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Assessment of insects diversity with influence of industrial pollutants in agricultural zones of District Sialkot, Pakistan

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Abstract. *Asghar A, Qadeer O, Mushtaq S, Maalik S, Majeed W, Bano N, Nargis S. 2022. Assessment of insects diversity with influence of industrial pollutants in agricultural zones of District Sialkot, Pakistan. Biodiversitas 23: 2047-2053.* Insects are the most important creatures on earth, due to their multifunctional role in the environment by providing different services such as biological indicator, decomposer, pollinator, scavenger, pest, predator in the agroecosystem. But with the passage of time due to anthropogenic activities such as, environmental pollution, industrial waste, use of effluents for agriculture purpose, the insect fauna has been facing the dramatic threat to their diversity particularly in most polluted areas. The present study was designed to find insects diversity variation under polluted environments (heavy metals) in agriculture fields having brassica and Rice crops. Two different sites, tannery and non-tannery area were selected for the sampling of insects. The total number of insects found in tannery area fields were 2195 and 3753 from the non-tannery area. The abundance of *Melanoplus bivittatus* (Say, 1825) was highest in non-tannery area and *Acrida exaltata* (Walker, 1859) was highest in tannery area fields. From the tannery area and non-tannery area, the maximum abundance 32% and 37% was found for order orthoptera. The cadmium concentration in tannery rice crops was 0.626 ppm and brassica concentration were 0.315 ppm. The concentration of cadmium in non-tannery area samples was 0.115 ppm for rice and 0.101 ppm for brassica plants while 0.1231 ppm for rice and 0.104 ppm for brassica insects. The concentration of lead in tannery areas of rice crops was 0.637 ppm and brassica concentration were 0.672 ppm. The concentration of lead in non-tannery area samples was 0.324 ppm for rice and 0.267 ppm for brassica plants while 0.214 ppm for rice and 0.266 ppm for brassica insects. The diversity and richness were recorded 3.11 and 3.525 for rice while 3.124 and 3.913 for brassica fields, respectively. The species maximum was seen in the non-tannery area and less abundance was recorded in the tannery area. It is further suggested that preventive measures should be taken to protect the insect diversity for balancing the ecological balance in the nature.

Keywords: Agricultural landscapes, diversity, heavy metals, insects.

INTRODUCTION

Sialkot is a major city of Pakistan, well known for its leather industry, cutlery and surgical instruments worldwide (Abdullah et al. 2015; Ali et al. 2015). There are 3229 industrial units; over 250 are tannery units, producing 547-814 m^3 of tannery effluents per day. Due to the discharge of untreated industrial effluents, soil contamination have emerged as severe environmental problem (Ali et al. 2015). Due to continuous exposure to these chemicals, tannery workers may face a variety of health issues, including eyes irritation, respiratory tract problems, and various types of cancers such as buccal, lung, bladder, and pancreatic (Hashmi et al. 2017). The tannery industry is considered among the primary pollutant of the environment. Discharge of untreated effluents from tanneries has become a potential threat to soil and water pollution. Tanneries use over 250 chemicals for leather production and thus release a complex mixture of lethal chemicals such as chlorinated organic phenols, heavy metals chromium, cadmium, nickel, lead and azo-dyes etc. (Bosnic et al. 2000; Shukla and Vankar 2014).

The level of chromium and cadmium is observed many times higher in tannery wastewater than the permissible values suggested in Pakistan (Murtaza et al. 2010). These heavy metals are toxic for human health, even in low concentrations. Worldwide heavy metals contamination due to industrial effluents is gaining attention. Because of the indiscriminating discharge of metal-rich effluents, toxic or lethal sludge, and poisonous gases into the surrounding environment, the tanning industry is getting particular concern which is ultimately causing considerable damage to the environment (Ali et al. 2013). It is crucial to monitor these heavy metals in tannery effluents regularly, and its remedial measures are necessary to protect the environment as well human health in tannery areas (Bukhari et al. 2012).

Insects are diverse due to their variety of shape, function, considerable biomass, diversity, and relationships with other species and their surrounding environment (Zhang et al. 2001). Even though insects are ubiquitous and have extensive connections with plants and other animals.

