilution of the pure soil material, but the usual reference soil material Lufa 2.2.

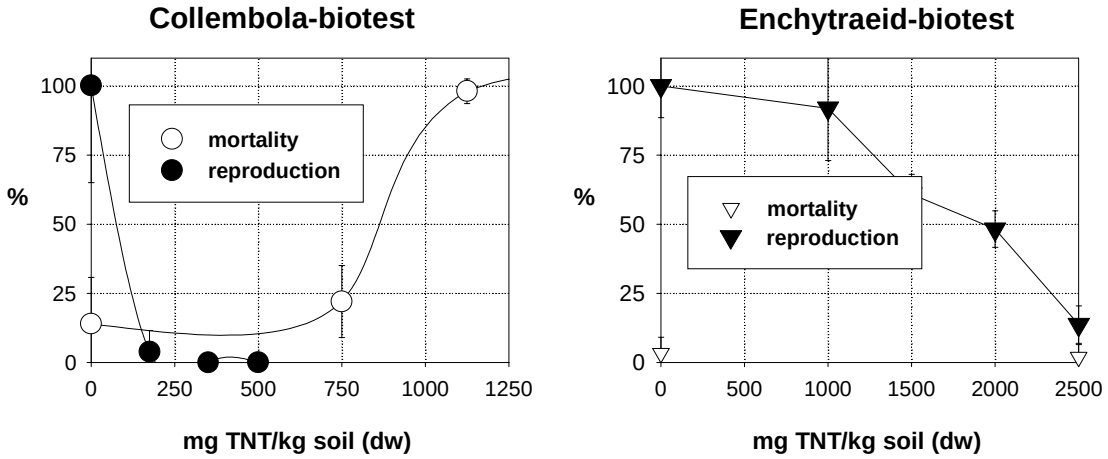

Fig. 7.4-2: Concentration-effect relationship with CTNT2a in dilution with the standard soil material Lufa 2.2 for *F. candida* and *E. crypticus.* Symbols: mean ± SD.

7.4.1.3 CTNT4a

The toxicity of CTNT4a, which was contaminated with 3100 mg TNT/kg soil (dw), was determined with mortality and reproduction tests. It caused 100% mortality and a 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 in two different tests.

Table 7.4-3: Mortality and reproduction rate with CTNT4a for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2*;* mean ± SD, n 5

Collembola-biotest		Enchytraeid-biotest	
% mortality	% reproduction rate	% mortality	% reproduction rate
100 ± 0.0*	0.0 ± 0.0*	12.0 ± 11.7	10.6 ± 12.5*

* significant difference to control with Lufa 2.2

The LC50(7d)- and EC50(28d)-values were determined in dilutions with Lufa 2.2. A reference soil material CTNT04a was available, however, it significantly reduced the reproduction of the enchytraeid. Thus Lufa 2.2 was preferred for the dilutions. In the collembola-biotest the LC50(7d) was 830.7 mg TNT/kg soil (dw) and the EC50(28d) 310.0 mg TNT/kg soil (dw). The EC50(28d) for *E. crypticus* was evaluated at 2541.4 mg TNT/kg soil (dw).

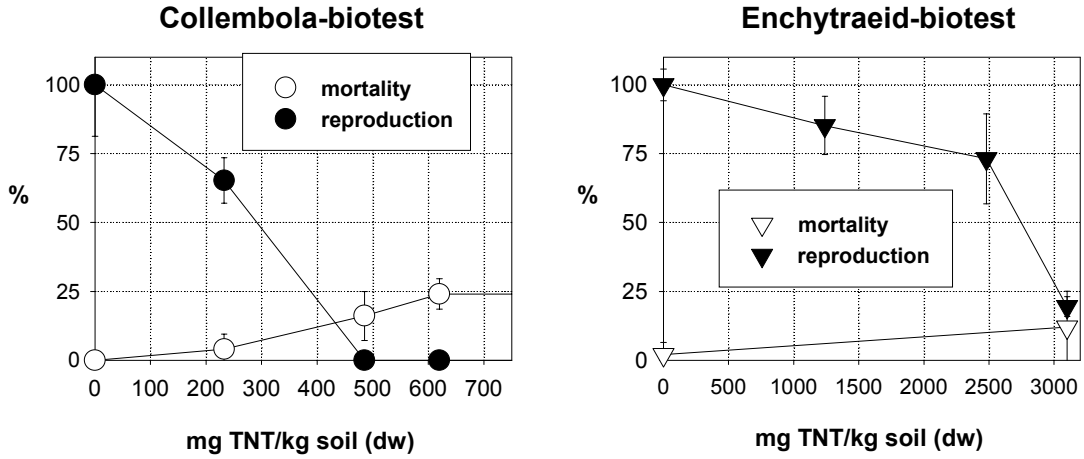

Fig. 7.4-3: Concentration-effect relationship with CTNT4a in dilution with the standard soil material Lufa 2.2 for *F. candida* and *E. crypticus.* Symbols: mean ± SD.

