ECOTOXICOLOGICAL TRIALS WITH BAK 1095 (BAYER AG, LEVERKUSEN) ACCORDING TO DIN V 54900

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1. ABSTRACT

Within the frame of examinations with BAK 1095 (small-scale test according to DIN V 54900 part 3 and "controlled composting test " DIN V 54900-Part 2, No. 3), the following working hypothesis as postulated by J. Fritz (1999) in his PhD-Thesis was tested: the "negative ecotoxicological impact " of the biodegradable material BAK 1095, produced by Bayer AG, mainly bases on the emission of heavily toxic NH_3. Fritz used the "controlled composting test" for the production of his compost and reached reproducibly negative results in bioassays (inter alia Daphnia, algae (Scenedesmus sp., Chlorella sp.) and cress) with compost containing 15 to 17 weight % of BAK - polymers.

As a result of the applied method "controlled composting test", especially when using base materials with high nitrogen contents, NH_3 and immature compost develop. According to Fritz, the compost with BAK showed considerably higher contents of total N, strongly increased pH-values and a higher elutable proportion of organic matter when compared to the control, as well as extremely high NH_4-N-contents (55 to 78 times higher than the control without BAK). The working hypothesis could be backed up by our examinations, in pilot-scale tests (PlanCoTec) as well as under the conditions of the controlled composting test (OWS).

In both tests, extremely high NH_4-N contents were recorded in early stages of the composting of BAK 1095 as compared to the control without BAK addition. During the pilot scale composting NH_4-N values were reduced by 99 %. In the controlled composting test, however, the NH_4-values did not decrease. Thus, toxic impacts measures in bioassays with sensitive testing organisms (cress test for gaseous phytotoxic substances, daphnia) took a parallel course to the ammonium contents. However, these impacts occurred independent from the addition of BAK 1095, when NH_4-N levels were artificially raised to the same level as in treatments with BAKs by the addition of ammonium to the control treatments. Tests with test plant barley which is less sensitive against NH_4-N/ NH_3 showed no negative effects with the same compost. Therefore, it has to be assumed that the results as found by Fritz are mainly, if most probably not entirely caused by a pure NH_3-effect. An additional cause is the chosen testing method.

2. INTRODUCTION

In contrast to the field of chemicals, standards for ecotoxicological examination of compost is mostly still in the stage of development. Attempts made in this context to transfer standard testing methods for ecotoxicity used in the field of chemicals to the field of composting proved to be far more difficult than initially assumed. Experts are aware of the problem and therefore, for example, only the barley test for plant phytotoxicity according to RAL GZ 251 (BGK, 1998) has been included in DIN V 54900 as an approved testing method. It is applied for treatments with a proportion of 10 % of the biodegradable polymers to be tested. Other testing procedures with daphnia, algae, the earthworm test as well as the cress test have shown methodical weaknesses. They are not yet sufficiently adapted or in principal not suitable for the assessment of toxic impacts of biodegradable materials in the compost matrix. Frequently, the direct impacts of the compost to be tested are already lethal for a number of organisms (e.g. due to the commonly high salt content of biocompost). Further work in this area is needed until a satisfactory solution is found.

Fritz (199) carried out tests with biodegradable materials to develop the methodology. During the course of these tests, he observed massive negative impacts of composts amended with BAK-polymers produced by Bayer AG on the testing organisms cress, *Daphnia magna* and algae (*Scenedesmus sp., Chlorella sp*.). He subsequently postulated a "negative ecological impact", especially of BAK 1095, and recommended to rate this biodegradable material as "not suitable for composting" until more data are available.

Beyond the postulated general "ecotoxicological impact" of the respective compost, however, a cause-and-effect chain was not proved experimentally (Fritz 1999).

Ecotoxicological tests carried out by PlanCoTec with BAK 1095 and 2195 according to DIN V 54900, using the plant phytotoxicity test according to RAL-GZ 251 (BGK, 1998) did not shown any negative impacts of BAK amended compost in the time period between 1996 and 1998.

After screening the results obtained by Fritz (1999), PlanCoTec came to the following conclusions:

- The negative impacts of compost with BAK-polymers on the testing organisms as found by Fritz (1999) are understandable and reproducible under the given methods protocol. The chemical analysis data, however, strongly indicated that the test results were caused by toxic effects of NH_3 which was emitted from the BAK amended compost. NH_3-development due to the ammonium content and pH was probably influenced by the chosen method of "laboratory composting" (controlled composting test) and the "immature" compost obtained contained BAK-polymers which due to its chemical structure had a high nitrogen content.

