Short Communication

Biological Evaluation of Soil Contamination Around a Non-Operating Pesticide Tomb

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Abstract

Our study was a biological evaluation of soil contamination near a non-operating pesticide tomb. The soil contaminated with pesticides had a negative effect on the growth of crustaceans such as *Heterocypris incogruens* (Ostracodtoxkit test) and the development of plants such as *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum* (Phytotoxkit test). This adverse effect persisted up to 5 years following the liquidation of the facility. The crustaceans under study were considered more useful in the evaluation of chronic toxicity than the root elongation test.

Keywords: pesticide tomb, soil contamination, Ostracodtoxkit, Phytotoxkit

Introduction

Among the wide variety of soil pollution by xenobiotics, chemical plant protection agents, also known as pesticides, play an important role [1]. It has been found, for example, that organisms living outside the treatment area may be exposed to a detectable adverse effect, even in a soil washout containing 1% of the applied dose [2]. This problem is associated to the largest extent with pesticide tombs. These concrete wells were constructed according to instruction 1/71 issued by Central Union of Agricultural Co-operatives and stored expired plant protection agents. Following the EU requirements, all registered facilities of this type should be eliminated by 2010. The agents deposited in the tombs included: chloroorganic insecticides (e.g. DDT, HCH and lindane, diene derivatives, chlorinated camphens), phosphororganic insecticides, carbaminians, dinitrophenols, phenoxy acids, s-triazines and mercury, arsenic, zinc, sulfur, and copper compounds [3]. Many of these banned compounds are Persistent Organic Pollutants according to the Stockholm Convention of 2001. They are highly toxic with acute or chronic toxicity, highly persistent in the environment and able to bioacumulate in the food chains – which constitutes a serious threat to biocenoses and human health [3]. After only a few years of operation, these facilities started leaking harmful substances to the environment. This was caused by inadequate selection of their locations, flaws in their construction, and the fact that in some cases pesticide waste was buried unprotected in the ground. As a result of chemical reactions occurring between organic solvents, water, pesticide active substances, and their metabolites, the tomb construction elements quickly corroded, the insulation layers degraded, and the facilities started leaking. Eventually, sometimes after only 3-4 years of operation, toxic substances migrated to soil, ground and surface waters, and to the adjacent areas. Many years later, some of the pesticide tombs under liquidation caused such extensive contamination of the nearby land and underground waters that the content of individual pesticides in the underground waters caused their degradation [4]. Some of these compounds, such as 2,4-D, MCPA, and simazines, were found to form durable complexes with copper and

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Table 1. The effect of soil contamination near a non-operative pesticide tomb on mortality (in %) length (in μm) and growth inhibition (in %) of crustaceans.

Feature	Sampling places						$LSD_{0.01}$
	control	center of the tomb	base of the tomb	25 m	50 m	125 m	
Mortality (in %)	11.7 a	23.3 a	18.3 a	18.3 a	8.3 a	13.3 a	15.8
Mean length in all replicants (in μm)	713.8 a	510.9 cd	505.3 cd	542.0 bc	562.1 b	473.2 d	44.0
Growth inhibition (in %)	-	39.5 bc	40.6 bc	33.4 ab	29.5 a	46.8 c	8.0

Means in the same line with a common letter are not significantly different (P<0.01).

mercury cations [5] which also occur in pesticide tombs. The measurement of the total xenobiotic content in the soil as a part of chemical monitoring does not provide, however, a full picture of the real threat they may pose to biocenoses. To measure this threat, the determination of the correlation between xenobiotic bioavailability and their ecotoxic effect on the environment is required [6]. The bioavailability of organic hydrophobic pollutants is one of the key issues in the evaluation of real threats associated with their presence in the soil. The main limiting factors in this regard are soil sorption properties, which can be significant even at contents of 0.1% of organic substance [7]. Bioanalytical techniques have been applied in recent years to better understand and evaluate the processes that occur in chemically contaminated soils [8]. It is stressed that a single evaluation criterion may be insufficient [9]. Apart from the traditional floristic, faunistic, and biocenotic studies based on the presence or the absence of particular species, a 2nd generation biological test (known as microbiotests) are becoming increasingly popular. Ready-to-use rapid test kits containing dormant organisms that can be easily resuscitated are increasingly being employed to assess environmental toxicity effects on a quantitative basis. These include single-cell organisms, complex organisms in early developmental stages, or small invertebrates and vertebrates. These can measure chemical threats – even those undetectable by traditional analytic methods. Many authors have reported on the usefulness of phytotoxicity tests in the analyses of soil contamination [10-13]. For this purpose, ready-to-use kits such as Phytotoxkit and Ostracodtoxkit can be applied [14, 15].

