5 Toxicity of TNT in different reference soil materials

5.1 Abstract

By sorption the bioavailability of a pollutant and hence its toxicity is decreased. Thus, the soil matrix has to be considered in order to evaluate the toxicity of a compound in soil. In the case of TNT the organic matter as well as the clay minerals can act as sorbents. Therefore, the toxicity of TNT was determined for four different soil materials. The three reference soil materials from the LUFA Speyer Lufa 2.3, Lufa 3 and Lufa 4 differed from the usual standard soil material Lufa 2.2 in their clay and organic matter content. The commercially available potting soil was characterised by a very high organic matter content. TNT was the least toxic in this soil material for both test species in regard to mortality and reproduction. On the other hand in Lufa 2.3 with the lowest content of organic carbon TNT was the most toxic. The three other soil materials differed mainly in their clay content. The toxicity of TNT decreased with an increase of the clay content in the soil materials. Thus in Lufa 2.2 TNT was more toxic than in Lufa 3 and finally Lufa 4.

5.2 Theoretical background

The fate and impact of chemicals in the environment is affected by their ability to become associated with solid phases by sorption. Thereby the behaviour of the chemical changes depending on whether it is dissolved in water or clinging onto the exterior of solids or being buried in the soil matrix. In addition, the rate of biodegradation of a substance is influenced, since the dissolved form is more easily taken up by microorganisms and hence more easily available (SCHWARZENBACH et al, 1993: 255).

However, sorption is not a simple single process, but a combination of various interactions of the particular chemical (sorbate) with any particular solid (sorbent) (SCHWARZENBACH et al, 1993: 258) such as ionic or covalent binding, Van der Waals attractions, hydrogen bonding, charge transfer or hydrophobic bonding (BOLLAG & LOLL, 1983). Which one dominates in the end depends on the structural properties of the sorbate and the sorbent (SCHWARZENBACH et al, 1993: 258). In the case of soils the organic matter and the clay minerals can act as sorbents.

5.2.1 Organic matter

The organic matter or humus is a complex and rather resistant mixture of brown or dark brown amorphous, colloidal substances synthesised from the tissues of various organisms (BOLLAG & LOLL, 1983). It is divided in three fractions according to their solubility in acids or dilute alkali: humic acid, fulvic acid and humin. These are large aromatic polymers, made up of nitrogen heterocycles, quinones, phenols and benzoic acids. Hence, they contain functional groups like carboxyl-, hydroxyl-, carbonyl- and thiol-groups, which may act as binding sites. On the other hand they also have aliphatic moieties, some of which are hydrophobic (BOLLAG & LOLL, 1983). Hence, polar as well as unpolar compounds may interact with the humus.

5.2.2 Clay

The clay minerals are phyllosilicates (KUNTZE et al, 1994), crystalline aluminium-magnesium-ion-silicates. They have functional groups like the hydroxyl groups of silicon (SiOH), aluminium (AlOH, $AlOH_2$), ion (FeOH, $FeOH_2$) or carbon (COH), which act as Lewis-acids and can thus exchange ions depending on the pH-value. As a result of the very common isomorph exchange of Si^{4+} against Al^{3+}, clay minerals usually have a permanent negative charge, too. Therefore they can act as a cation exchanger with a given cation-exchange-capacity.

In soil clay minerals are the main component of the clay fraction, the finest soil fraction with grain sizes below 2 µm (SCHEFFER & SCHACHTSCHABEL, 1998: 137). In contrast TNT does not adsorb easily to sand, which is indicated by the high hydraulic conductivity for TNT in sand, which is with > 10^{-5} m/s more than a factor of three faster than in clay with < 10^{-8} m/s (HILDENBRAND, 1995).

5.2.3 Sorption to organic matter and clay

Since humus as well as clay minerals may interact with substances, the two sorbates compete for the binding of xenobiotics. However, if the organic matter content surpasses 6% the competition is reduced, as the humus saturates the exchange sites of the clay (BOLLAG & LOLL, 1984).

5.2.4 Sorption of TNT

For uncharged organic substances with a low aqueous solubility (< 10^{-3} M) and no susceptibility to specification changes or complex formation, the sorption can mainly be attributed to hydrophobic interaction and is thus controlled by the organic carbon fraction of the soil (KARICKHOFF, 1981). If the ratio of mineral to organic carbon is < 30 the mineral contribution to sorption is usually masked, regardless of the mineral content (KARICKHOFF, 1984). For these substances a correlation between the grade of sorption and the hydrophobicity given by the octanol-water-coefficient can be drawn (HADERLEIN et al, 1996).

This is not the case for the slightly polar explosive TNT, for which the sorption is more correlated with the cation exchange capacity, extractable ion and the clay content (PENNINGTON & PATRICK, 1990). As a result of the electron-withdrawing nitro-groups the aromatic ring of TNT is electron deficient (SPAIN, 1995: 2) and hence a possible sorbent to negative charged sites. Thus, TNT was found to adsorb easily to clay minerals by forming electron donor-acceptor

(EDTA) complexes. The surfaces of the clay minerals serve thereby as $e^{\ominus}$-donors and the nitroaromatic compound as an $e^{\ominus}$-acceptor (HADERLEIN et al, 1996; WEISSMAHR et al, 1997; WEISSMAHR et al, 1998). As these surfaces are easily accessible no slow diffusion into micropores is necessary and the sorption occurs within minutes (HADERLEIN et al 1993).