The task for PlanCoTec/ OWS now was to prove the working hypothesis on the "NH_3-effect" of the "immature" BAK amended composts. The following measures were taken:

- Experimental testing of the ecotoxicological effects of an addition of 10 mass percent BAK 1095 by weight to a compost produced under laboratory conditions (controlled composting test according part 2 of DIN V 54900) and under standard conditions according to the test on compostability at a pilot scale level (part 3 of DIN 54900). In this testing procedure, the impacts of the compost were assessed using a standard test with regard to plant compatibility (barley test) and to gaseous phytotoxic effects as well as its impact on daphnia during the composting periods.

3. MATERIALS AND METHODS

3.1 composting

3.1.1 Pilot scale Composting

The set-up of the experiment was carried out according to the guidelines of DIN V 54900. The behaviour of defined biowaste-compost with an addition of 10 % by weight (BAK —treatment; 90% powder < 500µ m, 10 % semicircle testing bodies with a 2 mm layer thickness, base area 25 cm^2) were compared with a treatment without BAK 1095 (control). Samples were taken on three occasions (after 5, 10 and 15 weeks composting time) in order to determine the dynamics of the ammonium content and its impact on the test organisms. Additionally, ammonium in the form of ammonium sulphate was added in two increasing steps to the compost samples each time the samples were taken, which were then compared to untreated samples in the ecotoxicity tests (barley test, cress test on gaseous phytotoxic substances, daphnia (OWS)). The aim was to demonstrate the impact of NH_3/NH_4-N on the testing organisms by artificially increasing the ammonium content, independent from an addition of BAK 1095. Treatments with additional ammonium fertilisation are described as F0, F1, F2 or together with the other treatments, for instance, as C1 F0 (Control treatment, fertilisation level 0) and B1 F0 (BAK treatment, fertilisation level 0), respectively.

The ammonium content of both treatments was determined using separately implemented reference samples (5 l with 10 % BAK 1095, powder and bodies), which were removed two days before the sampling date. These data from these samples served as a base for the adjustment of the two-step increase of the ammonium content in the test samples. As a gradation scheme, the contents of the samples were adjusted according to values in Table 1. The actual values are listed in Table 5.

Composting time	after 5 weeks			after 10 weeks			after 15 weeks		
Ammonia fertilisation level	F0*	F1	F2	F0*	F1	F2	F0*	F1	F2
	mg NH_4-N/l compost								
Control	238,46	400,00	900,00	6,34	40,00	150,00	1,28	3,18	12,72
BAK	405,21	900,00	1500,00	40,46	150,00	300,00	3,02	12,72	25,44

Table 1: Test set-up for ammonium addition to samples taken after 5, 10 and 15 weeks of composting in the pilot scale composting test (F = Fertilisation level)

3.1.2 Lab-scale composting - Controlled composting test

The controlled composting biodegradation test was run according to ISO 14855, "Evaluation of the Ultimate Aerobic Biodegradability and Disintegration of Plastics under Controlled Composting Conditions" which is identical to test 3 in part 2 of the DIN V 54900 and CEN method prEN WI 261 085. In principal it is an optimised simulation of an intensive aerobic composting process where the biodegradability of a test item under dry aerobic conditions is determined. The inoculum consists of stabilised and mature compost derived from the organic fraction of municipal solid waste. The test item (BAK 1095 particle size 500 µm) was mixed with the inoculum and introduced into static reactor vessels where it was intensively composted under standard oxygen, temperature (58 °C) and moisture conditions (Table 2). The carbon dioxide production was continuously monitored and integrated to determine the carbon dioxide production rate and the cumulative carbon dioxide production. After determining the carbon content of the test item, the percentage of biodegradation was calculated as the percentage of solid carbon of the test item, which had been converted to gaseous, mineral C under the form of CO_2. Also the kinetics of the biodegradation could be established.

6 * blank/inoculum :	1500 g compost
3 * BAK 1095 :	1500 g compost + 100 g BAK 1095 + 50 g water
3 * blank + carbon source	1500 g compost + 60 g straw, 30 g wood shavings and 10 g of dextrose
3 * BAK 1095 + C	1500 g compost + 60 g straw, 30 g wood shavings and 10 g of dextrose + 100 g BAK 1095 + 50 g water

Table 2: Test set up for the controlled composting test

3.2 ANALYTICS

3.2.1 Ecotoxicity tests

Plant compatibility test with barley (BGK, 1998)

25 and 50 vol. % of the test compost, respectively, were mixed with standard soil 0 (EE0 = Einheitserde) and cultured with summer barley in three repetitions for 12 days under controlled climatic conditions. The test parameter was the fresh weight of barley in the test treatments compared to EE0 as the reference soil. If the fresh mass yield reaches 90 % of the yield of EE0, the compost is rated as plant compatible. In this case, the three samples from the control and the BAK treatment from the small-scale test were tested in a threefold repetition as original material and in both ammonium increase steps each time the samples were taken.

