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Biodegradation of Lindane by *Pleurotus sajor-caju* and Toxic Effects of Lindane and Its Metabolites on Mice

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Many toxic or carcinogenic organopollutants like Lindane, heptachlor, persist in the environment and tend to accumulate in the body fat of animals occupying higher trophic level (Addison et al. 1984). One reason for the environmental persistence of these compounds is that microorganisms are either unable to degrade them or do so very slowly (Alexander 1981). On the other hand white-rot fungi, capable of degrading lignin are able to degrade a wide variety of structurally diverse and environmental persistent organopollutants to CO_2 (Aust & Bumpus 1987). Included among the compounds degraded by these microorganisms are: DDT, Lindane; benzo (a) pyrene, organohalides. The ability of the white-rot fungi is dependent upon the lignin degrading system of these microorganisms (Shah et al. 1992).

Lignin degrading systems are commonly known as ligninases. The ability to degrade such a diverse group of compounds has been shown to be dependent on the nonspecific and nonstereoselective lignin-degrading system which is expressed by these microorganisms under nutrient (nitrogen, carbon, or sulfur)-limiting conditions (Bumpus et al. 1988). CO_2 released from organochlorinated pollutant by ligninases first occurred between the third and sixth days of incubation and showed a maximal rate between the time interval of 3 and 18 days, after which CO_2 production has continued in decreasing rates until the end of 30 days (Bumpus et al. 1985).

In this study, the physiological conditions that cause the degradation of Lindane (1,2,4,5/3,6-hexachlorocyclohexane) by **Pleurotus sajor-caju,** which take part in white-rot fungi have been determined. Following the identification of the LD_{50} dose of this substance its effects on cells in the blood of mice were investigated, related to toxic evaluations.

MATERIALS AND METHODS

Pleurotus sajor-caju used in the experiment were obtained from Dr. Zadrazil (Institut für Bodenbiologie, Bundalsalle 50 D-33, Braunsweigh, Fed.Dep. of Germany). The stock cultures of the organism were maintained on malt agar slants. The culture medium is a modification given by Eaton (1985).

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Culture medium contained (g/L) 0.12 NH_4Cl; 2.6 K_2HO_4; 0.5 $MgSO_4$; 0.1 $CaCl_2$; 10 glucose; 0.001 thiamin. The culture medium was prepared in either 0.1 M citrate buffer or 0.1 M tris buffer. After dissolving (1:1) in acetone, Lindane was added to the medium.

The growth of the organism in the culture was determined gravimetrically by measuring the dry weight of mycelia.Lindane was extracted by a process modified by Kaya (1982).

Obtained extracts were analyzed by using chrompack 438: a model gas chromatography system equipped with a CP-SIL-5CB column and E.C.D (Electron Capture Detector). The temperature of the injection port was 160°C, exit port was 80°C and nitrogen (40 mL/min) was used as carrier gas. The decrease in the initial and final value of Lindane was determined at the end of the analysis.

In toxicity experiment, 10 female albino mice weighing 18-22 g. were used in each treatment group. Control mice were fed with wheat material and Lindane free water, while mice in the test groups were fed with wheat material and water, containing Lindane and its metabolites. Lindane and metabolites were given to the mice orally at a time.

For determination of LD_{50}, method described by Behrens and Karber (1935), was used. Results were calculated by the formulation below :

$$LD_{50} = L\ D_{100} - \frac{\Sigma\ (a.b)}{n}$$

n= number of mice in each group.
a= the difference between the two doses following each other subsequently
b= the arithmetic average of the death caused by the two doses following each other.

At the beginning of the experiment, blood samples of the control groups of mice were analyzed so as to compare with the blood samples of test groups.The blood samples of test groups were collected 48 hours after Lindane was given. A similar experiment was achieved at the same conditions with a different group of mice with the metabolites of Lindane. The blood samples were collected from the tail of mice for the determination of the amount of Lymphocyte, Notrophyl, Monocyte, Eosinophyl, Reticulocyte. Blood preparations were stained by May-Grund Wald staining technique (Erkoçak 1980).
For statistical analysis, one side variance analysis and Kruskall-Wallis analysis methods were used (Dobson 1990).

