# Effect of inhibitors on Zygophyllum dumosum plant litter decomposition

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#### **ABSTRACT**

The contribution of soil fauna to decomposition processes was studied by elimination of specific biotic elements using chemical inhibitors. Changes in overall activity of the microbial community were studied in *Zygophyllum dumosum* leaves treated with the inhibitors Nemacur (nematocide), Edigan (biocide), and water (control). At the end of a one-year study, the decomposition of leaves treated with Nemacur or Edigan indicated a loss of about ten percent mass compared to leaves treated with water. The kinetic constant for mass loss exhibited a bi-phasic decomposition process (typical for the Negev Desert) for inhibitor- and water-treated leaves. However, the water-treated leaves exhibited a turnover by 30% higher than the other treatments on a yearly basis. The Shannon-Weaver (H') index of the microbial community in the decomposing leaves was found to be higher in the water-treated leaves only in the first sampling period, after which no differences between inhibitor- and water-treated leaves were observed. This study elucidates the importance of the biotic element in soil to decomposition processes in an arid climate, with focus on microbial communities.

Keywords: biocide; microbial functional diversity; nematocide; soil

The involvement of soil biota in nutrient uptake and transformation processes emphasizes the close relationship between decomposition, which is biota-dependent, and nutrient availability (Whitford 2002). Moreover, studies by Coleman and Crossley (1996) have shown that macro-climatic variables, e.g., rainfall and temperature, play an important role in affecting the community of decomposer organisms, which are also regulated by resource quality and the physico-chemical environment.

Studies by Whitford et al. (1988) have elucidated the indirect effect of abiotic variables such as rainfall and evapotranspiration on the decomposition of organic matter, while a recent work by Austin and Vivanco (2006) emphasized the important role of photodegradation in above-ground litter decomposition in semi-arid ecosystems. The decomposition rate has, thus, been shown to vary according to the type of ecosystem, ranging from a decay constant of 0.03/year in tundra to 6.0/year in tropical forests.

The bacterial component is known as being able to regulate plant litter decomposition, nutrient cycling, and flow of energy to higher trophic levels (Ladd et al. 1985). Because the interactions within

the microbial community in soil are complex from a functional, structural, and compositional point of view, they are commonly studied as a 'black box', and the importance of the interactions between specific components of the microbial community is often ignored.

Biological activity in terrestrial ecosystems is regulated mainly by climate and organic matter content. Desert ecosystems have biological activity which is limited mainly to those periods of 'windows of activity' which will, in turn, affect decomposition rates. Therefore, biological activity, as well as decomposition rates, in a Negev Desert system, exhibits a bi-phasic pattern with relatively high rates of weight loss during short winter periods and low rates of weight loss during dry seasons (Steinberger and Whitford 1988).

Elimination of certain biotic factors is a common method used to study certain biological processes. It is carried out either by physical means, using litterbags of varying mesh sizes, or by chemical elimination, where various types of microand meso-fauna are removed, leaving the rest of the biotic community to function without them (Mayzlish and Steinberger 2004).

The goal of the present study was to evaluate the contribution of the microbial community in soil to the decomposition of organic matter in an arid climate. Our hypothesis was that elimination of specific populations will alter the food webs in soil, thus changing the kinetics and pathways of decomposition, as expressed by the mass left at the end of the experiment as well as by changes in structural composition of the microbial community.

This research aims contribute to a better understanding of the structure and function of the biotic components in soil, while gathering more information on biotic interactions in arid environment.

#### MATERIALS AND METHODS

**Study site**. The study site is situated at the M. Evenari Runoff Research Farm (30°47′, 34°36′E) in Avdat, in the Negev Desert, Israel. The elevation is approximately 600 m above sea level, with an average annual rainfall of 90 mm (Avdat station), ranging from 24 mm in an extremely dry year to 183 mm in a wet year. The rain season begins in October and ends at the end of April, with most of the rainfall occurring in scattered showers between December and February. An additional source of moisture is approximately 35 mm/year of dewfall occurring heavily during approximately 200 nights of the year (Evenari et al. 1982).