Test on gaseous phytotoxic effects with cress (BGK, 1998)

Cress seed were exposed to gases from test compost in a preserving jar. The seeds were made to germinate on a cotton wool swab fixed 3 cm above the test compost in the preserving jar. The jars were closed apart from a 1mm air slot. After seven days culturing time, the fresh mass of cress was compared to samples with EE0. If samples from the test compost reach 80 % of the fresh mass of EE0, the test had been passed. In this case, compost from both treatments were tested according to the division described above and in Table 1. In addition to the parameter fresh mass and root length of the cress were determined.

Daphnia test

The Daphnia, acute toxicity test was according to the OECD Guidelines for Testing of Chemicals # 202 "Daphnia sp., Acute Immobilization Test and Reproduction Test". It was applied after the preceding composting tests (controlled composting test and pilot scale test). The compost produced at the end of the test could contain residuals of the original test item such as metabolites, undegraded components and inorganic components. The purpose of the Daphnia acute toxicity test was to evaluate any toxic effect of the test compost containing the test item residuals in comparison to the control compost to which no test substance was added at the start of the preceding biodegradation. The toxicity of the test compost is expressed as the percentage mortality of the organisms in the test compost compared to the control compost.

3.2.2 Chemical Analysis

The analysis on water content, carbon content or organic matter, total N, C/N ratio, NO_3-N, NH_4-N, pH, composting degree as well as the differentiation of the composting balance were carried out according to the Book of Methods of the German Federal Association for Compost Quality (BGK, 1998). As not stated otherwise contents of NO_3-N and NH_4-N are given in mg per kg wet weight of compost.

3.2.3 Extinction

In order to determine dissolved organic carbon (DOC) the extinction of eluate samples (1/5: 1 part compost in 5 part of distilled water) derived from the different treatments of the controlled composting test was determined. The eluate was centrifuged before use. The extinction was determined at 485 nm.

3.3 Results and Discussion

Composting was conducted in both test runs according to the validity criteria of DIN V 54900 (PlanCoTec, 2000 a, 2000 b, OWS, 2000).

3.3.1 Degradation Rate of BAK 1095

In the controlled composting test the net CO_2 production and the biodegradation percentage after 60 days of incubation were 2027 mg CO_2 (net production)*g^{-1} test item 89,3 % for BAK 1095, respectively. During the first weeks of the composting process a strong ammonia smell was noticed in the reactors containing the BAK material, both in the 1095 and in the series without or with an extra carbon source.
In the case of the pilot scale test, the observed degradation behaviour of BAK 1095 showed high correspondence to the results of the earlier DIN-tests under pilot scale conditions or under field conditions. The material was characterised by a very fast, 100 % degradation / disintegration during the 15-weeks composting time.

3.3.2 Nutrient Dynamics

Within the frame of the examinations, it could be shown that, especially concerning nitrogen dynamics and the development of NH_4-N when BAK 1095 was added, a high dependence existed with regard to the chosen composting method, carbon availability, C/N ratio as well as the composting time (point in time).

The proportion of ammonium - N, for example, was significantly higher than the control values during the course of the controlled composting test of the BAK treatment (Table 3). Even after a testing period of 60 days, which included a 15-days "maturing period" with ambient temperatures, the ammonium content of the material could not be reduced and subsequently additional nitrate did not develop as a feature for completed composting. The extinction as a feature for high proportions of "water-soluble" organic matter showed in the same direction. The pH value in all treatments was at least 0,5 units higher than in the control. That means that conditions for a high release of ammonia were given in this laboratory composting method during the entire testing phase. Treatments with an addition of an extra C-source behaved, apart from small level differences, similarly.