The specific interaction of TNT with the clay minerals is prevented by strongly hydrated cations such as protons (H^+), potassium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), aluminium (Al^{3+}) or ammonium (NH_4^+). Thus, the mobility of TNT can be controlled by the concentration of weakly hydrated ions such as K^+ or NH_4^+ (HADERLEIN et al, 1996). For TNT and other uncharged nitroaromatic carbons the mobility and thus the sorption is not pH-dependent between pH 3 and 9 (HADERLEIN et al, 1996), but not very resistant to desorption. Hence, the soil sorption to clays will not effectively prevent mobility through surface soils in the solution phase (PENNINGTON & PATRICK, 1990). Increased sorption in time is usually attributed to the formation of transformation products, which are incorporated in the organic matter (HUNDAL et al, 1997).

5.3 Materials and methods

In order to compare the toxicity of TNT four different uncontaminated reference soil materials were chosen. To investigate the influence of clay and of organic matter three soil materials with a varying content of clay and of organic matter were selected. As a representative of a soil material with a very high organic matter content a commercially available potting soil was chosen. Only TNT was tested as a contaminant, since it is the most common explosive on contaminated sites and the most toxic one of those tested in this thesis.

The toxicity of TNT in the different soil material was assessed with the mortality and the reproduction tests (see p. 6, chapter 3.3.1.1). No choice tests were performed as it cannot be excluded that the test organisms are attracted or repelled by different soil parameters.

5.3.1 Reference soil materials

Four soil materials distributed by the Lufa Speyer and one commercially available potting soil were used. No information was available for the potting soil purchased from "Pluta", apart from that it was a peat product according to DIN 11540-F20 with all the necessary nutrition for plants.

All Lufa soil materials were taken from a depth of 20 cm and are thus part of the A-horizon. Lufa 2.3 was a silty sand and the used batch F2.340000 was taken from a site in Offenbach a. d. Quick (Rhineland-Palatinate, Germany) in “Bildgarten 508”. Lufa 3 and 4 are no longer distributed by the Lufa. Lufa 3 was a loamy sand, whose batch RB 32300 was taken from a site at Altlußheim (Rhineland-Palatinate, Germany) in “Kleine Pfraum 6063”. Lufa 4 was a sandy loam and the used batch LB42300 was taken from Römerberg-Mechtersheim (Rhineland-Palatinate, Germany) in “In der Speyerer Hohl 977”.

These soil materials varied in their clay content and in the content of total organic carbon. Table 5.3-1 gives an overview of the chemical and physical properties of the tested soil materials in comparison to the usual standard soil material Lufa 2.2. The complete list of the soil properties is given in appendix B.

Table 5.3-1: Comparison of chemical and physical properties of the different reference soil materials with the usual standard soil material Lufa 2.2 indicated by shading

properties	**Lufa 2.2**	**Lufa 2.3**	**Lufa 3**	**Lufa 4**	**pot soil**
clay (Ø < 0.002mm)	6.7%[1)]	7.4%[1)]	9.6%[1)]	24.4%[1)]	n.d.
silt (Ø 0.002 - 0.063 mm)	15.4%[1)]	29.0%[1)]	35.2%[1)]	37.0%[1)]	n.d.
sand (Ø 0.063 – 2.0 mm)	78.1%[1)]	63.7%[1)]	55.2%[1)]	38.8%[1)]	n.d.
soil type[3)]	loamy sand[1)] Sl2	silty loam[1)] Su	silty-loamy sand[1)] Slu	sandy loam[1)] Ls2	n.d.
pH	5.8[2)]	6.5[2)]	7.1[2)]	7.4[2)]	6.5[2)]
%C_{org}	2.2[1)]	0.7[1)]	1.1[1)]	1.6[1)]	23[2)]
ratio mineral - C_{org}	3.0	3.1	8.7	15.3	
MWC per 100 g soil (dw)	50.0[2)]	36.0[2)]	40.4[2)]	62.1[2)]	366.4[2)]

1) determined by the Lufa according to DIN
2) determined by the institute of Ökotoxikologie und Biochemie of the FU Berlin
3) according to the German soil classification system (AG BODEN, 1994); for full forms see abrevialtions
n.d. not determined

5.3.1.1 pH-value

The pH-values of all soil materials were neutral to slightly acidic. For *F. candida* it was shown that the mortality was not affected by a variation of the pH in the range of 3.4 to 6.9. The reproduction, however, was significantly reduced in more acidic soil materials with a pH of 4.3 (ACHAZI et al 2001: 89-90). In another study with artificial OECD-soil (70% sand, 20% Kaolin clay and 10% sphagnum peat), however, the reproduction was affected in soil materials with a higher pH, but not significantly (CROUAU et al, 1999).

For the enchytraeid a pH-optimum was determined between 5.3 and 6.8. Outside this range the reproduction was reduced and the mortality increased in particular below pH 4.7 (ACHAZI et al, 1996). In artificial OECD-soil neither the mortality nor the reproduction were affected in the range of pH 4.0 to 7.0 (RÖMBKE et al 2000: 117).