RESULTS AND DISCUSSION

For the degradation of Lindane and growth of **Pl. sajor-caju,** optimum pH was found to be 5, incubation period 20 days, and

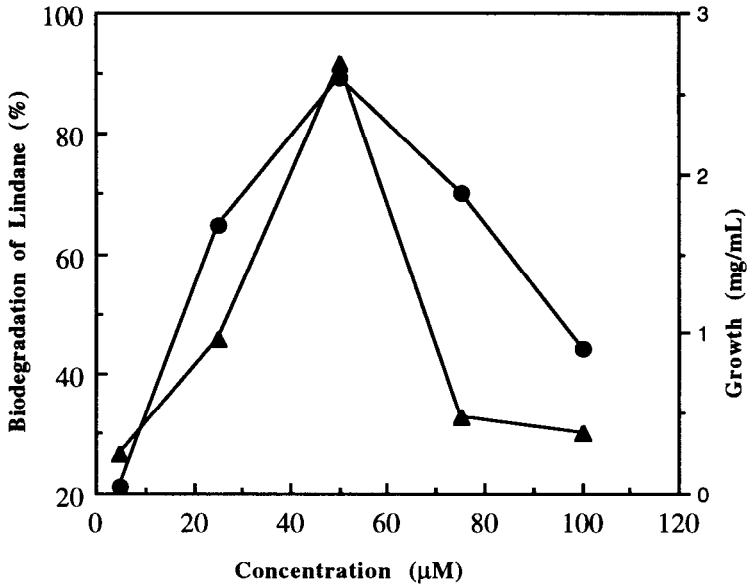

Figure 1. The effect of Lindane concentrations on degradation and growth of **Pleurotus sajor-caju** (•) : Biodegradation of Lindane (%). (▲) : Growth (mg. dry weight of mycelia /mL media). Culture was incubated at 30°C, 150 r.p.m., pH: 5, for 20 days.

incubation temperature 30°C, while optimum concentration was determined as 50 µM.

According to the available researches, the optimum Lindane concentration was found to be 50 µM in the degradation of Lindane by **Pl. sajor-caju** and in the growth of this fungus (Figure 1).

This situation makes us think that high Lindane concentration might produce a toxic effect on white-rot fungi. On the other hand, it was determined that the growth of fungus and degradation of Lindane were not large while the amount of Lindane concentration was so small (Figure 1). Other similar researches have shown that organic compounds like Lindane may be used as carbon and energy sources by white rot fungi (Bumpus & Aust 1985).

It was determined that amount of degradation of Lindane started to increase after 10 days of incubation time and reached the maximum level in 20 days where the effect of incubation time on biodegradation was verified (Figure 2).

Similar studies on biodegradation of other organic compounds by white rot fungi show us that the degradation begins between the time period of 3 - 6 days, and is maximal between 12 and 18 days after which this degradation continues at a reduction rate until the end of 30 days (Bumpus & Aust 1987).

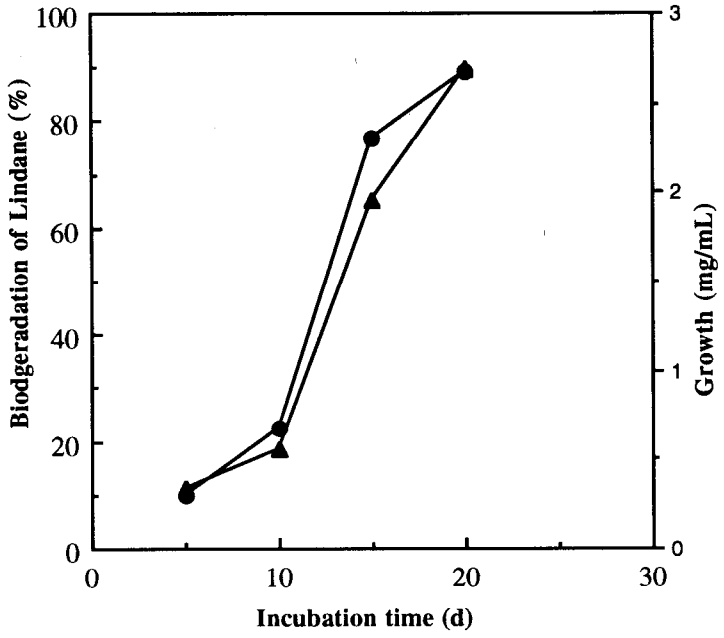

Figure 2. The effect of incubation times on degradation and growth of **Pleurotus sajor-caju** (•) : Biodegradation of Lindane (%). (▲) : Growth (mg. dry weight of mycelia /mL media). Culture was incubated at 30°C, 150 r.p.m., pH: 5 and 50 µM Lindane concentration.