The analysis of total N, NH_4-N and NO_3-N, respectively, for the compost during the small-scale test, however, showed the typical characteristics of normal behaviour of the nitrogen turnover during composting (Table 4). The impact of BAK 1095 was already reflected in the total nitrogen content. The values of the BAK - treatment always remained above the values for control. During composting, values constantly decreased 20 and 22 %, respectively, between sampling dates in the 5th and 15th week for both control and the BAK - treatment. Such nitrogen losses are commonly observed in practice and occur as gaseous losses under the precondition that nitrogen is not lost via the leachate. Thus, a strong smell of ammonia was often observed in the thermophilic phase of composting. The ammonium content showed a dynamic behaviour similar to total nitrogen content. For example, ammonium contents of the BAK - treatment decreased 95 % between the 5th and the 15th week and thus showed a close correspondence to the values of control with a decrease of 99 % within the same time period. However, after 5 weeks of composting control values were found to have reached only half the value compared to the BAK - treatment.

	pH	E.C. (µS/cm)	NH_4-N (mg/kg compost)	NOx-N (mg/kg compost)	Extinction 485 nm
	After 30 days				
Blank BAK 1095	7.4 ± 0.2 8.6 ± 0.1	5233 ± 94 4957 ± 129	91 ± 34 1198 ± 21	1067 ± 23 270 ± 141	2.18 ± 0.39 11.39 ± 2.83
Blank + C BAK 1095+C	8.1 ± 0.5 8.7 ± 0.1	4277 ± 240 4437 ± 272	299 ± 242 1074 ± 40	313 ± 385 -4 ± 5	3.99 ± 0.91 17.56 ± 2.60
	After 45 days				
Blank BAK 1095	7.5 ± 0.1 8.6 ± 0.1	4458 ± 334 4583 ± 161	69 ± 34 1147 ± 52	1103 ± 75 357 ± 113	2.40 ± 0.35 14.05 ± 1.85
Blank + C BAK	8.2 ± 0.7 8.7 ± 0.1	4103 ± 266 3743 ± 503	349 ± 301 959 ± 36	360 ± 421 30 ± 11	5.50 ± 2.49 13.76 ±

1095+C					0.79
	After 60 days				
Blank BAK 1095	7.0 ± 0.2 8.5 ± 0.1	5357 ± 193 4717 ± 61	96 ± 48 1048 ± 54	1270 ± 61 351 ± 70	3.32 ± 0.31 9.63 ± 1.43
Blank + C BAK 1095+C	8.1 ± 0.9 8.8 ± 0.1	4317 ± 215 3960 ± 92	475 ± 418 962 ± 59	366 ± 475 18 ± 11	5.47 ± 2.36 13.46 ± 0.54
Blank+NH_4-N		5020	629 ± 63		

Table 3: Intermediate and final analyses from the controlled composting test

While the NH_4 - N contents decreased to very low levels during the course of composting under optimal conditions and a sufficient maturation period, NO_3 - N levels, as the final link of nitrogen mineralisation, increased at the same time. From a relative point of view, similar differences in concentration were observed for the nitrate content between treatments at the end of composting compared to NH_4 -N content after five weeks of composting .

The trend, however, was similar for both treatments, i.e. high initial total N and NH_4-N contents are later on reflected in high NO_3-N contents. At the end of the composting process, the BAK treatment showed a significant difference to the control (around 50%), whereas only a marginal difference was recorded for the sampling date after 10 weeks. During the last 5 weeks, under mesophilic temperature conditions (and therefore very favourable for nitrification), a high proportion of nitrogen in the BAK-treatment was mineralised (compare total nitrogen content).

The results presented here are in agreement with the results obtained by Fritz (1999)for the controlled composting test. On the other hand, however, it was shown that during proper composting (according to the standard guidelines of the DIN V 54900) BAK 1095 behaved very typically like a material with a low C/N ratio.

	BAK - Treatment			Control - Treatment		
	Composting time (weeks)			Composting time (weeks)		
Parameter	5	10	15	5	10	15
pH [1)]	8,00	7,03	6,63	8,30	7,45	7,80
Salt content [2)]	4,90	7,00	7,20	5,60	8,23	8,48
Nt. (% dm)	2,21	2,14	1,71	1,69	1,54	1,35
NH_4-N [3)]	460,07	29,82	23,74	265,34	18,87	2,85
NO_3-N [3)]	1,02	571,38	1037,69	37,62	520,99	471,15

Table 4: Analyses of samples from the pilot scale composting plant
[1)] 3 replicats per sampling date; [2)] g KCl/l fresh matter; [3)] mg / l fresh matter

When using materials with a low C/N ratio, the temperature increases very fast, the proportion of carbon compared to nitrogen is too low and therefore nitrogen is released from the degradation of organic raw materials is to a lesser extent fixed in microbial biomass. Mineralised N is then mainly found in the form of NH_4-N as the first step of the mineralisation of organic nitrogen. Under certain temperature and pH conditions, heavy losses of nitrogen may occur in the form of NH_3 release. Meyer (1979, 1982), for example, could show that during composting of cow manure and straw with a C/N = 28 in aerated barrels (30 l/h), and at temperatures > 55° C, high NH_3 losses occur fast. When the proportion of straw was higher (C/N = 39) and aeration was reduced, NH_3 release was not observed.