7.4.2 Elsnig near Torgau

The 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). As a synergetic effect of the different explosives could not be excluded the toxicity of Hexyl, Hexogen and Octogen in combination with TNT was tested in mortality and a reproduction tests.

Table 7.4-4: Mortality and reproduction rate of TNT in combination with other explosives for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest		Enchytraeid-biotest	
contaminants	**% mortality[1)]**	**% reproduction rate[2)]**	**% mortality[1)]**	**% reproduction rate[2)]**
TNT	12.0 ± 7.5	31.0 ± 18.0	20.0 ±25.3	48.5 ± 24.3
Hexyl	n.t.	20.8 ± 5.2	68.0 ± 14.7	2.7 ± 0.7
TNT + Hexyl	58.0 ± 20.4*	32.1± 18.1	96.0 ± 8.0*#	32.4 ± 19.2#
TNT + Hexogen	32.0 ± 19.4	20.8 ± 14.2	58.0 ± 22.3	49.4 ± 14.6
TNT + Octogen	26.0 ± 8.0	23.9 ± 19.6	52.5 ± 29.5	65.5 ± 13.2

1) LC10 or 2000 mg/kg soil (dw) of the substances
2) EC50 or 2000 mg/kg soil (dw) of the substances
* significant difference to TNT
\# significant difference to other explosive
n.t. not tested

Hexogen and Octogen showed no significant synergetic effect up to 2000 mg/kg soil (dw) either in the mortality or in the reproduction test for both species. Hexyl had no such effect in the reproduction test with the collembola, but the mortality in the soil material containing TNT and Hexyl differed from the soil material containing only TNT. The mortality of the enchytraeid in the soil material with TNT and Hexyl, too, differed from the soil material containing only TNT as well as from the soil material containing only Hexyl. The reproduction of the soil material with both explosives differed just from the one containing only Hexyl, as the reproduction was very low in this soil material.

In an additional test with various Hexyl-concentrations it was confirmed that the mortality was already significantly increased at 250 mg Hexyl/kg soil (dw) in combination with TNT for the collembola as well as for the enchytraeid. The mortality for the different tested concentrations is given in Table 7.4-5.

Table 7.4-5: Mortality of the LC10(7d) of TNT in combination with different Hexyl-concentrations for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

	Collembola-biotest	Enchytraeid-biotest
contaminants[1) 2)]	**% mortality**	**% mortality**
TNT	26.0 ± 15.0	28 ± 7.5
Hexyl 250	12.0 ± 4.0	2.0 ± 4.0
Hexyl 500	8.0 ± 4.0	2.0 ± 4.0
Hexyl 1000	14.0 ± 4.9	4.0 ± 4.9
Hexyl 2000	14.0 ± 8.0	48.0 ± 21.4
TNT + Hexyl 250	52.0 ± 19.4*	74 ± 32.0#
TNT + Hexyl 500	30.0 ± 11.0*	100 ± 0.0*
TNT + Hexyl 1000	52.0 ± 20.4*	100 ± 0.0*
TNT + Hexyl 2000	62.0 ± 21.4*	100 ± 0.0*

1) LC10 of TNT
2) Hexyl in mg/kg soil (dw)
* significant difference to TNT and corresponding Hexyl-concentration
\# significant difference to corresponding Hexyl-concentration

Thus, for Hexogen and Octogen a synergetic effect in ETNTa can be excluded, as their concentrations in this soil material were below the ones tested. The concentration of Hexyl with 296 mg Hexyl/kg soil (dw) was above the concentration with a synergetic effect for both test species. Hence, an synergetic effect of TNT and Hexyl in ETNTa cannot be excluded for the mortality test in the pure soil material.

The toxicity of the pure soil material was assessed with mortality and reproduction tests. It had a 100% mortal effect on both test species and did also completely reduce the reproduction.

Table 7.4-6: Mortality and reproduction rate with ETNTa for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

Collembola-biotest		Enchytraeid-biotest	
% mortality	% reproduction rate	% mortality	% reproduction rate
100 ± 0.0*	0.0 ± 0.0*	100 ± 0.0*	0.0 ± 0.0*

* significant difference to control with Lufa 2.2

In a dilution with Lufa 2.2 for the collembola a LC50/7d)-value of 199.3 ± 27.3 mg TNT/kg soil (dw), and an EC50(28d)-value of 123.1 mg TNT/kg soil (dw) were evaluated. In the enchytraeid-biotest the LC50(7d) was 685.3 ± 91.9 (3 tests) mg TNT/kg soil (dw), and the EC50(28d) 315.9 mg TNT/kg soil (dw) (2 tests).