The study site has a temperate desert climate, with hot summers (18–32°C in June) and cold winters (5–14°C in January). The soil is a deep, fine-textured, loessial sierozem (calcisol) with a high amount of carbonates (40%). The plant community is a mixture of perennial shrubs, mainly *Hammada scoparia* and *Zygophyllum dumosum*, and a large variety of annuals, dominated by *Stipa capensis* (Evenari et al. 1982).

**Experimental details**. Leaves of *Z. dumosum* were collected at the end of the growing season near the study site. The plant material was airdried for 72 h and  $4 \pm 0.01$  g of it was placed in  $10 \times 10$  cm fiberglass litterbags (mesh size 1.5 mm). Each 100 litterbags were treated by one of the following: Nemacur (nematocide), Edigan (biocide), or water (control). The efficacy of Nemacur was proven in Pen-Mouratov et al. (2004). Edigan is a biocide used to inhibit bacteria, soil fungi, and nematodes. Its efficacy in eliminating nematodes and protozoa was shown by Pen-Mouratov et al. (2004).

The solutions were prepared as recommended by the manufacturers: (a) the Nemacur [ethyl-(3methyl-4-methylthiophenyl)-isopropylamido-\*phosphate]:

40 ml in 10 l water; and (b) Edigan (sodium N-methyl dithiocarbamate): 3.5 ml in 10 l water.

The litterbags were soaked for 2 days in the treatment solutions. After the soaking period, the litterbags were air-dried. In November 2000, the litterbags were inserted into the -10 cm soil layer at the study site. Six litterbags (2 from each treatment) were retrieved randomly at time zero and taken to the laboratory to calculate the handling loss. Eight litterbags from each treatment were collected monthly during the study period.

The rationale for placing the litter bags at 10 cm depth and above-ground came from the understanding of the natural telescopic movement of soil biota in arid regions, they remove above-ground litter and move it to deeper soil layers (Steinberger 1991).

Four soil samples were collected from the 10 cm soil layer of the open field during the same sampling period. The samples were kept in the container until arrival at the laboratory, where they were sieved through a 2-mm mesh in order to remove root particles and other organic debris.

Chemical and biological analyses were conducted in the laboratory as follows: four out of eight litterbags of each treatment from every sampling date were used to determine plant litter dry weight; the remaining 4 litterbags from each treatment were used to determine microbial functional diversity.

Soil moisture was determined gravimetrically by drying a known weight of subsample at 105°C.

Mass loss determination: to determine the dry weight of the litter in each litterbag, the litterbag content was dried at 60°C for a minimum of 72 h before weight determination. Organic matter weight loss was obtained using the Elkins and Whitford (1982) equation. The first order kinetic constant (k) for mass loss was calculated as follows:

$$A_t = A_0 e^{-kt}$$

Where:  $A_t$  is the mass remaining at time t,  $A_0$  is the initial mass, t is time and k is a negative coefficient.

The Biolog (Biolog, Hayward, CA, 1993) plate identification method was used to determine microbial functional diversity (Gram negative) (Zak et al. 1994). To each of 96 Biolog wells, a 100- $\mu$ l aliquot from dilution of each sample was added: the content of litter bag + 90 ml 0.2% water agar; 15 min shaking at 160 rounds per minute; 30 min sedimentation. The microplates were incubated at 22°C. Bacterial community utilization of substrates was quantified by measuring the intensity of the color change (590 nm after 48 h, 72 h, and 96 h) caused by reduction of the tetrazolium dye. The

number of categories of utilized substrates resulting from microbial activity constituted the data set from which functional diversity was assessed.

**Data analysis**. All data were subjected to analysis of variance (ANOVA). Significance was observed at the P < 0.05 level, and Tukey's values were calculated for separation of means (Sokal and Rohlf 1969).