Hence, for the reference soil materials the pH should not affect either test species.

5.3.1.2 Grain size

Considering the grain size the amount of sand in the Lufa soil materials decreased in the order Lufa 2.2, Lufa 2.3, Lufa 3 to Lufa 4, whereas the amount of clay increased in the same order. The grain sizes have not been analysed for the potting soil and cannot be compared.

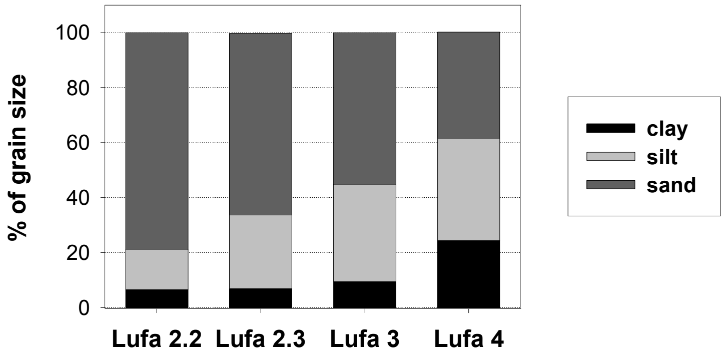


Fig. 5.3-1: Distribution of grain size in the Lufa soil materials.

5.3.1.3 Organic carbon: C_{org}

The C_{org} represents the content of carbon in the organic matter (DUNGER & FIEDLER, 1997: 54). It was with 23% outstandingly high in the potting soil, which consisted mainly of peat. For the Lufa soil materials the content of organic carbon was much lower, with Lufa 2.3 having the lowest (0.7%) and Lufa 2.2 the highest (2.2%). The two other soil materials were in between with 1.1% for Lufa 3 and 1.6% for Lufa 4.

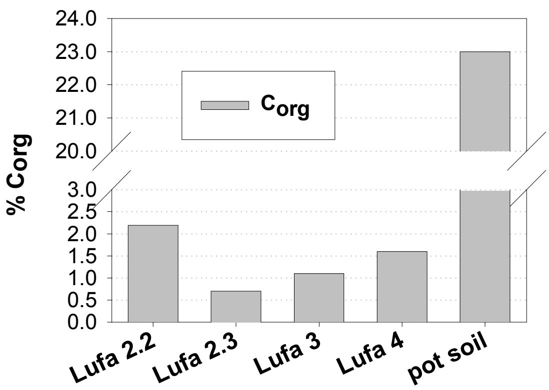


Fig. 5.3-2: Content of organic carbon in the reference soil materials.

5.3.2 Treatment of soil materials and tests

Before the soil materials were used in the tests they were defaunated by freezing them twice at -18°C with an intermediate phase at room temperature to allow the hatching of cocoons, eggs or survival stages. From the potting soil only 5 g soil (fw) were used in the mortality test and 15 g soil (fw) in the reproduction test, since this soil material was very voluminous.

5.3.3 Validity of tests and treatment of results

The mortality in the uncontaminated soil material was not in all soil materials below 20% and the tests therefore not valid. However, if a repetition of the test showed the same high mortality, these tests were considered as valid, assuming that the higher mortality might be caused by the structure of the soil material itself.

For a comparison with the usual standard soil material Lufa 2.2 the means of the mortality and the reproduction rate for Lufa 2.2 were calculated from the controls of all the tests performed during the course of this thesis. These values were used for a statistical evaluation. They were compared with the mortality or the reproduction rate in the reference soil materials without TNT in a t-test (see p. 9, chapter 3.3.3).

For a comparison of the toxicities of TNT in the different reference soils the LC50(7d)- and EC50(28d)-values were calculated via a progression analysis according to FINNEY (1971). If the mortality was high in a test this might lead to an apparent contradiction with the concentration-effect relationship, as for the calculation the mortality of the control was taken into account.

In order to determine whether the organic carbon or clay content was correlated with the 50%-values a linear regression for each, as well as for the ratio of mineral to organic carbon was performed. The correlation coefficient r^2 evaluates the relationship: the closer it is to 1, the stronger is the linear relationship.

5.4 Results

5.4.1 Comparison of the uncontaminated reference soil materials

No control with the usual standard soil material Lufa 2.2 was performed in parallel to the reference soil materials. Thus, the means of the mortality and the reproduction rate for the usual standard soil material Lufa 2.2 were calculated from the controls of all the tests performed during the course of this thesis in order to compare them with the other reference soil materials.