Studies that include faunal biomass assessments and status reviews of key indicator groups like butterflies and charismatic individual species show a decline in insect diversity and abundance (Forister et al. 2019; Wagner 2020). The loss of threatened insect species previously has been predicted many times although it is a giant dilemma until now. The major cause of species loss is habitat degradation, environmental pollutants, industrial raw waste etc. The situation has worsened as recent reviews have revealed declines across multiple continents that outweigh the loss of individual species in terms of functional and ecological impact (Dirzo et al. 2014; Janzen and Hallwachs 2019; Montgomery et al. 2020; Sánchez-Bayo and Wyckhuys 2019). Although Tessaro et al. (2016) stated that beetles are biological indicators of the soil when chemical and organic fertilizers are used to enhance the quality and productivity of the soil.

From different areas of the globe, the decrease in the total biomass of the insects is being stated in multidecadal studies. During large scale monitoring in Ohio, United States 33% decrease in the abundance of the butterflies was observed in approximately 21 years (Wepprich et al. 2019); in Scotland from 1975 to 2014 in Rothamsted insect survey, 20% of the reduction was observed (Dennis et al. 2019). The reduction of 70% total biomass of flying insects was observed (Hallmann et al. 2017) from 1976 to 2012.. Many studies have also revealed that heavy metals cause soil pollution, which negatively affects the soil macrofauna (Nahmani et al. 2005; Tessaro et al. 2016).

Basically, Pakistan is an agricultural country but due to increasing urbanization the rapidly development in industrialization have ultimately resulted in the discharge of toxic effluents, polluting water bodies and making them unsuitable for consumption in different practices, including the agricultural sector. Generally, heavy metals are present in low levels in agricultural soils, but their toxicity cause hazardous effects on plants and soil insects diversity. Increasing the number of heavy metals in the environment by industrialization and frequent use of fertilizers in agricultural practices and domestic activities has become the main problem, leading to harmful effects on the agro-ecosystem (Khan et al. 2016). Due to excessive agricultural activities and uncontrolled industrial spread, Sialkot and its populations are extremely exposed to hazardous environmental pollutants (Junaid et al. 2016, 2017).

The present study is an interesting combination of these facts, which aims to determine the insect's diversity of region Sialkot with influence of tanneries wastewater, especially heavy metals like Cadmium and Lead, and their concentration in plants and insects. The present study is a key step to take notice of these activities to reduce environmental problems concerning future perspectives.

MATERIALS AND METHODS

Study area

An initial survey was conducted for selecting fields for insects collection. As most tanneries are present in the Sialkot Tannery zone, this area was selected as a "tannery area sampling site". Secondly, we selected Pasruras "non-tannery area sampling site". In these areas, brassica and rice crop fields were selected to sample insects. Sites were selected at the nearest possible distance to the functioning industrial units. Characteristics of the study are presented in (Table 1).

Insect collection

The samples were collected for six months in the year 2019. Sweep net and quadrat method were used for insects collection. The net was swept in 1m quadrats selected on the sides and in each field's center. Hand-picking with forceps was also used in the quadrats and picking insects from the net. The collected insects were temporarily stored in glass jars and polythene zip lock bags.

Insect preservation and Identification

The collected insects were brought to the Zoology departmental lab of Government College Women University Sialkot. Distilled water was used to wash the insects to remove dust particles attached to their bodies. Insects were then preserved in the solutions of 70% ethanol and 2-3 drops of glycerin. The pinning method preserved soft-bodied insects that were prone to discoloration or degradation. Insects specimens were identified using the literature (Triplehorn et al. 2005), the fauna of British India, stereomicroscope and internet keys. Insects were labeled with their scientific name, crop name and date of collection.

Insects sample analysis for heavy metals Cadmium (Cd) and Lead (Pb)

For determining heavy metals Cd and Pb accumulation in insects, samples were prepared for atomic absorption spectroscopy. Freshly collected insect samples were washed with distilled water. Insects were dried for a week in a drying oven at 65-70°C. Dried insects were grinded for further process. Once the fine powder was obtained, it was weighed 0.3g on an electric balance. 4 ml HNO_3 was added to digest insect samples and heated on a hot plate for 50 minutes. 2 ml $HClO_4$ was added after digestion and the solution was again heated for one hour. After the color of the solution disappeared, 50 ml solution was prepared by adding distilled water. The prepared sample was stored at 4°C. It was used for heavy metal detection with an atomic absorption spectrophotometry.

