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Detection of Parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellín, Colombia

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ABSTRACT

High Performance Liquid Chromatography (HPLC) was used to determine and quantify Parathion in insects collected from decomposing rabbits previously injected with 5mg/kg of weight of commercial Methyl Parathion. Its effect on the succession of medically and legally important insects was studied.

Three rabbits were given a lethal dose of Parathion by intracardiac injection and a fourth was killed by cervical dislocation. Samples of liver were taken from each to verify the presence of Parathion in the tissue. A high concentration of the product was found with values between 1.379 mg and 1.68 mg per Kg of weight.

Presence of Parathion was detected in insects collected at each stage of decomposition, from fresh to dry remains and at different stadia of development of 10 species of arthropods from the orders Diptera, Coleoptera, Hymenoptera, Isopoda and Acari.

Introduction

Insects can be used as an alternative for toxicological analysis when it is not possible to obtain samples such as blood, urine or internal organs due to the advanced state of decomposition or skeletonisation of a corpse [1-4].

Several studies illustrate the great potential of entomotoxicology for providing additional information on cause of death, postmortem interval, and geographical area where the body was found [5], [1], [3]. Psychoactive substances and antidepressants have been detected by diverse methods in different stadia of insects, such as pupae of Phoridae, exuviae and faecal material of Dermestidae [6], *Cochliomyia macellaria* larvae [7] and other diptera [8, 9].

The majority of studies in entomotoxicology, either at experimental level or case studies, have dealt with barbiturates [5, 7, 10, 11], opiates [9, 12-18], diazepam [4], cocaine [8, 19], MDMA (ecstasy) [20], Amitriptyline, nortriptyline [6, 11, 21, 22] and heavy metals [23]. However, very few studies have focused on organophosphorus compounds [24].

It has been shown that toxicological analysis by liquid chromatography is more sensitive using insect larvae than body tissue [25]. This increases the importance of using insects in this type of research and investigation.

Parathion (C10H14NO5PS) is an insecticide and acaricide widely used in agriculture. It is strongly absorbed by the soil surface but is degraded by photolysis. It is estimated that it disappears from the soil within one week [26, 27]. It is broken down into pnitrophenol, diethyl thiophosphoric acid and paraoxon [28, 29].

Although this type of research is at its initial stages in Latin America [4] the number of cases of intoxication by toxic substances or drugs is considerably high. This study contributes to forensic research in Colombia in the field of entomotoxicology.

Methods

This study was carried out in urban conditions, within the campus of the Universidad de Antioquia, at an altitude of 1450 m above sea level, temperature between 17 and 26 ºC and average rainfall of 1031 mm [30].

Four rabbits were used (2.50 – 2.80 kg in weight). Three of them (S1, S2, S3) were used to evaluate the effect of Parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) and a fourth as the control (Sctrl). The four rabbits were first given 1 ml/kg of weight of Sodium Penthotal to anaesthetise them (suggested by the Universidad de Antioquia Committee on Ethics). The rabbits S1, S2 and S3 were given a lethal dose of 5 mg/kg of Parathion by intracardiac injection and 2 ml orally. The control rabbit (Sctrl) was killed by cervical dislocation. The rabbits were subsequently placed in metal cages (60 x 50 x 50 cm) with a 20 m distance between each.

Arthropod samples and data collection

Samples for the study of insect succession were collected over a period of 28 days, three times per day for the first 12 days, 2 times per day for the next three days and once per day for the last 12 days. The arthropods were collected in the following order: First, those flying over and/or landing on the carcasses; then those which were found in natural cavities (eyes, nose, mouth, anus) and in the wound; and finally those that were under the carcass and in the soil to a depth of 10 cm. The collected material was fixed in 70% alcohol in the case of immature specimens. Adults were killed with ethyl acetate and mounted on entomological pins. Environmental and body temperatures were noted at each sampling. Each carcass was weighed once a day.

Toxicological Analysis

A toxicological analysis was carried out on the liver of each rabbit and the insects collected.