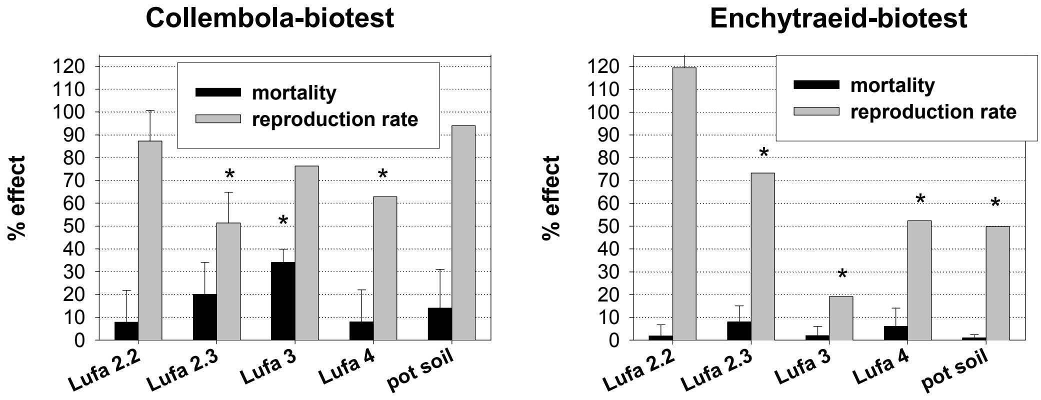
Table 5.4-1: Comparison of the mortality and the reproduction rate in uncontaminated reference soil materials with the usual standard soil material Lufa 2.2 indicated by shading

reference soil material	Collembola-biotest		Enchytraeid-biotest	
	mortality [%]	reproduction rate [juveniles/adult]	mortality [%]	reproduction rate [juveniles/adult]
Lufa 2.2	7.7 ± 14.0	87.3 ± 25.3	1.8 ± 4.9	119.4 ± 27.7
Lufa 2.3	20 ± 14.0	51.3 ± 13.4*	8.0 ± 7.0	73.2*
Lufa 3	34 ± 5.7*	76.2	2.0 ± 4.0	19.2*
Lufa 4	8.0 ± 12.0	62.8*	6.0 ± 8.0	52.4*
potting soil	14.0 ± 17.0	93.9	1.0 ± 1.4	49.9*

* significant difference to usual standard soil material Lufa 2.2

For *F. candida* the mortality was significantly higher in Lufa 3, but also in Lufa 2.3 it was much higher than in the usual standard soil material Lufa 2.2. The reproduction rates were significantly lower in Lufa 2.3 and in Lufa 4 in comparison to Lufa 2.2.

In the enchytraeid-test the mortality was in none of the reference soils significantly higher than in Lufa 2.2. The reproduction rates, however, were in all reference soil materials significantly lower than in Lufa 2.2. The difference was most marked in Lufa 3.



* significant difference to usual standard soil material Lufa 2.2

Fig. 5.4-1: Mortality and reproduction rate in different reference soil materials for *F. candida* and *E. crypticus*.

5.4.2 Lufa 2.3

The toxicity of TNT in the silty loam Lufa 2.3 was determined in mortality and reproduction tests. For the collembola-test the LC50(7d) was determined at 104.5 mg TNT/kg soil (dw). The EC50(28d) evaluated in two tests was much lower with 23.5 ± 4.7 mg TNT/kg soil (dw). For the enchytraeid TNT was much less toxic than for the collembola with an LC50(7d) of 640.5 mg TNT/kg soil (dw) and the much lower EC50(28d)-value of 277.0 mg TNT/kg soil (dw).

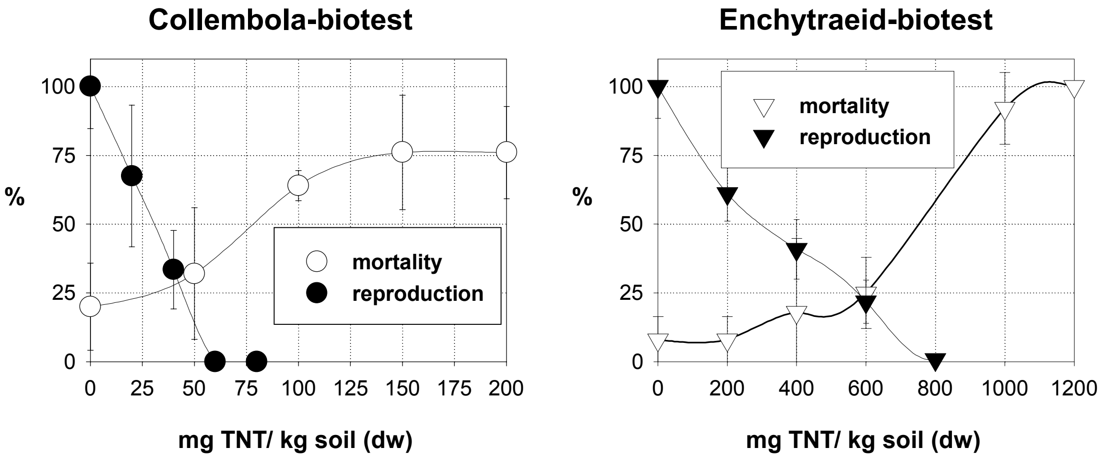


Fig. 5.4-2: Concentration-effect relationship for TNT in the reference soil material Lufa 2.3 with *F. candida* and *E. crypticus.* Symbols: mean ± SD.

5.4.3 Lufa 3

With mortality and reproduction tests the toxicity of TNT was assessed in the silty-loamy sand Lufa 3. The mortality of *F. candida* was twice evaluated due to the high mortality in the control, yielding to a LC50(7d) of 453.7 ± 17.2 mg TNT/kg soil (dw) (two tests). The value for the EC50(28d) was with 40.6 mg TNT/kg soil (dw) much lower than the LC50(7d). For the enchytraeid a LC50(7d) of 1374.8 and an EC50(28d) of 623.9 mg TNT/kg soil (dw) were evaluated.