At the concentrations of the LC5087d)-value the Hexyl-content was with 13-44mg Hexyl/kg soil (dw) much lower than the concentration with a known synergetic effect. Hence, the synergetic effect of TNT and Hexyl had not to be taken into account.

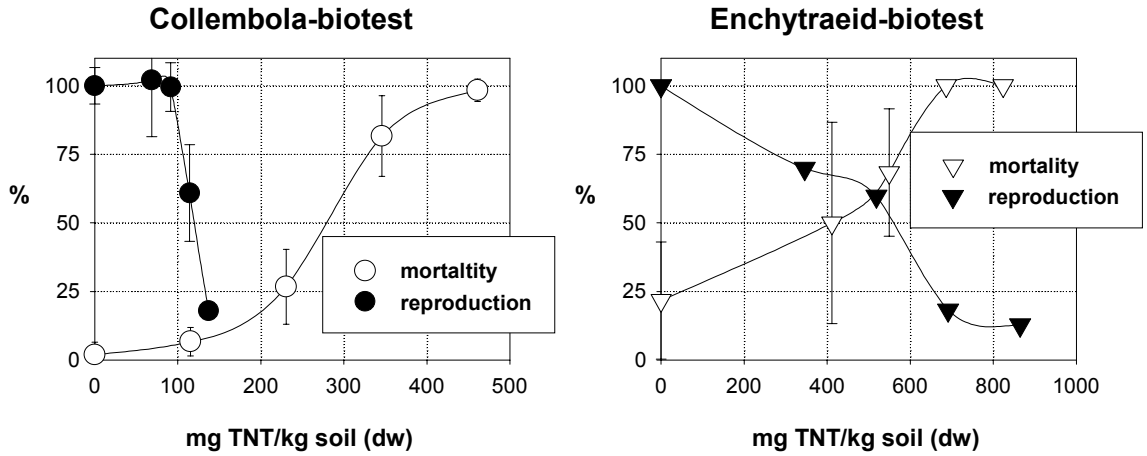

Fig. 7.4-4: Concentration-effect relationship with ETNTa in dilution with the standard soil material Lufa 2.2 for *F. candida* and *E. crypticus*. Symbols: mean ± SD.

The big differences in the standard variation were likely to be the result of a heterogeneous distribution of TNT in the soil material.

7.4.3 Hambühren

The toxicity of this soil material was tested in mortality and reproduction tests after some preliminary treatments, which were necessary for the following remediation (see p. 94, chapter 7.2.3). Then the soil material LTNT1a was contaminated with 350 mg TNT/kg soil (dw). It was 100% mortal for *F. candida* and no reproduction could be detected. For the enchytraeid the mortality increased insignificantly to 18% and the reproduction rate was significantly reduced to 25.5% in the pure soil material.

Table 7.4-7: Mortality and reproduction rate with LTNT1a for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

Collembola-biotest		Enchytraeid-biotest	
% mortality	% reproduction rate	% mortality	% reproduction rate
100 ± 0.0*	0.0 ± 0.0*	18.0 ± 18.3	25.5 ± 10.4*

* significant difference to control with Lufa 2.2

For LTNT1a a LC50(7d) of 145.0 mg TNT/kg soil (dw) and an EC50(28d) of 90.0 mg TNT/kg soil (dw) were evaluated for *F. candida* in a dilution with Lufa 2.2. In the enchytraeid-biotest the EC50(28d) was 272.5 mg TNT/kg soil (dw).

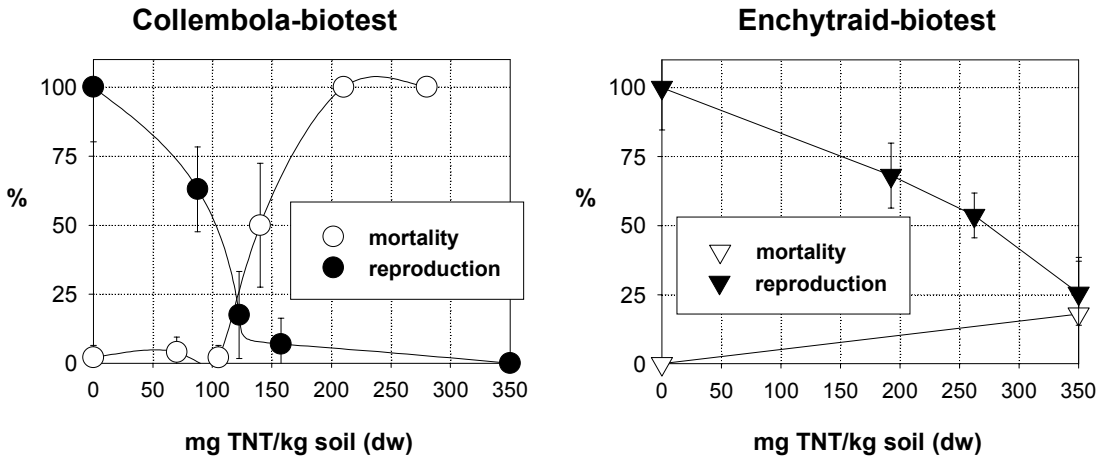

Fig. 7.4-5: Concentration-effect relationship with LTNT1a for *F. candida* and *E. crypticus.* Symbols: mean ± SD.