The aim of this paper was to evaluate soil contamination in the vicinity of the pesticide tomb in Warlity Wielkie near Ostróda, liquidated in 2004.

Material and Methods

In spring 2009, 5 soil samples were taken from a depth of 20-30 cm: one from the center of a non-operating pesticide tomb (placed on a gravel rise) and the others from the foot of the rise, at 25 m, 50 m, and 125 m distances from the base of the tomb. Previously, approximately 9.5 t of pesticides (mainly DDT) were deposited here and stored for 30 years. The loamy sand in this area was covered with hydrophilous vegetation. The soil was characterized by poor permeability and low organic content [16]. Soil samples taken for analysis were deprived of stone particles and remains of plants using sieves with 2 mm holes. Then they were dried and saturation of soil was checked according to pretreatment procedure. The experimental part of the study was carried out based on the OSTRACODTOXKIT chronic toxicity test and the PHYTOTOXKIT plant germination and early development test (distributor: TIGRET sp. z o o., Warszawa, producer: MICROBIOTESTS Inc., Belgium).

The crustaceans (*Heterocypris incogruens*) used in the OSTRACODTOXKIT test constitute a key component of the aquatic environment. They are common in freshwater bodies on all continents and were selected for the test due to their sensitivity to a wide variety of toxic substances. The test procedure involved 6-day exposure of young crustaceans covered with a standard medium to a thin layer of soil. The test was carried out in an All-Round-AL 185-4 incubator under controlled temperature and humidity in six replications. At the end of the test, two effects were determined: mortality and growth inhibition of the tested crustaceans. The laboratory experiment was carried out using a PHYTOTOXKIT test under controlled temperature and humidity conditions. The standard phytotoxicity test included one monocotyledon and two dicotyledon plants. The experiment was carried out in special transparent vessels that enabled direct observation and measurement of the root length. The plants used for the phytotoxicity tests such as sorghum (*Sorghum saccharatum*), watercress (*Lepidium sativum*), and mustard (*Sinapis alba*) germinate quickly and have high root and sprout growth rates. The observations and read-outs of the results were available within 3 days. The images were automatically registered using digital equipment.

Results and Discussion

Based on the data included in Table 1, a significant inhibition of crustacean growth without large differences in their mortality was found upon completion of the experiment. The greatest mortality (23.3%) was recorded among ostracods placed on the soil sampled from the tomb's center. Least significant difference ($LSD_{0.01}$) of crustacean length, statistically analyzed between mean results amounts to 44.0 μm. The largest organisms were noted in control (713 μm). The greatest growth inhibition (46.8%) was

Table 2. The effect of soil contamination near a non-operative pesticide tomb on germination of tested plants (in %).

Sampling places	Plant germination (%)					
	Watercress *Lepidium sativum*		Mustard *Sinapis alba*		Sorghum *Sorghum saccharatum*	
	3 days	6 days	3 days	6 days	3 days	6 days
Control	96.7 a	96.7 a	100 a	100 a	93.3 a	93.3 a
Center of the tomb	93.3 a	93.3 a	96.7 a	96.7 a	86.7 a	86.7 a
Base of the tomb	83.3 a	90 a	100 a	100 a	90 a	90 a
25 m	96.7 a	96.7 a	96.7 a	96.7 a	86.7 a	86.7 a
50 m	93.3 a	100 a	96.7 a	96.7 a	86.7 a	86.7 a
125 m	93.3 a	93.3 a	93.3 a	93.3 a	93.3 a	93.3 a
$LSD_{0.01}$	17.7	11.8	11.8	11.8	22.1	22.1

Means in the same column with common letter are not significantly different (P<0.01).
% – percent, µm – micrometer, mm – millimeter, LSD – least significant differences

Table 3. The effect of soil contamination near a non-operative pesticide tomb on growth of tested plants (in mm).