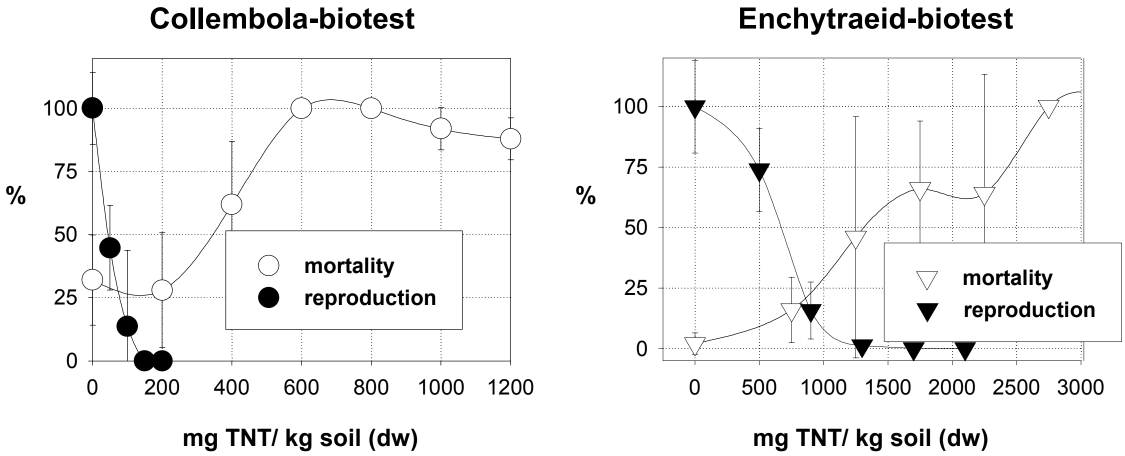


Fig. 5.4-3: Concentration-effect relationship for TNT in the reference soil material Lufa 3 with *F. candida* and *E. crypticus*. Symbols: mean ± SD.

5.4.4 Lufa 4

In the sandy loam Lufa 4 the toxicity of TNT was determined with mortality and reproduction tests. For the collembola a LC50(7d) of 415.8 mg TNT/kg soil (dw) was determined and the EC50(28d) was 171.1 mg TNT/kg soil (dw). The corresponding values for the enchytraeid were 1099.0 mg TNT/kg soil (dw) for the LC50(7d) and 918.8 mg TNT/kg soil (dw) for the EC50(28d).

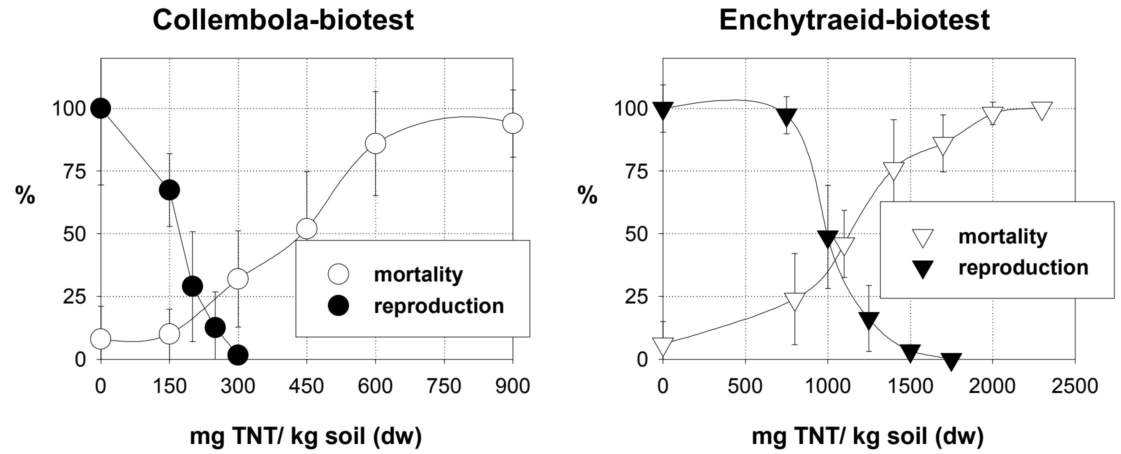


Fig. 5.4-4: Concentration-effect relationship for TNT in the reference soil material Lufa 4 with *F. candida* and *E. crypticus*. Symbols: mean ± SD.

5.4.5 Potting soil

The toxicity of TNT in the commercially available potting soil was assessed with mortality and reproduction tests. The LC50- and EC50-values were very high for both test species in this soil material with a very high content of organic carbon. For *F. candida* the values were 1571.7 mg TNT/kg soil (dw) for the LC50(7d) and 619.7 for the EC50(28d). For *E. crypticus* the LC50(7d) was 5843.3 mg TNT/kg soil (dw) and the EC50(28d) 3111.1 mg TNT/kg soil (dw).