Table 1. Topography of selected areas for study

Characteristics	Sialkot	Pasrur
Coordinates	32.4945° N, 74.5229° E	32.2625° N, 74.6576° E
Vegetation/Crops	Brassica and Rice crops	Brassica and Rice crops
Soil type	Clay loam	Clay loam
Average Annual Temperature (°C)	27.72	28.16
Average Annual Humidity (mm)	69.41	74.23

Plant analysis

Plant samples were taken from brassica and rice fields. They were washed under tap water and then distilled water to remove dust. After this, plants were cut into 2-inch pieces using a sharp blade. Cut pieces were air-dried for a week to remove all the moisture. After the drying procedure completed, plants were grinded into fine powder. To prepare the solution for atomic absorption spectrophotometry, 0.4g plant powder was added in a flask along with 4.5 ml HNO_3 for digestion and heated for one hour on a hot plate. After digestion 2 ml $HClO_4$ was added and heated for another hour. Once the solution became colorless, it was diluted up to 50 ml with distilled paper. The prepared solution was used in atomic absorption spectrophotometry for heavy metal detection.

Statistical analysis

Values obtained from monthly samples were treated for statistical analysis purposes. The insects' diversity was measured using the Shannon diversity index (Magurran 1988). The mean, maximum and minimum values were tabulated to measure the concentration of lead and cadmium. The individual rarefaction curve and species abundance distribution was measured to find the significance of data and flow of species diversity in both the selected sites. All the data were analyzed at the level of $\alpha = 0.05$ using PAST software and Excel.

RESULTS AND DISCUSSION

Results

The total number of insects found in tannery area fields were 2195 out of which 1203 were collected from rice while 992 specimens were collected from brassica crops. These specimens were belonged to 33 species, 21 families and 7 orders. The number of *Melanoplus bivittatus* was highest in tannery area fields. There were 11 species which were different in both crops. The total number of insects found in non-tannery area fields were 3753, out of which 1739 were collected from rice, while 2014 specimens were collected from brassica fields. The number of *Acrida exaltata* was highest in tannery area fields. There were 10 species that were different in both crops (Table S1).

From the tannery area, the maximum abundance (32%) was found for order orthoptera and Lepidoptera (19.69%), while the least was found for the Hymenoptera (Figure 1). Moreover, there were species from six orders recorded from rice crop and seven orders in brassica crops. No species belonging to order Hymenoptera was recorded in the rice crop. Order orthoptera showed the highest relative abundance in both crops, i.e., 34.75% for rice and 29.94% for brassica crops (Table 2).

In the non-tannery area, the maximum abundance was found for order orthoptera (37%), Lepidoptera (15%) and Coleoptera (15%), while the least was found for the Hymenoptera (1%) (Figure 2). Moreover, six orders were recorded from rice and brassica crops. No species belonged to order Odonata was recorded in rice crops and Hymenoptera in brassica crops. Order orthoptera showed the highest relative abundance in both crops, i.e., 30.76% for rice and 42.65% for brassica crops (Table 3).

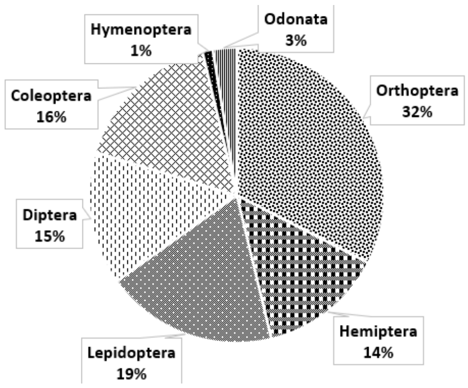

Figure 1. Order wise insect diversity in tannery area of Sialkot, Pakistan

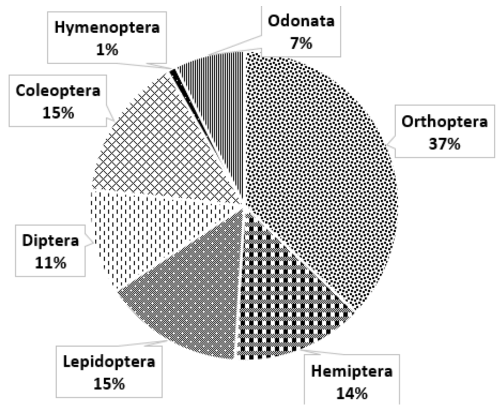

Figure 2. Order wise insect diversity in non-tannery area

Table 2. Order wise % relative abundance in tannery area

Order	Rice (%)	Brassica (%)
Orthoptera	34.75	29.94
Hemiptera	14.21	13.00
Lepidoptera	18.37	19.69
Diptera	12.05	17.74
Coleoptera	16.96	16.03
Hymenoptera	0.00	2.52
Odonata	3.66	1.31