As soon as the rabbits were killed, a small incision was made in the abdomen and a 20 g liver sample was taken. This was stored in a dry tube and frozen at -4ºC.

Every 48 hours an additional sample of the different species of insects was collected for toxicological analysis. The same process was followed as for the liver samples.

The method used to quantify the amount of Parathion was High Performance Liquid Chromatography (HPLC). The following HPLC condition was used in the chromatographic analysis: Mobile Phase: water/acetonitrile; Programme: 15:85 (ACN:H2O) 5 minutes at a flow rate of 1 ml per minute, then changed to 70:30 (ACN:H2O) for 15 minutes; DAD Detector: λ = 274 nm; Solvent: 70:30 ACN:H2O; Column: Waters µBondapak S18: 3.9 x 300 mm; Retention time for Parathion: 12.89 minutes.

Preparation of Samples

The samples were prepared following Thompson *et al* [31]. The sample was weighed, macerated and 0.5 ml of HPLC grade water was added. It was sonicated in order to liberate the analyte, centrifuged to separate the supernatant fraction (to obtain the greatest amount of analyte) and isolated by decantation. This extraction process was carried out in triplicate and the supernatant was collected in one container. The analyte was then extracted with solvents – three extractions of 0.5 ml each time. It was dried in the dark and reconstituted with 1.5 ml of ACN:H20 (70:30) and transferred directly to an HPLC vial for analysis.

Validation of the method

Calibration Curve

The standard solution of Parathion was prepared from pure Parathion, diluted with HPLC grade acetonitrile. Standards in the elution solvent (ACN: Water: 70:30) were prepared from the diluted Parathion. In the table 1 shows the raw data used for the calibration curve (tab. 1, fig. 1). The calibration curve was produced by plotting Area (UA) against Concentration (ppm) of the pure standard using the statistics package, Statgraphic 5.0. To establish the quantifiable limits, linearity was evaluated at concentration values at both extremes of the curve. It was found that Parathion is quantifiable within a range of 0.1 ppm to 20 ppm (tab. 1, fig. 2). Figure 2 clearly shows a linear relationship between the variables area and concentration. Correlation coefficient = 0.998765 and R2 = 99.7532%.

Conc. (ppm)	Area	Area / Conc.	log conc.
0.4	1734150	4335375	-0.397940008672
0.4	1812787.75	4531969.375	-0.397940008672
0.6	2468823.25	4114705.41667	-0.221848749616
0.6	2539573.50	4232622.5	-0.221848749616
0.8	3359754.5	4199693.125	-0.0969100130081
0.8	3405949	4257436.25	-0.0969100130081
1	4112264	4112264	0
1	4105239.75	4105239.75	0
1	4194453	4194453	0
5	20208050	4041610	0.698970004336
10	42588016	4258801.6	1
15	62593800	4172920	1.17609125906
20	92452288	4622614.35	1.30102999566
0.192	808193.5	4209341.14583	-0.716698771296
0.192	806933.19	4202777.03125	-0.716698771296
0.122	533457.56	4372602.95082	-0.913640169325
0.122	553866.44	4539888.85246	-0.913640169325

Tab. 1. Raw data for calibration curve

Detection of Parathion in arthropods

A total of 53 specimens, collected at different stages of decomposition of the rabbits were analysed for parathion: 29 diptera (24 larvae, 3 pupae, 1 pupa case, 1 adult), 13 coleoptera (adults), 6 hymenoptera (adults), 1 hemiptera (adult), 1 isopod and 3 acarids (adults).

Parathion was detected in the following diptera: *Phaenicia sericata, Musca domestica, C. Albiceps, Fannia scararis*, coleoptera: Tenebrionidae, Dermestidae and Staphylinidae, and in ants of the genus *Doymyrmex*, in 1 isopod and acarids. However, the small amount assimilated only allowed its detection, not quantification (Tab. 3 and 4).