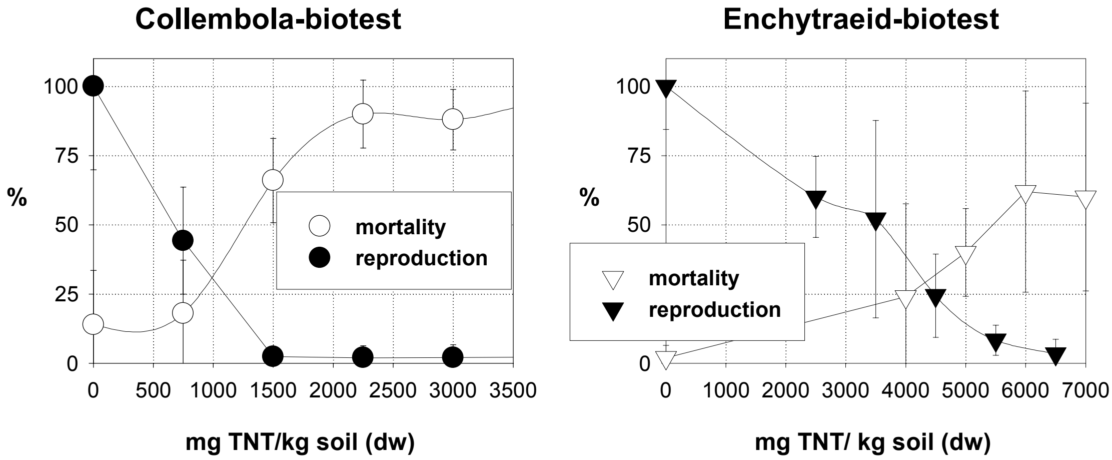


Fig. 5.4-5: Concentration-effect relationship for TNT in the potting soil with *F. candida* and *E. crypticus.* Symbols: mean ± SD.

5.4.6 Comparison of the toxicity of TNT in different reference soil materials

The toxicity of a compound in different soil materials can best be compared by looking at the LC50(7d)- and EC50(28d)-values.

Table 5.4-2: LC50(7d)- and EC50(28d)-values for TNT in different reference soil materials for *F. candida* and *E. crypticus* in comparison to the usually used standard soil material Lufa 2.2 indicated by shading

	Collembola-biotest		Enchytraeid-biotest	
Reference soil material	**LC 50 (7d) in mg/kg soil (dw)**	**EC 50 (28d) in mg/kg soil (dw)**	**LC 50 (7d) in mg/kg soil (dw)**	**EC 50 (28d) in mg/kg soil (dw)**
Lufa 2.2	139.9 ± 8,2	64.3 ± 20.0	949.9 ± 617.8	501.2 ± 227.2
Lufa 2.3	104.5	23.5 ± 4.7	640.5	277.0
Lufa 3	453.7 ± 17.2	40.6	1374,8	623.9
Lufa 4	415.8	171.1	1099.0	918.8
Potting soil	1571.1	619.7	5843.3	3111.1

The toxicity of TNT varied very much between different soil materials. The order was for both test species the same, but different in the mortality and the reproduction test:

Mortality test: Lufa 2.3 < Lufa 2.2 < Lufa 4 < Lufa 3 < Potting soil

Reproduction test: Lufa 2.3 < Lufa 2.2 < Lufa 3 < Lufa 4 < Potting soil

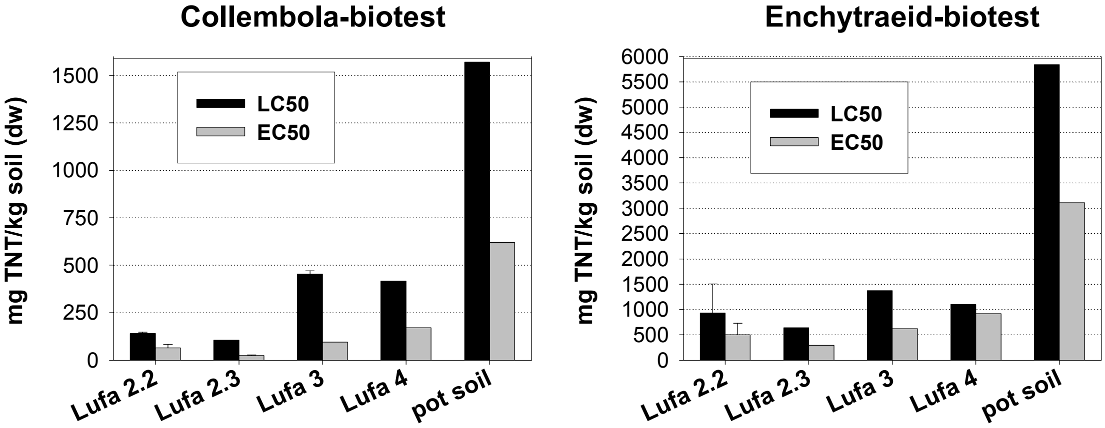


Fig. 5.4-6: LC50(7d)- and EC50(28d)-values in the different reference soil materials with *F. candida* and *E. crypticus.*

5.5 Discussion

5.5.1 Comparison of uncontaminated reference soil materials

Since the mortality for *F. candida* was very high in the two tests with the loamy sand Lufa 3 (see p. 62, Table 5.4-1), this effect cannot be attributed to a lack in fitness of the animals. In addition, Lufa 3 was the only soil material to cause a significant increase of the mortality for the collembola in comparison to the usual standard soil material Lufa 2.2. Hence, it has to be assumed that the soil material itself induces the mortality. It was observed that the soil material became very lumpy when water was added to adjust the water content to 60% of the MWC. As the lump of soil did not cover the whole bottom of the test vessels the animals might have fallen on the glass floor when introduced into the test vessel and not on the soil material. Thereby they might have been damaged. In addition the lump of soil material might have squashed the animals, if the test vessel was shaken. Even at the end of the test the animals were still visible on the surface of the soil lump, since they were unable to find any holes to get into the soil material.