According to Meyer, temperatures above 55° C should be avoided during composting, as NH_3 - losses then show a considerable increase. These losses were, according the same author, the higher the greater the proportion of manure (i.e. the lower the C/N ratio) was in the mixture and the more redundant available nitrogen was bound as NH_4-N, caused by the unstable N-compounds in manure and urine.

With increasing availability of carbon compounds, as straw powder, N-losses decreased despite the increase of the organic nitrogen fraction in the compost, even when temperatures exceeded 50° C to 55° C. Similar conditions were observed with liquid cattle manure, which had only a small reservoir of easily metabolisable carbon. The addition of easily metabolisable carbon in the form of silage leachate during the fermentation of liquid cattle manure fixed N in the biomass and the content of free ammonia of approx. 2.3 g NH_3-N / l was drastically reduced to approx. 0.3 g NH_3-N / l liquid manure (Grabbe, 1978). Grabbe included the additional aspect that degradation by the nitrifying flora is prevented at temperature levels > 40° C, thus the oxidation of ammonia to nitrite and nitrate, respectively, does not occur and that, depending on the aeration intensity considerable ammonia losses through gas emissions may occur. In studying horse manure for the production of button mushroom substrata Grabbe (1975)stated that ammonia losses were only limited when the dissimilation performance of the microflora was in equilibrium with the assimilation performance. This was only the case when the C/N ratio was increased to values above 20:1.

If these results are called to mind now for the actual case of composting on laboratory scale under the described process conditions with the controlled composting test and using BAK 1095, the following picture results:

- The "controlled composting test" with a continuously controlled temperature (at 58° C) is critical with regard to NH_4-N/NH_3 development for two reasons. Firstly, the microflora in the composting material shifts from thermophilic fungi increasingly towards thermophilic bacteria at temperatures above 45° C to 50° C. At temperature levels above 65° C, virtually only spore producing thermophilic bacteria metabolise the material. In contrast to thermophilic fungi, thermophilic bacteria metabolise significantly less carbon and fix continuously relatively lower amounts of nitrogen in their biomass compared to the fungi (Müller, quoted after Gottschall, 1984). Secondly, nitrifying conditions (mineralisation of NH_4 to NO_3) cannot be established because of the temperature.
- The continuos temperature control at 58°C during the "controlled composting test" leads altogether to a process management with focus on degradation, with respective N-release from organic matter. This is also hinted at by the high DOC-contents in both BAK-treatments, and possibly also by the extinction value of 485 nm (Table 3). The BAK-compost treatments were not stabilised. Thus the results obtained by Fritz were further confirmed.
- BAK 1095 has a rather low C/N ratio of 9. Additionally, the compost used by Fritz already showed a low C/N ratio of 14. Considering the high amount of BAK added to the compost, this nitrogen surplus became especially important. It can be assumed that easily available carbon sources, both from biowaste and BAK, were not available to a sufficient extent for the N-assimilation of the micro-organisms. As digestion of carbon sources from the organic matter of the compost (cellulose, lignin) only takes place very slowly, microbial incorporation of nitrogen into protein compounds stagnated. In the case where a fresh carbon source is added to the mature compost, conditions are similar to those of the BAK treatment in the controlled composting test (Table 3).

In the values presented for the controlled composting test and from the results of Fritz (1999), high NH_4-N contents were measured even after an additional two-week secondary treatment at ambient temperature at temperatures at which nitrification can take place. It can therefore be assumed that even higher NH_4-N contents in the compost would be found directly after the "controlled composting test" was stopped. In this context, a well-known phenomena occurring in fresh compost has to be referred to. Fresh compost contains a high proportion of NH_4-N as part of the total nitrogen content because of the unfinished composting process, and when pH-values are high, ammonia is released. The ammonium proportion Fritz found in the BAK treatments were up to 10- 14 % of the total nitrogen. Usually, mature composts contain 0 — 3 % NH_4-N as a proportion of the total nitrogen.

In the examinations carried out by Fritz, the pH of 7.8 of the BAK-compost compared to the control with a pH of 7.2 favoured the gaseous emission of NH_4-N in the form of ammonia. According to Kinzel (1992, in Hadwiger-Fangmeier *et al*., 1992), NH_4^+ dissociates at pH-values of the soil solution > 7.0 increasingly into NH_3 and H+ (pH-dependent steady-state equilibrium NH_4^+ / $NH_3 + H^+$).