7.4.4 Stadtallendorf

The toxicity of the low contaminated soil material STNT with 15 mg TNT/kg soil (dw) was evaluated with mortality and reproduction tests. It did not effect the mortality of either test species, but the reproduction rate was significantly reduced to 14.2% ± 7.6 (2 tests) for *F. candida* and to 16.1% ± 5.4 for *E. crypticus*.

Table 7.4-8: Mortality and reproduction rate with STNTa for *F. candida* and *E. crypticus* in relation to control with Lufa 2.2; mean ± SD, n 5

Collembola-biotest		Enchytraeid-biotest	
% mortality	% reproduction rate	% mortality	% reproduction rate
4.0 ± 4.9	14.4 ± 7.6*	2.0 ± 4.0	16.1 ± 5.4*

* significant difference to control with Lufa 2.2

7.4.5 Toxicity of TNT-contaminated soil materials

The toxicity of the tested TNT-contaminated soil materials differed very much. For a better comparison only those are listed for which LC50- or EC50-values have been evaluated.

Table 7.4-9: Toxicity of the TNT-contaminated soil materials in reference to their LC50(7d)- and EC50(28d)-values in dilution with Lufa 2.2

	Collembola-biotest		Enchytraeid-biotest	
	LC50(7d)	EC50(28d)	LC50(7d)	EC50(28d)
soil material	mg TNT/kg soil (dw)		mg TNT/kg soil (dw)	
CTNT1a	601.1	136.6	> 1600	1042.4
CTNT2a	888.2	186.1	> 2500	1780.3
CTNT4a	830.7	310.0	> 3100	2541.4
ETNTa	199.3 ± 27.3	123.1	685.3 ± 91.9	315.9
LTNT1a	145.0	90.0	> 350	272.5

A comparison of the EC50(28d)-values with the ones determined in the standard soil material Lufa 2.2 or the other reference soil materials is not practical, as the other soil materials have been contaminated for years. Thus, sorption of TNT to the soil matrix has already occurred resulting in a reduced bioavailability and hence in a lower toxicity as already described (see p 57-59 chapter **5.2** and p. 72, chapter **6.2.1**).

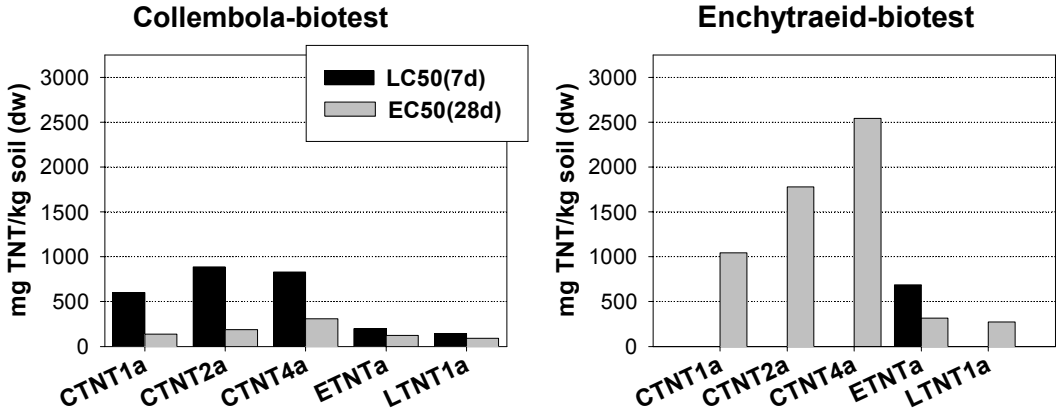

Fig. 7.4-6: LC50(7d)- and EC50(28d)-values for the TNT-contaminated soil materials in dilution with Lufa 2.2 for *F. candida* and *E. crypticus.*

7.4.6 Summary of results

For the evaluation of the toxicity of soils special criteria have been established, as the reproduction varies a lot even in uncontaminated soil materials (see p. 60, Table 5.5-1). With

the collembola-test 20 soil materials were tested, of which four soil materials had a significantly higher reproduction than the standard soil material Lufa 2.2. Six soil materials showed a significantly lower reproduction, for four of them even below 50%.