The Shannon-Weaver index (H') was used to determine bacterial functional diversity (BFD) based on the number of different substrates utilized by the microbial community. Data obtained were processed as follows:

- 1. Raw difference (RD): X-X<sub>O</sub>, where: X is the raw value for each well and X<sub>O</sub> is the color intensity at 590 nm (OD<sub>590</sub>) of the blank well used for calibration. Negative score readings were set to zero.
- 2. Average well color development (AWCD): *SRD/* 95 (since there were 95 wells used as substrates): the number of utilized substrates are the substrates with *RD* > *AWCD*.
- 3. Diversity was calculated using a population ecology equation in which the raw difference data were incorporated as follows:

$$H = -SP_i (lnP_i)$$

Where:  $P_i$  is the ratio between the activity of a particular substrate and the sum of the activities of all substrates.

The Shannon-Weaver index was calculated both for the leaves that were treated with the various inhibitors and for the soil control samples taken from the open field. In order to detect changes in the net activity of the microbial community of the leaves, control soil H' values were subtracted from the H' values of the treated leaves. The rationale behind this adjustment has two purposes: (1) by omitting the microbial activity in the soil, we get the net microbial activity in the treated leaves; (2) the adjustment creates an equal 'starting point' for all the treatments, that is, a point from which a comparison can be done.

The data were tested by computing redundancy discriminate analysis (RDA) to provide more infor-

mation taking into account environmental factors such as seasonality and treatments as the main factors determining soil abiotic parameters and substrate utilization by the soil microbial community, and also taking into account properties proportionally related to the results obtained [Program CANOCO, Version 4.54, October 2005 – written by Ter Braak (C) 1988–2005, Biometris – quantitative methods in the life and earth sciences, Plant Research International, Wageningen University and Research Centre, Box 100, 6700 AC Wageningen, the Netherlands]. The Monte Carlo permutation (499) test was used to calculate the significance of given environmental factors and their relevance to community structure (ter Braak 1995).

#### **RESULTS**

The amount of rainfall during the study period totaled 56.2 mm, while the total amount that year was 80.3 mm (Figure 1). This amount is less than the mean multi-annual rainfall of 90.0 mm. Forty-seven percent of the total rainfall occurred in January (26.5 mm), while the remaining 53% was distributed throughout the rainy season ending in May 2001.

Soil moisture (Figure 1) was found to follow the rainfall pattern, reaching a maximum of 7.9% during the rainy season, followed by a gradual decline to a minimum of 1.7% during the dry season. Soil moisture content increased significantly (P < 0.01) in the rainy season compared to the results obtained for the three seasons that followed (spring, summer, and autumn).

Litter mass loss of Z. dumosum leaves under the various treatments is presented in Figure 2. A bi-phasic pattern in weight loss was obtained in all treatments, where significant (P < 0.0001) differences in litter mass loss over time were demonstrated. Significant (P < 0.01) differences in the decomposition of the leaves were observed between the control and the other two treatments

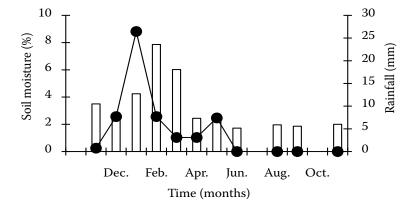


Figure 1. Rainfall ( $\bullet$ ) and soil moisture ( $\Box$ ) at the study site from November 2000 to November 2001 at the Avdat station

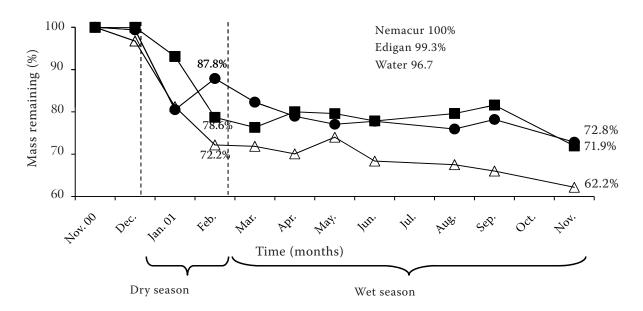


Figure 2: Remaining mass (%) of leaves treated with  $\blacksquare$  Nemacur,  $\bullet$  Edigan, and  $\Delta$  water in soil at the depth of 10 cm, throughout the study period

(Nemacur and Edigan). At the end of the study period, a larger amount (approximately 10%) of inhibitor-treated leaf mass was left in the soil.