Table 3. Order wise % relative abundance of insects in non-tannery area

Order	Rice (%)	Brassica (%)
Orthoptera	30.76	42.65
Hemiptera	16.04	11.92
Lepidoptera	18.46	11.52
Diptera	14.03	8.29
Coleoptera	18.63	11.97
Hymenoptera	2.07	0.00
Odonata	0.00	13.65

The concentration of cadmium in tannery area of rice crops was 0.626 ppm while 0.315 ppm for brassica. The concentration of cadmium in insects found in rice fields of tannery area was 0.3531 ppm while 0.187 ppm from brassica. While the concentration of Cadmium was high in samples of tannery area than the non-tannery area. The concentration of cadmium in non-tannery area samples was 0.115 ppm for rice and 0.101 ppm for brassica plants while 0.1231 ppm for rice and 0.104 ppm for brassica insects. The lead concentration in non-tannery samples was lower than its concentration in the tannery area (Table 4). As the table indicates, the concentration of lead was high in samples of tannery area than the concentration in non-tannery samples. The concentration of lead in tannery areas of rice crops was 0.637 ppm and brassica concentration was 0.672 ppm. The concentration of lead in insects found in rice fields of tannery area was 0.6979 ppm while 0.605 ppm for the insect samples collected from brassica crops of tannery area. The concentration of lead in non-tannery area samples was 0.324 ppm for rice and 0.267 ppm for brassica plants while 0.214 ppm for rice and 0.266 ppm for brassica insects (Table 4).

The diversity indices were estimated for the tannery and non-tannery areas along with their sub-sites (rice and brassica fields). In the tannery area, the taxa was found 26 for rice fields and 28 for brassica fields. The diversity and richness was recorded 3.11 and 3.525 for rice while 3.124 and 3.913 for brassica fields, respectively. In the non-tannery area, taxa was found 27 for rice fields and 29 for brassica fields. The diversity and richness were recorded as 3.23 and 3.49 for rice and 3.02 and 3.68 for brassica fields. In the total area wise species diversity, the Shannon diversity index was noted maximum 3.40 compared to the 3.13 in the tannery area. (Table 5).

The species accumulation curve showed that maximum species were noted in the non-tannery area and less richness was recorded in the tannery area. The growing phase of the non-tannery area was observed higher compared to the tannery area (Figure 4). The species abundance model explained that the data is less varied in the tannery area with the observed values (k=0.075, Chi^2= 74.68, $p < 0.05$) (Figure 5.A). In the non-tannery area, the data is significantly found, and maximum variation and flow of species was observed (k=0.044, Chi^2= 88.81, $p < 0.001$) (Figure 5.B).

Table 4. Determination of cadmium and lead concentrations in Plants and Insects

Sample Id	Unit	Cadmium	Lead
T. Rice plants	ppm	0.626	0.637
NT Rice plants	ppm	0.115	0.324
T Rice Insects	ppm	0.3531	0.6979
NT Rice Insects	ppm	0.1231	0.214
T Brassica Insects	ppm	0.187	0.605
NT Brassica Insects	ppm	0.104	0.266
T Brassica Plants	ppm	0.315	0.672
NT Brassica Plants	ppm	0.101	0.267

Note: *T refers tannery; *NT refers Non tannery

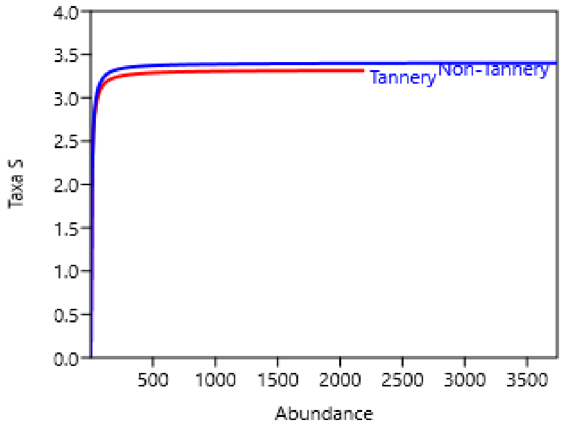

Figure 3. Individual rarefaction curve showing the species rank concerning both sites (tannery and non-tannery area)