With *E. crypticus* 17 soil materials were tested, of which one had a significantly higher reproduction and 11 soil materials a significantly lower reproduction than Lufa 2.2. In the artificial standard soil material – the OECD-soil with 70% sand, 20% Kaolin clay and 10% peat – the reproduction of both test systems was significantly reduced (ACHAZI et al, 2001: tables 4-5). Hence, special criteria have been established for the evaluation of soils (ACHAZI et al, 2000).

Table 7.4-10: Toxicity criteria for the evaluation of soils in relation to a control with the reference soil material Lufa 2.2 (ACHAZI et al, 2000)

toxicity	mortality	reproduction rate
non-toxic(--)	no significance	no significance
ecotoxicologically critical (+)	≤ 20% and significant difference to control	≥ 50% and significant difference to control
toxic +	20% - 98%	49% - 1%
very toxic ++	≥ 99%	≤ 1%

For contaminated soils the differentiation between toxic and very toxic is not really necessary. As the reduction of the reproduction is more important in an ecosystem than an increased mortality, the habitat function of a soil material can be judged on the basis of the reproduction test only, if the soil materials are classed differently in the mortality and the reproduction tests.

Table 7.4-11: Classification of the contaminated soil materials according to the guidelines established for contaminated soils

soil material	mg TNT/kg soil (dw)	Collembola-biotest		Enchytraeid-biotest	
		mortality	reproduction	mortality	reproduction
CTNT1a	1600	**+**	**+**	**(+)**	**+**
CTNT2a	2500	**+**	**+**	**--**	**+**
CTNT4a	3100	**+**	**+**	**--**	**+**
ETNTa	4577	**+**	**+**	**+**	**+**
LTNT1a	350	**+**	**+**	**--**	**+**
STNTa	15	**--**	**+**	**--**	**+**

-- non-toxic
(+) ecotoxicologically critical
+ toxic

7.5 Discussion

The toxicity of the soil materials differed very much and cannot be explained with the TNT-content of the soil material alone, as the soil materials STNT and LTNT1a with a relatively low TNT-content were more toxic than the other soil materials with a higher TNT-content.

7.5.1 Toxicity of the soil materials

In the collembola-biotest all contaminated soil materials from the ammunition plant “Werk Tanne” at Clausthal-Zellerfeld had to be classed as toxic (see p. 105, Table 7.4-11). For the enchytraeid, however, they were only toxic in respect to reproduction and non-toxic according to mortality with the exception of CTNT1a which was ecotoxicologically critical. However, for CTNT1a a negative effect of the relative low pH-value of 4.5 in combination with the high concentration of the heavy metals zinc (Zn) and lead (Pb) (see p. 96, Table 7.3-1) on the reproduction and the mortality cannot be excluded (ACHAZI et al, 1996). The original soil material ETNTa from the open-burning site at Elsnig near Torgau was toxic in both test systems. The contaminated soil material from Hambühren LTNT1a was toxic to *F. candida*, but not toxic to the enchytraeid on the basis of the mortality test and toxic in regard to the reproduction test.

The very low contaminated soil material from the ammunition plant at Stadtallendorf STNTa was not toxic in the mortality test for both species, but toxic in the reproduction test. This toxicity was probably not caused by the low TNT-content of 15 mg/kg soil (dw), but might be the result of the soil texture as the soil material becomes very lumpy if adjusted to the water content of 60% of the MWC. As already shown, both animals do not reproduce very much in this kind of soil material (see p. 66). Therefore, this soil material was not further investigated.

As the soil type, the soil texture and probably other unknown factors influence the reproduction of the test species (see p. 66-68, chapter 5.5.1), the comparison with just one standard soil material is not feasible. Unfortunately, the reference soil materials provided for these tests could not be used as they affected at least one of the test species in one of the tests. Hence, it would be useful to establish a whole set of reference soil materials belonging to different soil types. The results of a contaminated soil material could then be compared with the reference soil material of the same soil type. In addition, it might be necessary to use a different water content than 60% of the WHC, as this was evaluated as best for Lufa 2.2 and OECD, but might not be suitable for other soil materials (see p. 67).

In general, the mortality test was sufficient for a quick determination of toxicity. If a soil material was toxic in the mortality test, it was also so in the reproduction test. However, if a soil material was not toxic on the basis of the mortality test a reproduction test was necessary to ensure that the soil material was really not toxic (ACHAZI et al, 2001). Altogether six soil materials have been tested. On the basis of the reproduction test, as the more sensitive parameter, all six soil materials had to be classed as toxic either for the collembola and the enchytraeid.