3.4 Ecotoxicity Test

3.4.1 Test on Gaseous Phytotoxic Substances

After 5 weeks composting time at a substrate contents of < 300mg NH_4-N /l fresh matter a loss of 90 % of the root length of cress was observed in the control treatment compared to the reference EE0. Cress roots did not develop at all when ammonium contents were twice as high in the BAK-treatment or in the fertiliser increase levels of both treatments (Figure 1 a, Table 5). After 10 weeks composting time ammonium levels were reduced by approx. 93 % compared the results after 5 weeks. In both treatments conditions had developed favourable enough to reduce growth rate differences between the BAK and the control treatments to a relatively low extent, but the low NH_4-N concentration of the reference with EE0 were not reached.

The single parallel BAK treatments without additional ammonium fertilisation (F 0), however, still showed statistically effective differences to the control treatment although concentrations were only at 29 mg NH_4-N/l fresh matter (Figure 1 b, Table 5). The impact of the ammonium addition and the resulting increase of ammonia on the cress becomes independent from the addition of BAK. Growth rates of C1 F2 to C3 F2 (mean value of the ammonium concentration :

156 mg NH_4-N/l) were found at the same magnitude as B1 F1 to B3 F1 (ammonium content 174 mg NH_4-N/l) (Figure 1 b, Table 5). After 15 weeks, at the end of the composting time, ammonium values had been reduced to such an extent that only very small differences could be observed between both treatments with regard to root growth. Between the single parallel treatments of control and the BAK treatment without fertilisation, no statistically relevant differences could be recorded. The ammonium concentration of the test substrata were low enough not to allow any significant effect of an additional relatively small ammonium fertilisation (Figure 1 c, Table 5). For green biomass growth of the cress, the ammonium/ammonia effect was less significant as compared to root growth. However, results of green biomass growth were nearly equal as the results for the root length after 5 weeks of composting.No differences between treatments, fertilisation levels and EE0 were recorded for the following sampling dates (results are not shown).

Thus, in the available ecotoxicological examinations, the high toxicity of ammonia was confirmed as it has been described in the literature. Data on compost trials show that the main factors responsible for the degree of damage by ammonia are the dose of NH_4-N/-NH_3, the pH-value and the type of organisms. Katayama et al. (1985) already recorded 50 % growth depression of cress in substrata with sewage sludge compost at 1.48 mg NH_4-N/ g dry matter and a pH of 8.6 leading to an amount of 0.77 mg NH_3/m_. Van Haut (1979) observed a 25 — 50 % growth depression of cress at 1.08 mg NH_3/m_. Fritz measured in BAK-treatments up to 2.36 mg NH_4-N/ g dry matter, which means that his values were up to 60 % higher than the critical value given by Katayama. Therefore, simultaneously to the slightly lower pH in his substrates, even an increased ammonia impact was probably effective.

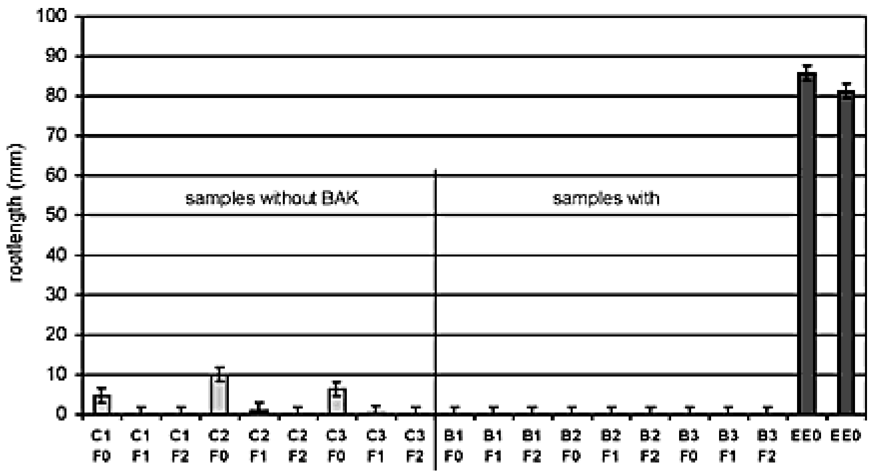
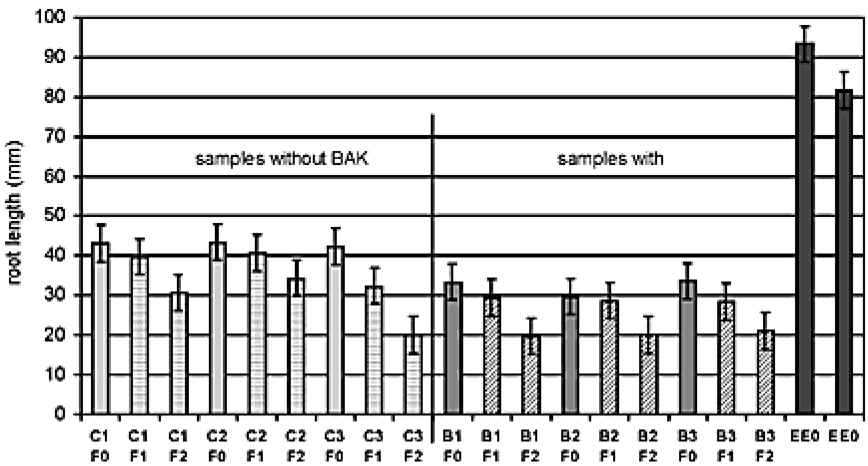