The optimum pH of medium for both growth and degradation activity of Lindane by **Pl. sajor-caju** was determined as pH 5 (Figure 3).

The suitable temperature of culturation for maximum growth and degradation activity of Lindane by **Pl. sajor-caju** was found as 30°C (Figure 4). On the other hand, temperature of incubation at 30°C was favorable and satisfactory condition for good degradation activity of **Pl. sajor-caju.** High temperature of incubation at 40°C has been found to be required by **Phanerochaete chrysosporium** which takes part in white rot fungi for maximum degradation of lignin part in organic toxic pollutants (Champell 1983). During last ten years, valuable efforts have been spent by some investigators to degrade some recalcitrant organopollutants by **Ph. chrysosporium** (Bumpus et al. 1988; Bumpus et al. 1985; Bumpus & Aust 1985). So far this is the first report on using of **Pl. sajor-caju** in degradation of Lindane. According to data obtained during the shake-flask experiments, **Pl.sajor-caju** which degrades lignin 89.11 % seemed to have a capability in degradation of Lindane.

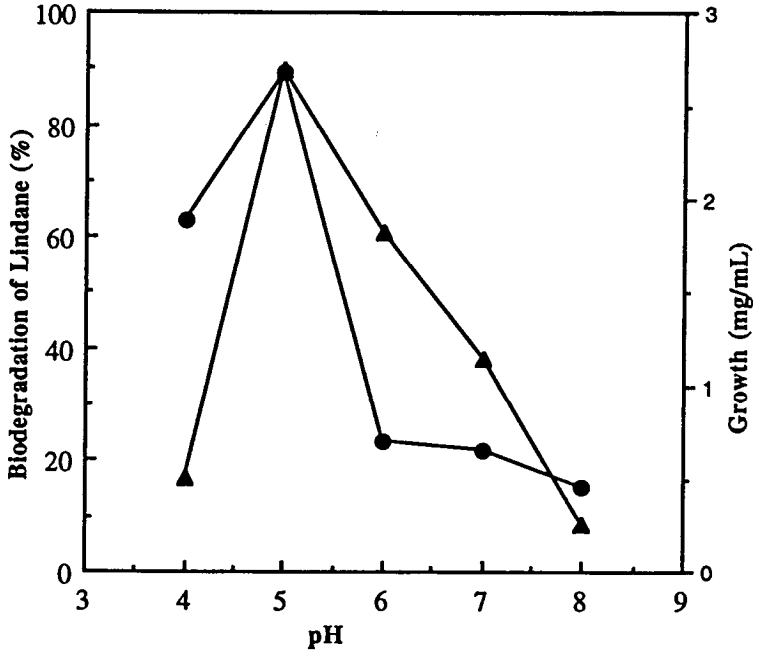

Figure 3. The effect of pH on degradation and growth of **Pleurotus sajor-caju** (•) : Biodegradation of Lindane (%). (▲) : Growth (mg. dry weight of mycelia /mL media). Culture was incubated at 30°C, 150 r.p.m., 50 µM Lindane concentration for 20 days.

Table 1. L D_{50}

Doses (mg/20)	0.75	2.25	3.75	5.25
Number of death	2	7	9	10
n	10	10	10	10
a	1.50	1.50	1.50	
b	4.50	8.00	9.50	
a.b	6.75	12.00	14.25	

$$LD_{50} = LD_{100} - \frac{\Sigma (a.b)}{n} = 5.25 - \frac{6.75 + 12.00 + 14.25}{10} = 1.95 \text{ mg/20 g}$$
$$= 97.50 \text{ mg/kg}$$

To verify the toxic effects of Lindane and its metabolites, primarily LD_{50} dose of Lindane was searched and LD_{50} found to be 97.50 mg/kg (Table 1).