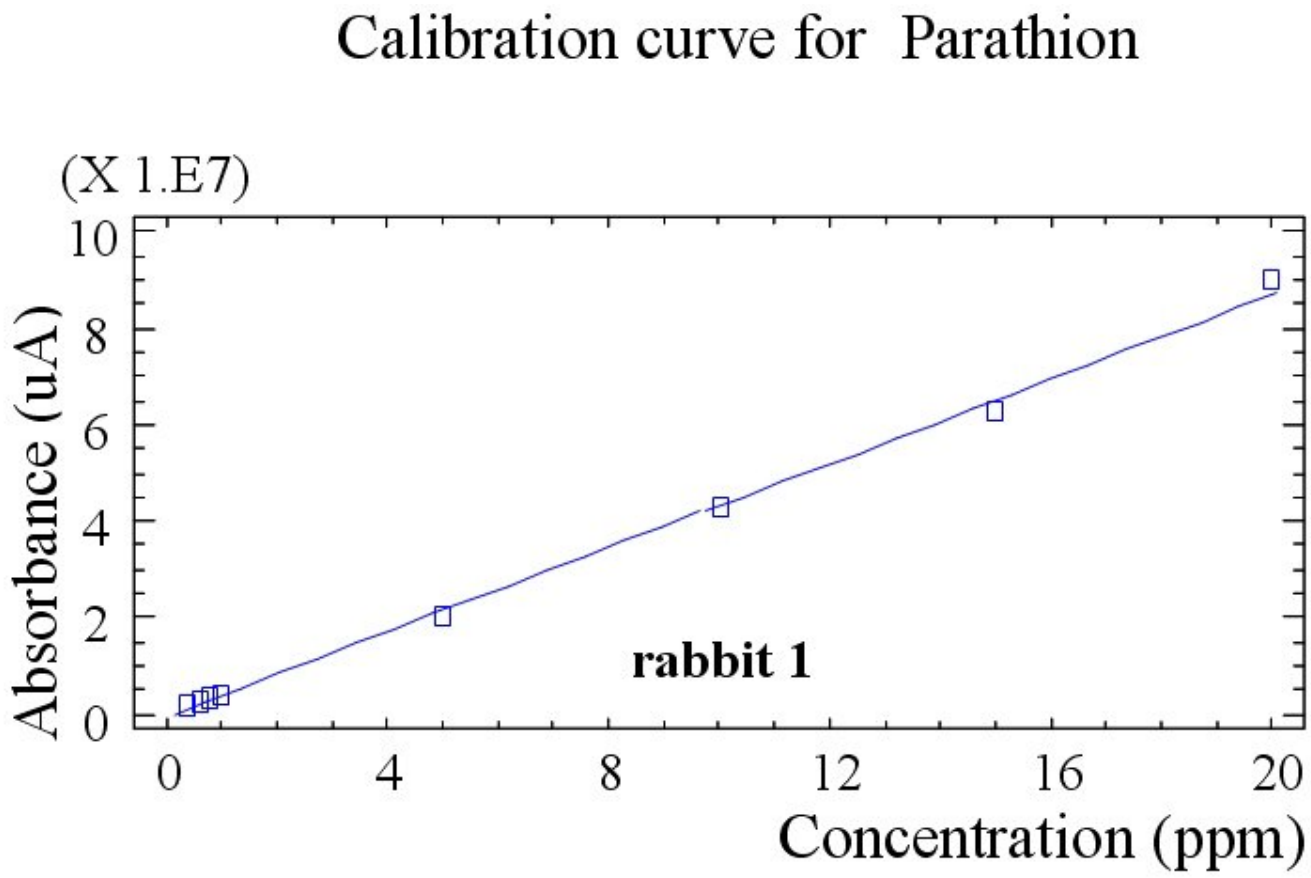


Fig. 1. *Calibration curve for parathion by HPLC*

Retrieval

An exact quantity of control larvae was weighed (6 in total), four of them were dosed with 200 µl of methyl parathion at 5 ppm. They were put through the same process as the initial sample. For the final extraction, in two of the dosed samples the analyte was extracted with 3 portions of 0.5 ml ethylic ether GR and the other two with the same quantity of reactive grade Toluene. The other two were treated as a control. A recovery of 82% was found for the dosed rabbits, showing that the extraction method was reliable.