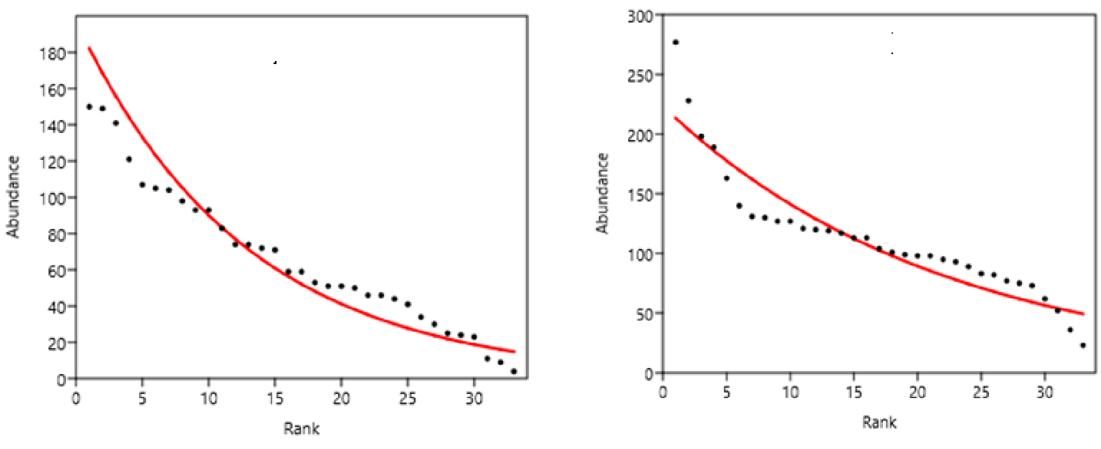

Figure 4. A. Species abundance distribution model of tannery area, B. Species abundance distribution model of non-tannery area

Table 5. Diversity indices of both tannery and non-tannery area

Diversity indices	Tannery area			Non-Tannery area		
	Rice	Brassica	Total	Rice	Brassica	Total
Taxa	26	28	33	27	29	33
Dominance_D	0.049	0.052	0.040	0.041	0.045	0.036
Simpson_1-D	0.951	0.998	0.959	0.958	0.954	0.963
Shannon_H	3.11	3.124	3.13	3.23	3.02	3.40
Evenness_e^H/S	0.863	0.811	0.832	0.937	0.852	0.908
Brillouin	3.055	3.055	3.272	3.187	3.167	3.374
Menhinick	0.7496	0.889	0.704	0.657	0.646	0.538
Margalef	3.525	3.913	4.159	3.49	3.68	3.89
Equitability_J	0.955	0.938	0.48	0.980	0.953	0.971

Discussion

As the results revealed, the discharge of industrial effluents, specifically tannery wastewater directly in the surroundings has affected the environmental quality. The discharge of heavy metals in the wastewater has affected the soil as well as plants through seepage. It has been reported in previously studies that heavy metals affect the producers, there are the chances of entry of these heavy metals into the food chains, thus affecting the life depending on these plants such as insects, as indicated by the present study (Azam et al. 2015; Chandra et al. 2009). Polluted water also had severe negative impacts on the macrofaunal populations. It causes the effects on the density, abundance, distribution and diversity of its population (Kanwal and Rana 2020). Heavy metals cause the contamination of the soil. This can results in the transfer of the pollutants in different levels of the trophic chain (Görür, 2006). Pollution is one of the most significant anthropogenic driver of environmental change. Heavy metals emitted by industry and transportation can pollute aquatic and terrestrial ecosystems, causing further ecotoxicological impacts on many insects species (Yang et al. 2018).

The capacity to tolerate heavy metals varies greatly among insects. Despite the global pollinator decrease, little is known about the impact of heavy metals on insects. Skaldina and Sorvari 2019) revealed that insect pests like aphids and butterfly larvae change their morphology and physiology in response to heavy metal pollution. Still, more research is needed to understand the general directions of adaptations in this functional group of economically important insects (Skaldina et al. 2018).

In our research, from the tannery area, a total of 2195 insects were captured, from which 1203 were collected from rice while 992 specimens were collected from brassica crops. Roy et al. (2016) studied brassica fields and collected 29 species from 16 families and 7 orders. Avgın and Luff (2010) also revealed that from rice plots 25 herbivore species of 12 families, 34 spider species of 8 families and 24 species of predatory insects from 12 families were found.

In our study, non-tannery fields contained 3753 specimens, out of which 1739 were collected from rice while 2014 specimens were collected from brassica crops. The maximum abundance was found for order orthoptera Lepidoptera and Coleoptera, while the least was found for the Hymenoptera. Another study (Tibbett et al. 2021) showed that predatory insects' abundance and species diversity were higher in rice fields. Many studies have recorded the worldwide extinction of insect populations and concluded that habitat degradation and pollution contributes to this dilemma (Hallmann et al. 2017; Lister and Garcia 2018). Over the last 40 years, several studies have confirmed the influence of pollution on insect populations and their behavior. Both direct and indirect effects of pollutions have disequilibria with higher or lower trophic levels of insects.