Sampling places	Length of roots (mm)					
	Watercress *Lepidium sativum*		Mustard *Sinapis alba*		Sorghum *Sorghum saccharatum*	
	3 days	6 days	3 days	6 days	3 days	6 days
Control	45.5 a	58.6 a	45.6 a	81.1 a	45.8 a	104.4 a
Center of the tomb	41.6 a	56.1 ab	48.3 a	67.3 ab	42.9 a	87.4 a
Base of the tomb	41.42 a	47.5 bc	42.9 a	67.9 ab	44.0 a	89.0 a
25 m	42.5 a	50.1 abc	34.5 a	53.7 b	45.2 a	91.1 a
50 m	38.8 a	46.1 c	50.7 a	66.7 ab	40.1 a	94.7 a
125 m	38.3 a	49.8 abc	51.5 a	73.0 a	42.3 a	85.4 a
$LSD_{0.01}$	13.0	9.4	10.9	18.7	10.1	32.7

Means in the same column with common letter are not significantly different (P<0.01).
% – percent, µm – micrometer, mm – millimeter, LSD – least significant differences

found for the test organisms living on the soil that originated from a 125-m distance from the base of the tomb. The results obtained from this part of the study indicate that the soil toxicity effects are still present up to 5 years following the liquidation of the tomb. The observed varied response of the indicator organisms could have been associated with the slow migration of toxic compounds that were gradually released from the soil sorptive complex following physical, chemical, and biochemical reactions [17]. Other authors [18] indicate that in the leachates, trace amounts of chemical contamination can be observed many years after elimination of pesticide tombs, whereas the accumulation of pesticide pollutants in the surface waters cannot be observed due to their rapid biological decomposition.

Based on the results from the PHYTOTOXKIT test, the contaminated soil did not inhibit the germination of the tested plants (Table 2). Seed germination could have been inhibited only at considerably high substance concentration in the soil [19]. The data included in Table 3 shows a significant reduction in root length after a 6-day exposure of watercress to the soil sampled from a 50-m distance from the base of the tomb ($LSD_{0.01}$=9.4 mm), as well as after the exposure of mustard to the soil sampled from a 25-m distance from the base of the tomb ($LSD_{0.01}$=18.7 mm). Statistical analysis showed that these differences were significant. This indicates that the degree of ground contamination by the remnants of the readily-available substances in the soil solution significantly affected the experimental plant growth [20]. Based on the study results, it became clear that crustaceans such as *Heterocypris inconguens* are more useful in the evaluation of soil contamination near the non-operating pesticide tomb than the plants such as *Lepidium sativum*, *Sinapis alba*, or *Sorhum saccharatum*. The above findings are also in the case of evaluation of compost and sewage sludge contamination with polycyclic aromatic hydrocarbons [15].

Conclusions

The study results indicate there is still a persistent environmental threat posed by pesticides that migrated to the soil in the vicinity of the liquidated pesticide tomb in Warlity Wielkie near Ostróda. An analysis of the results leads to the following conclusions:

1. The pesticides remaining in the soil in the vicinity of the non-operating pesticide tomb did not affect the tested crustaceans' mortality or the germination capacity of the tested plants.
2. The great susceptibility of the crustaceans to soil contaminated 125 m from the studied facility indicates that the remaining pesticides have migrated from the tomb to further areas.
3. The results of the study involving 6-day-old mustard and watercress seedlings indicate the phytotoxic effect of the remaining pesticides at distances of 25 and 50 m from the base of the non-operating pesticide tomb, with no effect on sorghum.
4. Among the chronic toxicity tests, the Ostracodtoxkit was found to be more useful than the Phytotoxkit for the evaluation of soil contamination by the remaining pesticides.
5. Based on the study results, it was found that the process of natural soil cleaning occurring after the elimination of such tombs is very slow and difficult to evaluate. The evaluation of the contamination requires systematic studies to monitor the contaminated areas, including the underground water leachates that contribute to accelerating the migration of contaminants. Use of well defined empirical water leachates tests with *Daphnia magna* and *Thamnocephalus platyurus* should also be considered.

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