7.5.2 Comparison of the toxicity of contaminated soil materials

A comparison of the toxicity of the contaminated soil materials can only be based on their LC50(7d)- and EC50(28d)-values for TNT in dilution with Lufa 2.2 (see p. 104, Table 7.4-9), as the TNT-contents of the soil materials differed very much. Unfortunately, these values could not be evaluated if the effect on the mortality or the reproduction rate in the pure soil materials was less than 50%, which was the case for the mortality of *E. crypticus* in CTNT2a, CTNT4a and LTNT1a. As the toxicity of the soil material from Stadtallendorf STNTa was more likely to be caused by the soil texture, this soil material is not considered further.

For the collembola-biotest the order for the mortality and the reproduction test is:

LC50: LTNT1a > ETNTa > CTNT1a > CTNT4a > CTNT2a

EC50: LTNT1a > ETNTa > CTNT1a > CTNT2a > CTNT4a

The lowest contaminated soil material LTNT1a was the most toxic one on the basis of the LC50(7d)- and EC50(28d)-values. This sandy soil material had the lowest content of organic carbon with 1.2% and also a very low clay content with 1%. Hence, the soil material had not many sorption sides for TNT.

The highest contaminated soil material ETNTa with 4577 mg TNT/kg soil (dw) was less toxic than LTNT1a. The content of organic matter was with 3% much higher than in LTNT1a and should explain its lower toxicity, although the clay content was even lower with 0%. For this soil material it can be assumed that all sorption sides for TNT were occupied due to the high TNT-content. Thus the soil material had to be diluted very much for the determination of the 50%-effect. They were achieved in dilutions with Lufa 2.2 containing only 4.3% of the contaminated soil material in the mortality test and 2.7% in the reproduction test.

The difference to the next toxic soil material CTNT1a is very large with a factor of 3.0 in the mortality test, but less remarkable with 1.1 in the reproduction test. This difference can be attributed to the increase in the clay content, as the content of organic matter was about the same (see p. 96, Table 7.3-1). The clay minerals are also possible sorbents for TNT, thus reducing its bioavailabilty.

The differences between the toxicity of the other soil materials are much less striking. The factors varied between 1.1 and 1.4 for the LC50-values and between 1.4. and 1.7 for the EC(50)-values. The increase in the organic matter content in the soil materials from “Werk Tanne” from CTNT1a to CTNT4a and CTNT2a might explain the relatively low toxicity of the later two (see p. 96, Table 7.3-1).

Although, all soil materials from “Werk Tanne” were also contaminated with heavy metals, especially Pb and Zn, for the collembola no toxic concentration for any of them could be derived from the calculation of threshold values (see Appendix D.2). However, CTNT1a had a rather

low pH of 4.5 which could induce a higher bioavailability of the heavy metals in this soil material and thus a higher toxicity in comparison to the other soil materials from ”Werk Tanne”. This could also be the case for CTNT2a with a pH of 6.9 as this soil material was more toxic in the reproduction test than CTNT4a with the higher pH of 6.7. The clay-content on the other hand was the same and the content of organic carbon was even higher in CTNT2a. However, the effect of the heavy metals and the pH can only be estimated and no clear assessment can be given, in particular not in combination with TNT.

For the enchytraeid-biotest the LC50(7d) could only be evaluated for the highest contaminated soil material from Elsnig, as the mortality in all other soil materials was below 50%. Therefore a comparison on the basis of the LC50(7d)-values was not possible. The order of toxicity for the EC50(28d)-values in the enchytraeid-biotest is:

EC50: LTNT1a > ETNTa > CTNT1a > CTNT2a > CTNT4a

Again LTNT1a, the soil material with the lowest content of organic carbon, was the most toxic one, followed by the highest contaminated soil material ETNTa. This soil material had to be diluted to 15.0% and 6.9% of its original content in the mortality and reproduction test, respectively, for the evaluation of the LC50(7d)- and the EC50(28d)-values.

As for the mortality test with the collembola the gap between ETNTa and CTNT1a is very big with a factor of 3.3. Again this might be an effect of the increased clay content, as the C_{org} was about the same (see p. 96, Table 7.3-1). The slightly lesser toxicity of the other soil materials from “Werk Tanne” CTNT2a and CTNT4a can also be considered as the result of the higher organic matter content (see p. 96, Table 7.3-1). In addition, the concentration for Zn at the EC50-value for TNT was in CTNT1a above the calculated threshold value for Zn in this soil material (Appendix D.2). In combination with the low pH of CTNT1a the Zn-content may enhance the toxicity in comparison to the other soil materials. The higher toxicity of CTNT2a in comparison to CTNT4a might again explain the lower pH of this soil material.