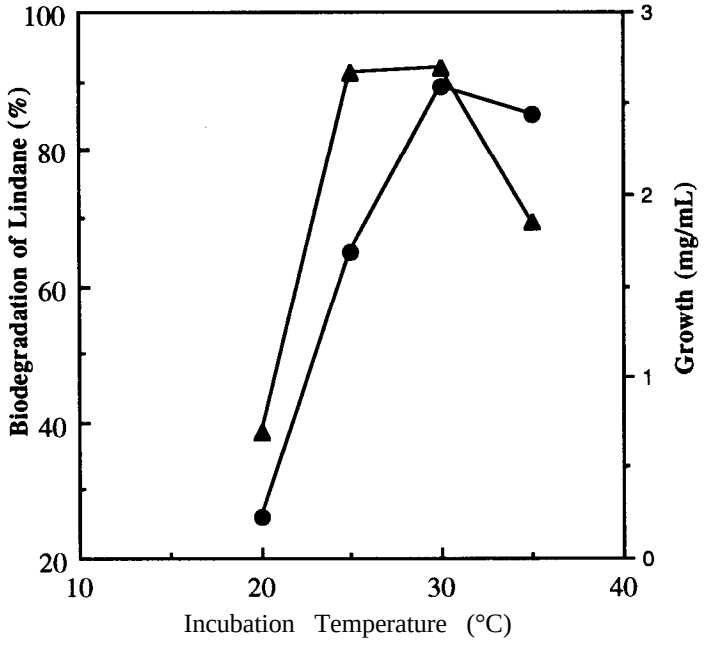

Figure 4. The effect of incubation temperatures on degradation and growth of **Pleurotus sajor-caju** (•) : Biodegradation of Lindane (%).(▲) : Growth (mg. dry weight of mycelia /mL media). Culture was incubated at 150 r.p.m., pH: 5, 50 µM Lindane concentration for 20 days.

When LD_{50} dose of Lindane is applied to mice, a decrease of lymphocyte counts is seen (as statistically significant), whereas an increase of Notrophyl counts is noticed (Table 2).

The decrease of lymphocyte counts in the blood may also be essential for the processing of antigens prior to the development of antibodies by immunocompetent lymphoid cells associated with them in the tissue (Fawcett 1986). Therefore, the reduction in lymphocyte counts caused by Lindane is important. It is seen that Lindane leads to the increase of reticulocyte counts. The increase of reticulocyte counts in the blood is explained as destruction of red blood cells.

When Lindane concentration which caused death in mice by 50 %, was degraded by fungi under optimum physiological conditions obtained metabolites, applied to mice caused no death. It was determined that the metabolites of Lindane lead to the decreased lymphocyte counts. Yet the metabolites of Lindane lead to lesser decrease of lymphocyte than Lindane.

Table 2. The effects of Lindane and metabolites on blood cells.

Groups	n	Control	Lindane	Lindane and metabolites
Lymphocyte	10	82.00±4.54	73.25±3.20*	78.25±1.25***
Notrophyl	10	16.00±2.94	26.25±3.09*	17.25±2.06
Monocyte	10	1.00±0.81	0.25±0.50	1.50±1.00
Eosinophyl	10	1.00±0.81	0.25±0.50	1.50±0.75
Young Nortophyl	10	0.00±0.00	0.00±0.00*	1.50±0.57***
Reticulocyte	10	4.25±0.47	4.87±0.28**	3.95±0.49

n : number of mice
* : important at the level of $p < 0.001$ statistically
** : important at the level of $p < 0.01$ statistically
*** : important at the level of $p < 0.05$ statistically

The reticulocyte counts were very similar to the control group, and there was not much statistical difference, when metabolites of lindane were applied to mice. On the other hand, taking the control group as base, it was observed that when lindane was applied directly, there is a quantitative effect on the cells in blood while no differential effects of metabolites are seen. Eventually lindane, which has toxic mutagenic and carcinogenic features, is accepted as an environmental pollutant and can be successfully degraded by **Pl. sajor-caju** which takes place in white rot fungi and after degradation of lindane no toxic effects of metabolites were observed due to acute toxicity.

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