Figure 1a: Root length of cress during the test of gaseous phytotoxic substances
a. composting time: 5 Weeks



Legend	C = Control Parallel 1 - 3	B = BAK-treatment Parallel 1 - 3	F0 bis F2 = Ammonia fertilisation level EE0 = reference soil (Einheitserde)

Figure 1b: Root length of cress during the test of gaseous phytotoxic substances
b. composting time: 10 Weeks

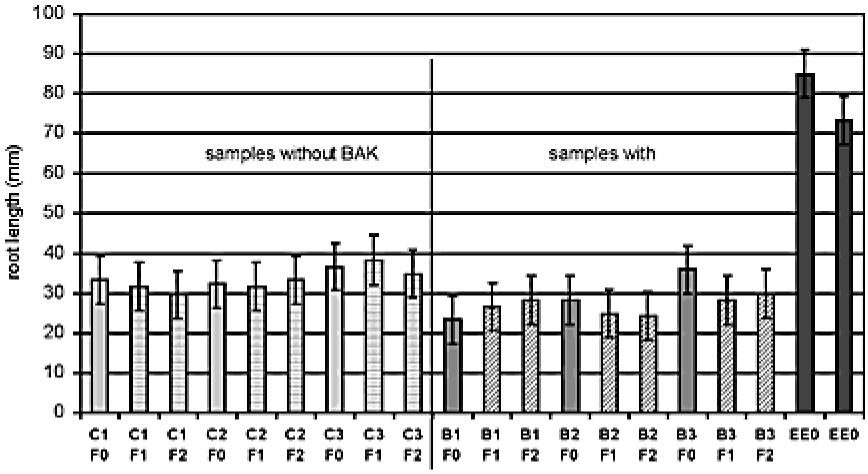


Figure 1c: Root length of cress during the test of gaseous phytotoxic substances composting time:15 Weeks

	pH Wert	Nt (% TS)	NH_4-N (mg/l medium)	NO_3-N (mg/l medium)
after 5 weeks composting				
Reference (EE0)	7,90	n. d.	0,39	0,69
BAK treatment (F 0)	8,00	n. d.	460,07	1,02
BAK treatment (F 1)	8,03	n. d.	1.120,81	0,32
BAK treatment (F 2)	7,90	n. d.	1.597,41	0,30
Control treatment (F 0)	8,30	n. d.	265,34	37,62
Control treatment (F 1)	8,00	n. d.	311,78	42,64
Control treatment (F 2)	8,03	n. d.	766,56	31,98
after 10 weeks composting				
Reference (EE0)	6,22	n. d.	< 0,2	-
BAK treatment (F 0)	7,03	2,14	29,82	571,38
BAK treatment (F 1)	7,02	n. d.	174,09	597,70
BAK treatment (F 2)	7,08	n. d.	319,79	587,39
Control treatment (F 0)	7,45	1,54	18,87	520,99
Control treatment (F 1)	7,42	n. d.	43,56	566,81
Control treatment (F 2)	7,42	n. d.	156,31	529,23
after 15 weeks composting				
Reference (EE0)	5,90	0,70	< 0,2	0,17
BAK treatment (F 0)	6,63	1,71	23,74	1037,69
BAK treatment (F 1)	6,50	1,88	47,17	1145,91
BAK treatment (F 2)	6,60	2,00	49,48	1054,60
Control treatment (F 0)	7,80	1,35	2,85	471,15
Control treatment (F 1)	7,77	1,32	5,88	426,25
Control treatment (F 2)	7,83	1,32	13,85	455,36