Results

Arthropod Succession

Over a period of 28 days, a total of 987 individuals were collected, belonging to 10 orders, 34 families, 17 genera and 7 species.

Each of the rabbits went through 5 stages of decomposition: fresh (day 0 to 2); bloated (day 3 to 5); active decomposition (day 6 to 9), advanced decomposition (10 to 13) and dry remains (day 14+). However, in S1 a mummification stage was seen from day 12 to 24, not observed in the other rabbits.

Detection of Parathion in the liver

The livers showed a high concentration of parathion. Values between 1.4 mg and 3 mg of Parathion per Kg of rabbit were detected. No presence of the product was found in the control (fig. 3 and 4, tab. 2).

Sample	Weight (g)	Area (UA)	mg / Kg
Rabbit 1 Liver	0,5312	60522390 ± 200097.08	1,38
Rabbit 2 Liver	0,6825	148278472 ± 239228.37	2.97
Rabbit 3 Liver	0,9374	87589624 ± 1066225.8	1,68

Tab. 2. Values for the detection of Parathion in the liver samples

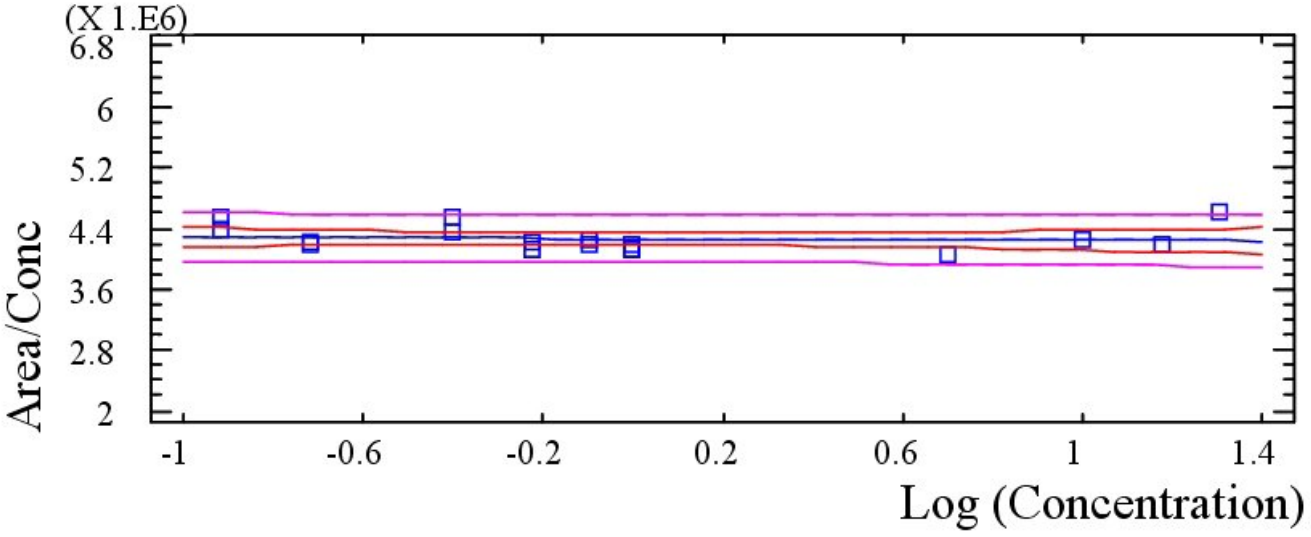


Fig. 2. *Evaluation of the linearity of the calibration curve (90-110% confidence limit)*

Conclusions

The presence of parathion only repelled arthropods or had insecticidal effects at the mouth of the treated rabbits. No relevant differences in insect succession were observed between the four rabbits. This contrasts to observations with Malathion, where oviposition was delayed and differences were seen in the number of taxa found on carcasses with or without the insecticide [24].

Phaenicia sericata was the first to arrive at the bloated stage and remained until the end of the active decomposition. The same was true of *Cochliomyia macellaria*, although it was observed in smaller quantities.