During the first phase of mass loss (Figure 2), and specifically from December to February (wet season), the decomposition rates were high and significantly (P < 0.001) affected by the treatment. At the end of the rainy season, the weight loss of the control water-treated litter reached 28% of its initial weight, whereas the Nemacur- and Edigan-treated litter weight loss reached 21% and 12%, respectively, for the same period (Figure 2). The second phase (dry season), starting from March and ending in November 2001 included spring, summer and autumn. During this period, there was a slow mass loss in all treatments. At the end of the study, a significantly (P < 0.05)higher decomposition rate (62%) was obtained in the water-treated leaves in comparison to 73% and 72% of the initial weight of the Edigan- and Nemacur-treated leaves, respectively.

The first-order kinetic constants calculated for mass loss clearly reflect bi-phasic decomposition (Table 1). The kinetic constants were high (0.15-0.37) in all treatments during the winter season and low (0.02-0.05) in the dry seasons. Within the wet period, the kinetic constants of the Edigan- and Nemacur-treated litters were lower than water-treatment leaves by 40% and 73%, respectively. During the dry seasons of the following period, the kinetic constant was found to be 13, 4, and 7 times lower in comparison to the wet season in the Nemacur-, Edigan- and water-treated litters, respectively. No significant differences in the kinetic constant were observed between the Nemacur and Edigan treatments on a yearly basis. However, the kinetic constant calculated for the water-control treatment demonstrated over 30% higher rates, elucidating the higher decomposition rate in the untreated litter.

Based on these results, the turnover period of organic matter treated by Nemacur and Edigan

Table 1. First-order kinetic constants for mass loss of the decomposing leaves were calculated for each treatment during the wet and dry seasons. The value of the constants throughout the year as well as for the turnover period (in years), is presented

Treatments	wet season Nov-Feb dry season Mar-Nov		over the year	turnover
		(years)		
Nemacur	0.27	0.02	0.09	3.2
Edigan	0.15	0.04	0.08	3.3
Water	0.37	0.05	0.13	2.2

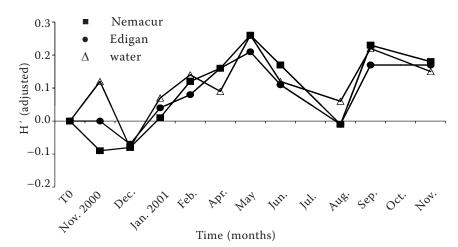


Figure 3. Microbial functional diversity (H') in treated leaves, throughout the study period. The values are expressed by the Shannon-Weaver index and are adjusted to the H' values obtained for the soil control samples from the open field

was prolonged by 45% compared to the control water-treated plant litter, resulting in a period of one additional year.

**Functional diversity (H').** Differences in H' were noticeable only in the first sampling period; during this time the index was the highest one in the water-treated leaf litter (Figure 3). At the same time, the adjusted microbial functional diversity index in the Edigan-treated leaves was similar to that of the soil samples, whereas the Nemacur-treated leaves yielded a lower index. From December to the end of the study period, no differences (P > 0.05) were apparent between the three treatments. The general trends were of an increase in the microbial functional diversity index that lasted until May, followed by a decrease during the dry summer. In September, high values were detected (Figure 3).