7.5.3 Comparison with literature

A very similar investigation was performed with *F. candida* for three different soil materials from the ammunition plant Hallschlag, (Rhineland-Palatine, Germany) (KRATZ & RIESBECK, 1998). The chronic toxicity was tested according to the draft of the ISO-Guideline 11268-2 and for the control Lufa 2.2 was used. No reproduction was found in the soil material H1 with the highest TNT-concentration of 6800 mg TNT/kg soil (dw) and 2000 mg DNT/kg soil (dw), since the soil material was also completely lethal. In H2 with 320 mg TNT/kg soil (dw) the reproduction rate was reduced to 96% of the control in Lufa 2.2 and in H3 with 0.23 mg TNT/kg soil (dw) to 77%, but neither reduction differed significantly from the control. The authors were not sure whether the reduction in reproduction should be attributed to an inhomogeneous distribution of the

contaminants in the soil material or to the variability of the toxicological endpoint. On the basis of the tests performed with *F. candida* in the course of this thesis the latter is more likely and the variability of the toxicity due to the soil type and the soil texture has to be considered, too (see p. 66-68, chapter 5.5.1). Hence H1 has to be considered as very toxic, whereas the other soil materials are non-toxic according to the guidelines for the assessment of soils (see p. 105, Table 7.4-10).

The US Army performed a very thorough study at Joliet Army Ammunition Plant (JAAP) (Illinois, USA) (PHILIPPS et al, 1994). Various samples from six different sites were tested for their phytotoxicity with cucumber (*Cucumis vulgaris*) and radish (*Raphamus sativus*) as well as for their toxicity to the earthworm *E. fetida.* The water extracts were investigated for their toxicity to the luminescent marine bacterium *Photobacterium phosphoreum* with a Microtox assay.

In the phytotoxicty tests the plant heights, the survival rates and the emergence rates were determined. As toxicity endpoints in the earthworm tests survival rates and the change in biomass were investigated, whereas for the bacterium the effective concentration at which the light output was reduced by 50% was evaluated.

Soil materials were classified as highly toxic if the survival rates were < 30% and the growth reduction was significant for the earthworm and phytotoxicity tests or if the EC50 was < 30% in the Microtox assay. In moderate toxic soil materials the survival rates for the earthworms or the plants were 30-70% or the growth reduction was significant, whereas for the Microtox assay the EC50 had to be 30-70%. For a classification as non-toxic the survival rates had to be > 70% and the growth reduction had to be not significant and for the bacterium the EC50 had to be > 70%.

At a site, on which lead azide explosives had been produced and on a demilitarisation area, no TNT at all was detected, but the soil materials were toxic. Also some soil materials of the other sites were toxic, although no TNT could be determined. Thus, the presence of other toxic contaminations cannot be excluded at this production plant. For the TNT-contaminated soil material a correlation of the toxicity and the TNT-content was observed (PHILIPPS et al, 1994). In the table below (Table 7.5-1) only the soil materials with a TNT-contamination are listed.

Table 7.5-1: Toxicity of TNT-contaminated soil materials form various sites at JAAP (modified from PHILIPPS et al, 1994)

site	TNT in mg/kg soil (dw)	*C. vulgaris*	*R. sativus*	*P. phosphoreum*	*E. fetida*
burning ground for waste explosives	10	--	--	++	--
	91	++	++	++	++
	218	++	++	++	++
burning ground on the load-and-pack side	1990	++	++	++	++
	519	++	++	++	++
	4594	++	++	++	++
	4518	++	++	++	++
	1435	++	++	++	++
	355	++	++	++	++
	31	++	++	++	++
	2417	++	++	++	++
	6025	++	++	++	++
	266	++	++	++	++
	19	--	--	+	--
	2655	++	++	++	++
	1158	++	++	++	++
	7487	++	++	++	++
load-and-pack operation side	655	++	++	++	++
	4207	++	++	++	++
	6.5	++	+	++	+
	1066	++	++	++	++
	7114	++	++	++	++
	15	++	+	--	--
	9123	++	++	++	++
	2092	++	++	++	++
	10679	++	++	++	++
	87087	++	++	++	++
TNT ditch complex	10138	++	++	++	++
	694	++	++	++	++

-- not significantly toxic
\+ moderate toxic
++ highly toxic

In general, the classification was very homogenous for the soil materials with a contamination of more than 20 mg TNT/kg soil (dw). Below this value one or more tests varied from the others, but this could also be the effect of other contaminations present in the soil material.

In another study at the same ammunition plant the toxicity of soil samples in relation to their content of TNT, Hexogen (RDX), Octogen (HMX) and degradation products of TNT was investigated (SIMINI et al, 1995). For *E. fetida* again the mortality and change in biomass were evaluated as toxicity endpoints. The concentration of TNT was correlated with these endpoints. Hexogen (RDX), Octogen (HMX) and degradation products of TNT did attribute little to the toxicity.