The high mortality in Lufa 2.3 for the collembola could also have been caused by the lumpiness of the soil material. However the tendency to become lumpy was not so marked in Lufa 2.3 as in Lufa 3. Hence, the mortality was not so strongly affected.

The reproduction of the collembola was reduced in all Lufa soil materials in comparison to Lufa 2.2, but only significantly in Lufa 2.3 and Lufa 4. For the soil materials Lufa 2.3 and Lufa 3 this can partly be explained by the tendency of these soil materials to become lumpy, yielding to a higher mortality. In addition, the collembola cannot find holes in the lumpy soil to deposit their eggs, which is not favourable for oviposition. However, in Lufa 3, in which this tendency to become lumpy was much more marked, the reproduction rate was not significantly lower than in Lufa 2.2. Hence, this cannot be the only factor influencing the reproduction. This is supported by the significantly lower reproduction rate in Lufa 4, which showed no tendency to become lumpy. However, it is not clear what this factor or these factors might be. In the potting soil on the other hand, the reproduction was even higher than in Lufa 2.2.

In all enchytraeid-biotests the mortality was not significantly different from the usual standard soil material Lufa 2.2 (see p. 62, Table 5.4-1). The reproduction on the other hand was significantly lower to Lufa 2.2 in all reference soil materials. For Lufa 2.3 and Lufa 3 this might again be caused by the tendency of the soil materials to become lumpy. Thus, the animals had to remain on the surface of the lump, which is unfavourable for the laying of cocoons. This is most clearly the case for Lufa 3 with the lowest reproduction rate. The low reproduction rate of Lufa 4 however, cannot be explained by this effect, indicating that other unknown factors might influence the reproduction rate, too.

In the case of the potting soil the low reproduction rate of 49.9 juveniles/per adult might be the result of a certain amount of dryness, as the WHC of the potting soil was enormous with 366.4 g H_20/100g soil (dw) (see p. 60, Table 5.3-1). Peat absorbs water very quickly and the soil material seemed to be dry even after it had been adjusted to 60% of the WHC. Thus, there might not have been enough soil pore water for the enchytraeid to feel comfortable in the soil material at 60% WHC and a higher WHC might be more suitable for this test species in this soil material.

The tendency of certain soil materials to become lumpy might also be reduced if the soil materials are not adjusted to 60% of the WHC, but to a lower water content. The water content of 60% of the WHC has been determined as best for the standard soil material Lufa 2.2 and the OECD-soil, an artificial soil substrate. Hence, a different water content might be much more suitable for clayey or peaty soils, which remains to be examined. In addition, the determination of the WHC is not very precise, even according to the ISO-guidelines. Thus, the time a soil material needs to become completely saturated with water is likely to depend on the soil texture and cannot be strictly fixed to one hour for all soil materials. The definition of the sand bath, too is very vague and thus a possible source of variations.

ACHAZI et al (2001) tested various uncontaminated soil materials in the reproduction test with *F. candida* and *E. crypticus*. In some of these soil materials the reproduction of one of the test organisms was significantly reduced in comparison to the reproduction in the control with Lufa 2.2. No correlation for the reduced reproduction could be found neither with the content of organic carbon, nor the clay content or the pH or any other soil property. In Table 5.5-1 the soil properties of the tested soil materials in which the reproduction of both tests species differed significantly from the control in Lufa 2.2 are given. The reproduction is given as the percentage of the one in the control with Lufa 2.2.

Table 5.5-1: Soil properties of different uncontaminated soil materials and mean of the reproduction rate in percent in relation to the usual standard soil material Lufa 2.2 for *F. candida* and *E. crypticus* (ACHAZI et al, 2001)

soil material	origin	% sand	% silt	% clay	soil type[1)]	pH	C_{org}	% reproduction rate *F. candida*	% reproduction rate *E. crypticus*
Lufa 2.2	Hahnhofen (G)	78.	15	7	Sl2	5.8	2.2	100	100
Berge	Berge (G)	65	25	11	Sl3	6.7	1.5	114.2	26.3
Blumberg	Blumberg (D)	55	37	8	Su3	5.6	0.7	139.7	29.9
Euro6	Normandy (F)	2	82	16	Ut3	7.3	0.3	117.2	2.5
Euro4	Normandy (F)	4	76	10	Ut4	7.1	1.6	6.6	52.6
Euro1	Sicily (I)	3	22	75	Tt	5.4	1.3	102.1	4.5
OECD	Artificial soil				St	6.0	5	31.8	68.0

1) according to the German classification system (AG BODEN, 1994); the full terms are given in abbreviations

The reproduction of the collembola and the enchytraeid was affected very differently, indicating that it is useful to perform both tests to evaluate the habitat function of a soil material. The tests also showed that a negative effect of a certain soil material on the test organisms cannot be excluded, although no contaminants could be detected by chemical analysis.