RDA analysis. The multivariate analysis of soil moisture, organic matter, and decomposition as affected by the three treatments on a seasonal scale, showed clear discrimination between the above variables. In RDA (Figure 4), a similar level of discrimination between all three treatments was observed. Seasonality was found to have a significantly (P < 0.002) higher outcome (elucidated by the length of the arrow), totaling 76% of the total variance exhibited by all the variables (81%) in comparison to treatment effect on soil moisture, organic matter, and decomposition process (Table 2). Soil moisture was found to be determined by winter and spring seasons rather than by treatment effects, while soil organic matter was found to be related to the winter and autumn seasons. Organic matter decomposition was found to be discriminated by the two high-moisture seasons, although they were also the determining factor resulting in the summer measurements. The three treatments were found to have a lower effect on the variables measured and discriminated from each other compared to seasonality. Multivariate analysis of microbial community functional utilization (Figure 5) expresses the effect of seasonality and treatment on the three main groups of substrates (carbohydrates, carboxylic acid, and amino acids), this in addition to the Shannon-Weaver index (H') – diversity based on the above substrate utilization (Table 3). In this case, the variance expressed by all the variables reached only 7%: Edigan accounted for a total of 3% of the changes, Nemacur -0.03%, and the seasons (winter, spring, and summer) accounted for 1.0, 1.0, and 2% of the changes, respectively. In all cases, the P value showed a non-significant (P > 0.05) effect of the above variables on substrate utilization and diversity. Collinearity was detected when an attempt was made to fit the autumn and control treatment variables. However, the Edigan treatment and the

Table 2. Results of RDA analyses, which estimated the values of environmental and treatment variables of soil moisture, organic matter and weight loss, at the study site

Variables	F-ratio	P values	Extra fit
Winter	41.75	0.002	0.290
Spring	11.61	0.002	0.650
Summer*		0.002	
Autumn	30.31	0.002	0.400
Edigan	1.22	ns	0.005
Nemacur	11.18	0.002	0.050
Control*			
Variance ex	0.810		

\*no additional variables can be added in order to improve the fit of the model. Collinearity detected when fitting marked variables; ns – not significant

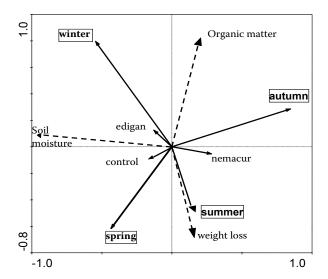


Figure 4. RDA analysis of seasonal and treatment data as an active environmental variable, and soil moisture, organic matter, and weight loss as supplementary variables. Variance explained by year: 0.81, P-value 0.0020; F = 11.98; number of permutation = 499

summer season were found to completely explain 70% of the changes, with strong discrimination regarding the other variables. The spring season was found to reinforce the significance of the H' diversity in substrate utilization.

#### DISCUSSION

The current research aimed to further test the contribution of the biotic components to the decomposition process. The results obtained in this study elucidated the following factors: (1) the effect of the abiotic environment on decomposition processes; and (2) the contribution of soil biota to decomposition processes.

Differences in decomposition patterns throughout the study period emphasized the important contribution of the wet season; during this time there was a 'window of activity' for biotic components, as reported for the Chihuahuan Desert in North America.

The use of inhibitors such as in the Nemacurtreated litter leaves did not change the bi-phasic pattern, which showed a rapid mass loss during the wet season followed by very slow decomposition during the dry season. However, the nematocide treatment caused bi-phasic decomposition to become more drastic in comparison to that caused by the water treatment. The decomposition in Nemacur was very slow during the dry period, which is a fact that implies a dependence on rainfall amounts. In this case, the use of the nematocide (Pen-Mouratov et al. 2004) may have increased the dependence of decomposition on soil water content, as was discussed above.

The use of Edigan as a biocide inhibiting a wide range of biotic components resulted in a decomposition process that was the slowest of the three treatments. Similar results that elucidate the dependence

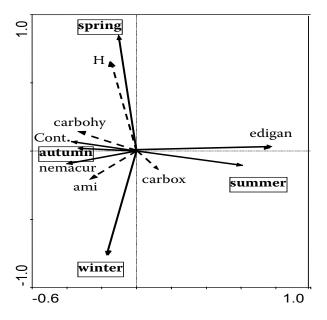


Figure 5. RDA analysis of seasonal and treatment data as an active environmental variable and microbial community substrate utilization (carbox – carboxyl; carbohy – carbohydrates; amin – amino acids) and their H' diversity – Shannon-Weaver index as supplementary variables. Variance explained by year: 0.07; P – value non-significant; F = 1.40; number of permutation = 499