The process of mummification observed in rabbit 1 meant that the dry remains stage began later than in the other rabbits. This may have been because the carcass was located in a sunnier area causing the body to dry out, avoiding the stages of putrefaction. It was possible to detect and quantify appreciable levels of parathion in the liver samples by means of HPLC. As was expected, parathion was not detected in the control.

With regard to the detection of parathion in the insects, its extraction was possible from 10 specimens of the following orders: Diptera, Coleoptera, Hymenoptera, Isopoda and Acari in different stages of

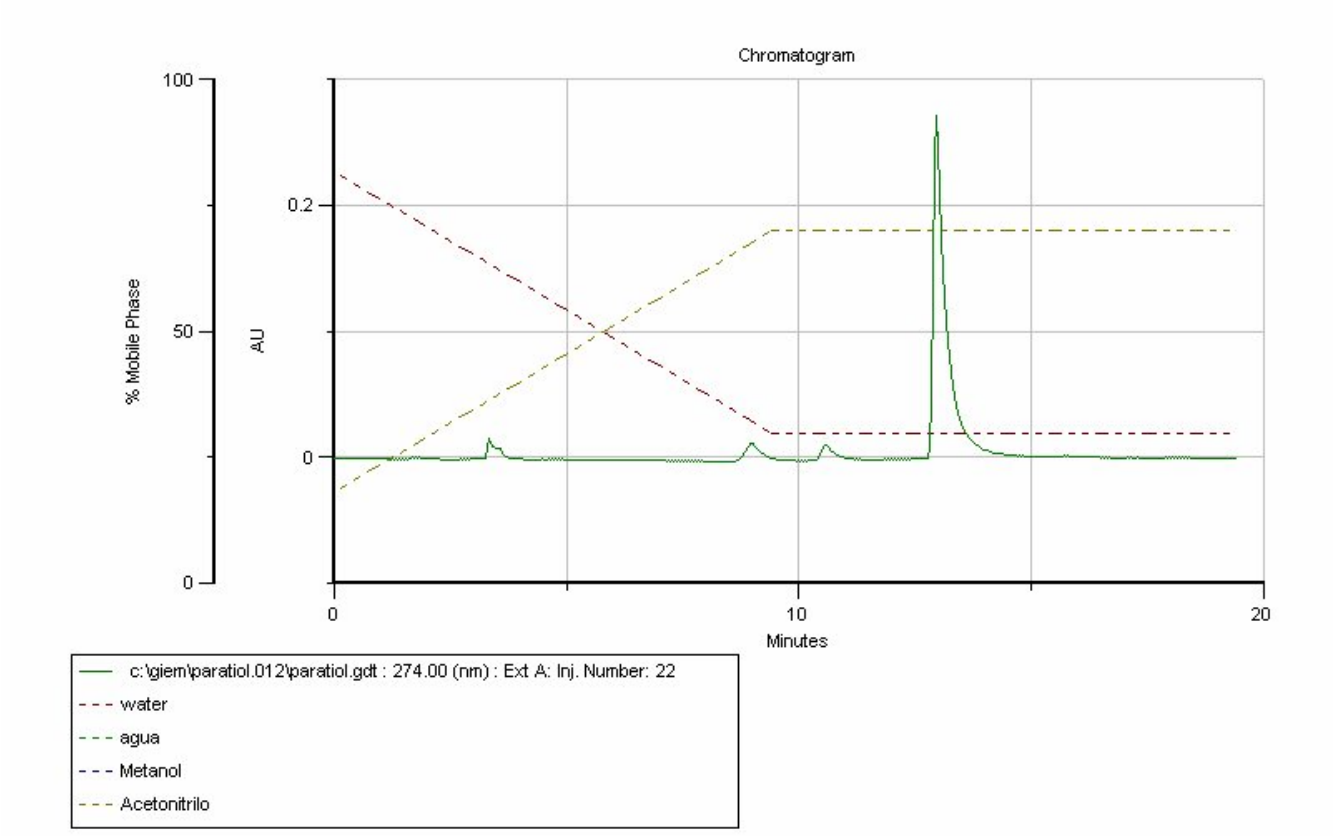


Fig. 3. *Chromatogram for Methyl Parathion extraction from the liver of rabbit 1.*