5.5.2 Comparison of the toxicity of TNT in the reference soil materials

As in the usual standard soil material Lufa 2.2, the collembola was more sensitive for TNT than the enchytraeid and the reproduction proved to be the the more sensitive test parameter than the mortality. The differences between the LC50(7d) and the EC50(28d), however, were in all soil materials higher than in Lufa 2.2. (see p. 65, Table 5.4-2). Especially for Lufa 3 and Lufa 2.3 in the collembola-biotest with the factor 11.2 and 3.9 compared to a factor of 2.2 in Lufa 2.2. Hence, Lufa 2.3 and Lufa 3 became more toxic with a longer test period, were more toxic for juveniles or were not favourable for reproduction. This might again be attributed to the tendency of the soil materials to become lumpy, as the adults do not find wholes in the lump of soil material, in which they usually lay their eggs. Hence, the reproduction was reduced in these soils. The two other soil materials were in the range of Lufa 2.2. with the factors 2.4 for Lufa 4 and 2.5 for the potting soil.

In the enchytraeid-biotest, too, Lufa 2.3 and Lufa 3 were the soil materials with the highest difference between mortality and reproduction with the factors 2.2 and 2.3. Again the tendency to become lumpy offers a possible explanation. In Lufa 2.2 the difference was 1.6 and thus in-between Lufa 4 (1.2) and the potting soil (1.9).

The order of toxicity differed in the mortality and the reproduction test, as TNT was more toxic in the mortality test with Lufa 4 than in the one with Lufa 3, but vice versa in the reproduction test. The difference between the two soil materials, however, was not very high in the mortality tests with a factor of 1.1 for the collembola and 1.3 for the enchytraeid. Thus the two soil materials can be considered as having the same toxicity for TNT in the mortality test. In the reproduction test the difference was higher with a factor of 2.4 for the collembola and 1.4 for the enchytraeid. Thus the order of toxicity has to be reconsidered:

Mortality test: Lufa 2.3 < Lufa 2.2 < Lufa 4 / Lufa 3 < Potting soil

Reproduction test: Lufa 2.3 < Lufa 2.2 < Lufa 3 < Lufa 4 < Potting soil

TNT was the least toxic in the potting soil for both test species in all tests, indicating that TNT was less bioavailable in this soil material. As the main difference between this soil material and the Lufa soil materials was the organic carbon content (see p. 60, Table 5.3-1), the reduction of the TNT-toxicity has to be attributed to the high content of organic matter. TNT adsorbs very easily and very strongly to the organic matter, thus making it less available for the organisms.

The toxicity of Lufa 2.2, the soil material with the second highest content of organic carbon, was higher than in Lufa 3 and 4 with a lower content. However, these two soil materials had a higher portion of clay (see p. 60, Table 5.3-1), which can also act as a sorbent for TNT. Especially Lufa 4 had a much higher clay portion than the other soil materials and also a slightly higher content of organic carbon than Lufa, resulting in a much higher ratio mineral C_{org} than in the other Lufa soil materials. In the reproduction tests Lufa 4 was the one with the second lowest toxicity of TNT after the potting soil. Hence, it must be assumed that the high content of clay reduced the bioavailability of TNT and thus its toxicity, although the sorption to clay is usually masked if the ratio of mineral to organic carbon is < 30, as it is the case for all the Lufa soil materials.

TNT was most toxic in Lufa 2.3, a soil material which was characterised by its very low content of organic carbon with 0.7%. Although the clay content of this soil material was higher than in Lufa 2.2 (see p. 60,Table 5.3-1), it was more toxic for both test organisms. The ratio mineral-C_{org} was about the same with 3.0 for Lufa 2.3 and 3.1 for Lufa 2.2. Hence, its high toxicity has to be contributed to its low content of organic carbon.

The toxicity of the soil materials decreased with an increasing content of organic matter or clay, but no linear correlation could be found, neither for the organic matter nor the clay content or the ratio of clay to organic matter. For all three factors the values for the regression coefficient r^2 were below 0.9.

In some of the soil materials the variances were very high. For *F. candida* especially in Lufa 3 (see p. 64, Fig. 5.4-3), which is probably the result of an inhomogeneous distribution of the toxicant. As the soil material tended to become lumpy, the mixing of the toxicant with the uncontaminated soil material might not have been so thoroughly. For *E. crypticus* the variations were very high in the potting soil and in Lufa 3. In Lufa 3 this can again be attributed to the mixing of the toxicant. In the potting soil there is also the possibility that some dryer areas existed, as peat absorbs the water very quickly and dryness is very harmful for the enchytraeid.

Another possibility for a comparison of the toxicity of a toxicant in different soils is by comparison with a reference value for a standardised soil with 10% organic matter and 25% clay, a method described in the Dutch List of Soil Quality Reference Values. In the case of organic compounds only the organic matter content (OM) is considered as necessary (VEGTER, 1995: 92). Since so far no reference value has been established for TNT, this method cannot be applied here. In addition the effect of the clay content on the toxicity of TNT would be completely neglected (see p. 58, chapter 5.2.2).