Table 5: Analyses of samples from the pilot scale composting plant used in the gaseous phytotoxic substances tests

3.4.2 Daphnia Test

Determination of Daphnia mortality performed with the samples of the controlled composting test after a duration of 60 days resulted in the 1/8 dilution level up to 100 % and 5 % for the BAK and control treatment, respectively (results are not shown). This could be related to the ammonium contents of the extracts (Table 3) . Ammonium/ammonia toxicity independent from the BAK amendment could be demonstrated by an artificial increase of the ammonium contents of the extracts. Therefore, the result obtained by Fritz could be confirmed and it was concluded that Daphnia mortality was almost - if not entirely — caused by NH_3 toxicity.

Samples from the pilot-scale experiments which are a much closer simulation of a real-life composting process showed a decreasing Daphnia mortality during the composting process. At the beginning of the test (after 5 weeks) parallel to the elevated levels of ammonium a higher mortality could be observed, but this level decreased significantly afterwards (Table 6). After 15 weeks no significant difference between blank and test compost was noted. Further evidence of the impact of ammonia content was again illustrated by the artificial increase of ammonium in the BAK or blank treatments.

After 5 weeks								
Lab code	Saample	Extinction 485 nm	Daphnia Toxicity (% mortality)					
Sample	NH4+-N	1/5 Eluate	1/4	1/8	1/16	1/32	1/64	1/128
Blank Level 1	17	3.68 ± 0.84	60	0	0	0	0	(0)
Blank Level 2	71		100	67	0	0	0	(0)
Blank Level 3	176		100	100	100	27	0	(0)
BAK Level 1	189	7.74 ± 0.39	100	100	23	0	0	0
BAK Level 2	261		100	100	33	0	0	0
BAK Level 3	407		100	100	100	73	27	0
After 10 weeks								
Lab code	Sample	Extinction 485 nm	Daphnia Toxicity (% mortality)					
Sample	NH4+-N	1/5 Eluate	1/4	1/8	1/16	1/32	1/64	1/128
Blank Level 1	33	3.36 + 0.57	100	27	0	0	0	(0)
Blank Level 2	13		80	33	0	0	0	(0)
Blank Level 3	41		100	27	0	0	0	(0)
BAK Level 1	8	4.90 + 0.97	73	33	0	0	0	0
BAK Level 2	45		100	53	0	0	0	0
BAK Level 3	78		100	33	0	0	0	0
After 15 weeks								
Lab code	Sample	Extinction 485 nm	Daphnia Toxicity (% mortality)					
Sample	NH4+-N	1/5 Eluate	1/4	1/8	1/16	1/32	1/64	1/128
Blank Level 1	3	2.97 + 0.88	60	0	0	0	0	(0)
Blank Level 2	2		60	53	13	0	0	(0)
Blank Level 3	6		93	27	0	0	0	(0)
BAK Level 1	10	3.13 + 0.38	93	7	0	0	0	0
BAK Level 2	13		80	20	0	0	0	0
BAK Level 3	17		80	27	0	0	0	0

Table 6: Results for extinction and Daphnia mortality with composts received from samples from the pilot scale composting plant.

3.4.3 Barley Test

The effects demonstrated with cress and daphnia could not be repeated for the test plant barley. The rather high salt content which was caused by additional ammonium fertilisation with ammonium sulphate had no impact on the results nor did the barley react significantly to the enhanced ammonium concentration in the BAK-treatment. Measured values were found to be within the magnitude of the reference treatment with EE0 at all sampling dates, and could thus be rated as plant compatible. All BAK-treatments reached the required threshold value of 90% of the yield (ecotoxicity test according to DIN V 54 900) obtained without addition of the biodegradable material.

4. CONCLUSIONS

After the reproduction of Fritz's experiments using the same methodology and additional testing under pilot scale conditions it was shown that the results found by Fritz were mainly caused by NH_3 toxicity affected by the chosen laboratory scale composting method and the chemical "nature" of BAK 1095. Under real composting conditions this would be irrelevant because ammonium/ammonia would have decreased to absolutely non-inhibitory values under proper composting conditions. There is no reason to doubt the already attested compostability of BAK 1095 according to DIN V 54900.

5. ACKNOWLEDGEMENTS
This research was funded by BAYER AG, Leverkusen.
A version of this paper was published in the Proceedings of an "ORBIT Special Event" on Biodegradable Polymers: production, marketing, utilisation, and residue management (ISBN 3-86068-143-5).

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