In some cases, pollutants may increase the prevalence of insect pests such as aphids, perhaps due to a loss of natural enemies (Brändle et al. 2001). In our study, the concentration of heavy metal cadmium and lead was high in samples of tannery area than the concentration in non-tannery samples. Ghannem et al. (2018) also revealed that the relative accumulations of each metal by each insect species is determined by the unique rates of absorption, the metal compositions of water and periphyton in each stream (Balistrieri et al. 2020). Siddig et al. 2016) examined that many insect species also used to monitor environmental changes and offer early warning signs of impending ecological changes.

Furthermore, many species are readily managed living creatures whose organization reproduces or forecasts the state of the environment in which they are found (Borowska and Pyza 2011). Geographical location, season, host plant, and agricultural management strategies all influence the diversity and abundance of arthropod species. Environmental change and pollutants may alter one or more species' abundance, variety, and growth rate in a given place (Conti et al. 2017). The study and evaluation of soil pollution due to heavy metals and the negative impacts of these pollutants on the living organisms and environment is of important concern (Rana et al. 2010).

In conclusion human activities have adversely affected the natural ecosystems all over the world. Industrialization is one of the major factors influencing the environment. The heavy metals released, and industrial wastes continuously get into the soil, water, and air, ultimately becoming part of the natural biogeochemical cycles. From the present study, it can be concluded that the discharge of tannery effluents into the water bodies and open environment directly affects life depending on it. Suppose the practices of disposing untreated effluents are not abandoned. In that case, it will lead to high risks for the insects and other organisms living in the surrounding areas.

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Table S1. Species diversity in rice and Brassica in tannery and non-tannery area

Order	Family	Species Name	Rice	Brassica	Total	Rice	Brassica	Total
Orthoptera	Acrididae	*Acrida exaltata*	111	39	150	15	112	127
		Hieroglyphus spp.	76	73	149	41	86	127
		Acrida spp.	50	24	74	100	98	198
		Melanoplus bivittatus	53	51	104	129	148	277
		Oxya spp.	55	43	98	71	157	228
		Omocestus spp.	0	23	23	0	119	119
		Mermiria spp.	11	0	11	57	47	104
	Gryllidae	*Acheta domesticus*	43	29	72	61	40	101
		Gryllus spp.	19	15	34	61	52	113
Hemiptera	Aphididae	*Schizaphis graminum*	35	11	46	54	109	163
		Rhopalosiphum spp.	0	30	30	60	33	93
	Aleyrodidae	*Bemisia tabaci*	9	15	24	52	23	75
	Pentatomidae	*Oebalus spp.*	76	31	107	45	44	89
	Lophopidae	*Pyrilla spp.*	51	42	93	68	31	99
Lepidoptera	Noctuidae	*Agrotis spp.*	34	17	51	24	28	52
		Spodoptera spp.	5	78	83	75	55	130
		Spodoptera spp.	68	37	105	75	45	120
	Crambidae	*Chilo spp.*	43	10	53	64	31	95
	Pieridae	*Pieris brassicae*	0	51	51	0	73	73
	Hesperiidae	*Pelopidas spp.*	71	0	71	83	0	83
Diptera	Syrphidae	*Episyrphus balteatus*	44	15	59	60	22	82
	Culicidae	*Anopheles gambiae*	77	44	121	89	51	140
	Culicidae	*Culex pipiens*	24	117	141	95	94	189
Coleoptera	Coccinellidae	*Cheilomenes sexmaculata*	51	42	93	60	53	113
		Coccinella septempunctata	0	59	59	0	98	98
		Coccinella spp.	46	0	46	62	0	62
	Curculionidae	*Lissorhoptrus spp.*	50	0	50	77	0	77
	Staphylinidae	*Paederus spp.*	43	31	74	55	43	98
	Chrysomelidae	*Chrysochus spp.*	14	27	41	70	47	117
Hymenoptera	Braconidae	*Cotesia spp.*	0	25	25	36	0	36
Odonata	Coenagrionidae	*Ceriagrion spp.*	44	0	44	0	23	23
	Libellulidae	*Sympetrum spp.*	0	9	9	0	121	121
	Aeshnidae	*Aeshna spp.*	0	4	4	0	131	131
Total			1203	992	2195	1739	2014	3753