The same correlations were given for other tests such as the Microtox assay with *P. phosphoreum* and the phytotoxicity tests with cucumber (*C. vulgaris*), radish (*R. sativus*) for germination and seedling. In general, it was proven that biotests can be used for the risk assessment of soil materials from contaminated sites (SIMINI et al, 1995).

In the project "Biological processes for soil remediation – ecotoxicological test batteries", which was sponsored by the German ministry of Education and Research (BMBF) from 1996-1999 grant number 1491032, various contaminated soil materials have been evaluated (MICHELS et al, 2001). In Table 7.5-2 an overview of the different tests with the TNT-contaminated soil materials from this project is given.

The soil materials CTNT1a, CTNT2a, CTNT4a and LTNT1a have already been described, as the evaluation of these soil materials with the collembola- and enchytraeid-biotests was performed as a part of this project during the course of this thesis. CTNT3a originated from the same ammunition plant and is described in the next chapter (see p. 121, Table 8.3-1). The soil materials HTNT1 and HTNT2 were taken from the ammunition plant Hallschlag (Rhineland-Palatine, Germany) and contained 13 400 and 12 800 mg TNT/kg soil (dw). All tested soil materials had to be classed as toxic for the applied criteria for toxicity given below the table.

In an international ring test with *F. candida*, *E. crypticus* and *E. fetida* for the evaluation of these test systems for the assessment of soil materials, another soil material from Clausthal-Zellerfeld was tested. This soil material, usually referred to as Clausthal (CT), contained only 259.1 mg TNT/kg soil (dw), but the concentrations of the heavy metals Cd, Cu, Pb and Zn were above the intervention values given by the German soil protection law (for further details see Appendix C).

For the collembola this loamy soil material was neither toxic in the mortality nor in the reproduction test. The mortality of the enchytraeid was not affected, too, but the reproduction rate was reduced with a 67% correspondence between the laboratories. In case of the earthworm neither the mortality nor the change in biomass was affected, but the reproduction rate was reduced with a 88% correspondence. This effect on the reproduction of the two annelids is likely to be the result of the heavy metals, in particular Pb with 820.8 mg/kg soil (dw) and Zn with 764 mg/kg soil (dw).

Table 7.5-2: Evaluation of the tested soil materials with other biotests; the results with the collembola and enchytraeid-biotest are given as a comparison and indicated by shading

		CTNT1a	CTNT2a	CTNT3a	CTNT4a	LTNT1a	HTNT1	HTNT2
phytotoxicity	*L. sativa*[1]	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	B. rapa[2]	+	n.t.	n.t.	+	n.t.	n.t.	n.t.
	S. saubspicatus[3]	+	n.t.	n.t.	+	+	+	+
microbiology	Q_R[4]	n.t.	n.t.	+	+	+	n.t.	n.t.
	nitrification[5]	+	+	+	+	n.t.	n.t.	n.t.
	C. inflata[6]	+	+	n.t.	+	+	n.t.	n.t.
genotoxicity	Ames-test[7]	+	n.t.	n.t.	+	+	+	+
	Umu-test[8]	+	n.t.	n.t.	+	+	+	+
water risk	*V. fischeri*[9]	+	n.t.	n.t.	+	+	+	+
	D. magna[10]	+	n.t.	n.t.	+	+	+	+
terrestrial invertebrates	*E. fetida*[11]	+	n.t.	n.t.	+	n.t.	n.t.	n.t.
	F. candida[12]	+	+	n.t.	+	+	n.t.	n.t.
	E. crypticus[12]	+	+	n.t.	+	+	n.t.	n.t.

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

1) fresh weight of soil mixture with Lufa 2.2 as control soil is more than 10% lower than the calculated value (RIEPERT et al, 2000)
2) 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)
3) $G_A > 4$ (G_A dilution in which the inhibition of growth is < 20%) (PFEIFFER et al, 2001)
4) respirative activation quotient (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 growth and hatching of encysted cells > 30% (PAULI et al, 2001)
7) $G_{EA} > 3$ with *S typhimurium* Ta98 or TA100 (G_{EA} dilution in which difference in induction < 80 (TA 100) or < 20 (TA 98)) (PFEIFFER et al, 2001)
8) $G_{EU} > 1.5$ with *S typhimurium* TA1535 or pSK1002 (G_{EU} dilution in which induction > 1.5) (PFEIFFEr et al, 2001)
9) $G_L > 8$ (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%) (PFEIFFER et al, 2001)
10) $G_D > 4$ (G_D dilution in which 9 out of 10 daphnia are still mobile) (PFEIFFER et al, 2001)
11) Reproduction rate < 50% (HUND-RINKE, 2001)
12) Reproduction rate < 50% (see p. 105, Table 7.4-10)