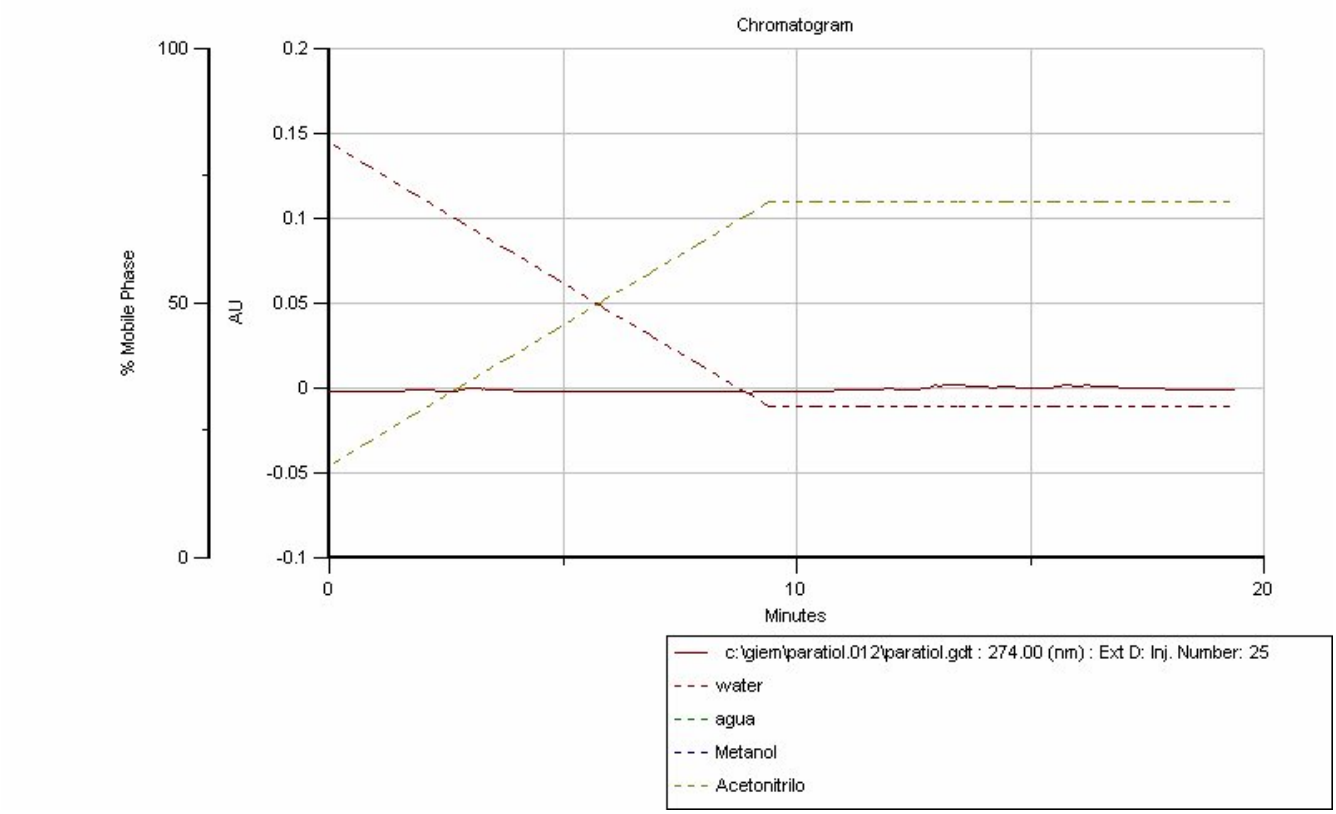


Fig. 4. *Chromatogram for Methyl Parathion extraction from the liver of the control rabbit.*

development (larva, pupae, pupae cases and adult) and at all stages of decomposition. At the bloated stage, parathion was detected in 3 larvae of *P. sericata*. At active decomposition in L3 of *C. albiceps* and in pupae and pupae cases of *M. domestica*. The highest number of species found containing parathion was at the dry remains stage; in pupae of *Musca* sp, in adults of Coleoptera (Dermestidae, Tenebrionidae and Staphylinidae), in *Dorymyrmex sp* ants, which were very abundant throughout the study. Parathion was also detected in isopods and acarids, which despite their small size accumulated high enough levels to enable its detection. This is probably due to their chitinous epidermis [3].