Table 3. Results of RDA analyses, which estimated the values of environmental and treatment variables of microbial community substrate utilization – carbohydrates, carboxylic, amino acids and H' diversity, at the study site

Variables	F-ratio	P values	Extra fit		
Winter	0.3	ns	0.01		
Spring	0.72	ns	0.01		
Summer	0.9	ns	0.02		
Autumn*	na	na			
Edigan	1.4	ns	0.03		
Nemacur	0.01	ns	0.0003		
Control*	na				
Variance explained by all variables 0.0					

\*no more variables can be added in order to improve the fit of the model. Collinearity detected when fitting marked variables. na – not applicable; ns – not significant

of decomposition on biotic activity were reported by Moorhead and Reynolds (1989), who showed that the use of a general biocide delayed biotic activity, causing a slower decomposition process. They concluded that in the Chihuahuan Desert, abiotic parameters play a relatively minor role in decomposition processes taking place in the soil.

The addition of water in the control treatment resulted in a larger amount of mass loss in comparison to the inhibitor-treated litter. This was the outcome of continuous and consistent mass loss during both the wet and dry seasons. As a result, enhanced biotic activity enabled a more intense process that yielded a lower mass that was left at the end of the study. These results are compatible with data reported by Beare et al. (1992). In their study, the use of fungicides and bactericides reduced the decomposition rate to as low as 36% of the mass remaining in the control plots.

Differences in the microbial functional diversity index (H') between the three treatments were found only in the first sampling period. At that time, the microbial functional diversity index in the water treatment was high relative to the Edigan and Nemacur-treated plant litter, which was the lowest among the three treatments. Similar results were obtained in studies conducted mainly in agricultural soil, in which the effects of inhibitors on the microbial community were tested. In a study by El Fantroussi et al. (1999), the prolonged use of the herbicide phenylurea was found to alter species

composition in soil and cause a decrease in species diversity. Another study that was conducted in agricultural soil for a short time range, showed that inhibitors were effective in inhibiting the microbial community for a time range of one week (for methyl bromide), after which time recovery was observed for a longer range. Our results differ from those of Wardle et al. (1994), who found that accumulation of a microbial community on litter from herbicide-killed plants occurs earlier than in unsprayed plants. This was assumed to be the outcome of herbicide-induced plant damage that increased the availability of readily utilizable microbial substrate.

The use of selective inhibitors, although tested under field conditions that are not as precise as laboratory ones, elucidates the importance of the biotic factor in decomposition, especially in arid environments.

We conclude that there is a significantly lower decrease in decomposition when biocides and nematocides are used, leading to a larger amount of mass remaining in the soil in comparison to the control treatment. The outcome of decreased decomposition may be harmful in the long run. Where large-scale and vast areas of lands are concerned, this alteration in biotic elements may lead to changes in soil composition and perhaps other soil parameters as well, due to the inhibition and change of crucial processes such as decomposition.

Environmental conditions were found to have a major effect on both soil physical components and decomposition processes. Their effect overlapped the treatment effects on a yearly basis. Analyses confirmed that substrate utilization levels were found to be related mainly to the winter and autumn seasons. This may confirm the substrate availability connected to the presence of the utilizing microbial community. These results may lead to the conclusion that microbial diversity can decrease and increase in biomass during the winter and autumn seasons, respectively, while during the other two seasons, an opposite trend will be obtained, leading to relatively high microbial diversity and low microbial biomass. This trend can be confirmed by Kubartova et al. (2007). According to them, in forests, seasonal changes lead to microbial community composition determination by substrate availability. Moreover, they emphasized that in summer, most of the existing microbial species prefer more extreme hot and dry summer conditions, leading to elimination of commonly abundant species and the appearance of other abundant species. The results obtained

in this study demonstrate the importance of seasonality and plant phenology interaction through their effect on organic substrate availability and functional diversity and structure in soil microbial community.

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