High Performance Liquid Chromatography (HPLC) is an efficient technique for detecting and quantifying parathion in tissues as well as in arthropods present from the initial to the final stages of decomposition, including dry remains.

Sample Material analysedStage Average areaObs.

Sample		Material analysed	Stage	Average area	Obs.
Rabbit 1	Diptera	P. sericata	L3		ND
		P. sericata	L1		ND
		M. domestica	L3	20540,43	NQ
		Muscidae	Pupa	393,50	NQ
		Stratiomyidae	L3		ND
		Stratiomyidae	L3		ND
		Stratiomyidae	L3		ND
	Coleoptera	Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult	23201,88	NQ
		Dermestes sp	Adult		ND
		Dermestes sp	Adult		ND
		Dermestes sp	Adult	194,96	NQ
		Staphylinidae	Adult	22423,72	NQ
	Hymenoptera	Dorymyrmex sp	Adult	437,13	NQ
		Dorymyrmex sp	Adult	335,45	NQ
	Hemiptera	Pentatomidae	Adult		ND
	Isopoda		Adult	535,85	NQ
	Acari		Adult	569,99	NQ
			Adult	1155,43	NQ
			Adult	645,94	NQ
Rabbit 2	Diptera	P. sericata	L1		ND
		P. sericata	L3		ND
		P. sericata	L3	6174,07	NQ
		M. domestica	L3		ND
		M. domestica	L3		ND
		M. domestica	L3		ND
		Muscidae	Pupa	102307,71	NQ
		Muscidae	Pupa case	115883,31	NQ
		Sarcophagidae	Adult		ND
		F. scalaris	L3		ND
		F. scalaris	L3		ND

Sample		Material analysed	Stage	Average area	Obs.
		F. scalaris	L3		ND
	Coleoptera	Dermestes sp	Adult		ND
		Dermestes sp	Adult		ND
	Hymenoptera	Dorymyrmex sp	Adult		ND
		Dorymyrmex sp	Adult	133869,04	NQ
		Dorymyrmex sp	Adult		ND
Rabbit 3	Diptera	P. sericata	L3		ND
		P. sericata	L3	22689,37	NQ
		P. sericata	L3		ND
		C. albiceps	L3	22937,76	NQ
		M. domestica	L3		ND
		M. domestica	L3		ND
		M. domestica	L3	22852,53	NQ
		M. domestica	L3	85753,965	NQ
		M. domestica	L3		ND
		Muscidae	Pupa		ND
		F. scalaris	L3	41883,86	NQ
	Coleoptera	Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult		ND
		Tenebrionidae (Strogylium sp)	Adult		ND
	Hymenoptera	Dorymyrmex sp	Adult		ND

Tab. 3. Arthropods analysed by HPLC to detect parathion

ND: not detectable, NQ: not quantifiable

Order	Species	Dev. Stage	Stage of decomposition
Diptera	P. sericata	L3	Bloated
	C. albiceps	L3	Active D
	M. domestica	L3	Active D/advanced
	F. scalaris	L3	Dry remains
	Muscidae	Pupa and pupa case	Dry remains
Coleoptera	Strongylium sp.	Adult	Dry remains
	Dermestes sp.	Adult	Dry remains
	Staphylinidae	Adult	Dry remains
Isopoda		Adult	Bloated
Acari		Adult	Active D/advanced/ Dry remains
Hymenoptera	Dorymyrmex sp.	Adult	Fresh/Dry remains

Tab. 4. Arthropods with positive detection of parathion, stadium of development and stage of decomposition at which it was collected.

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