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Fungal communities in bunker C oil-impacted sites off southern Guimaras, Philippines: a post-spill assessment of Solar 1 oil spill

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Abstract

Few studies have been conducted to determine how fungal populations respond over the long-term to oil spills in tropical coastal habitats. The present study aimed to provide information on temporal changes in a fungal community affected by an oil spill, and to determine whether there was any recovery of normal mycoflora in the oil-affected sites in Guimaras, the Philippines. Changes in fungal communities are described according to species composition and fungal load (CFUs ml^{-1} seawater or g^{-1} soil) by site and sample type. Samples were collected at the same eight sites in 2009, and comprised beach water, beach soil, mangrove surface soil and mangrove sub-surface soil. Fungi were enumerated and isolated using the spread plate serial dilution technique and identified based on colony and microscopic characteristics using available keys and monographs. In general, fungal density appears to have increased over the three-year period and there was a continuing dominance of members of the hyphomycetes, resembling previous data from 2006, though with some shifts in species composition. The observed changes in fungal community composition and density may be signs of initial recovery and re-establishment of a normal fungal flora among the disturbed areas.

Keywords: beach sediments; mangroves; marine fungi; Solar 1 oil spill.

Introduction

Fungi are among the most diverse microorganisms on earth. They are very important in human society, health and well-being (Walker and White 2005). Fungi, most especially the soil fungi, have been used widely in the pharmaceutical industry for the production of antibiotics (Manoch 2004), food, beverages, pigments, biofuels, industrial enzymes, biotechnological products, vitamins, organic and fatty acids and sterol (Walker and White 2005). Fungi are ubiquitous; they can be considered as primary decomposers of organic compounds found in soil or sediments and in water bodies (Kohlmeyer and Kohlmeyer 1979, Agate et al. 1988, Kavanagh 2005), which are very good sources of food for detritus, scavengers and filter feeders (Newell et al. 1987, Sarma et al. 2001, Maria and Sridhar 2002). Other fungi are considered as hydrocarbon-degraders (Atlas 1981, Leahy and Colwell 1990, Van Hamme et al. 2003) that are used for clean-up technology strategies or bioremediation in freshwater and marine habitats (Zhu et al. 2001, 2004). Indeed, the larger the fungal biomass in an ecosystem, the greater the amount of nutrients that will be recycled back into the ecosystem through the decomposition of leaf litter and wood (Pepper and Gerba 2004). However, due to anthropogenic activities, the fungal role in nutrient cycling and organic matter accumulation have become disturbed (Miller and Lodge 1997). Nevertheless, fungi have unique structures and physiologies that make them resilient in the face of disturbance be it natural or anthropogenic; the degree of resilience depends on the type and scale of disturbance (Morris et al. 2007).

Among disturbances experienced by microorganisms are accidental releases of oil (Zhu et al. 2001). Approximately $1.7–8.8\times10^6$ t of oil are dumped in the ocean annually (Harayama et al. 1999). During an oil spill event, there is a rise in the community of hydrocarbon-utilizing microorganisms to a proportion of about 100% of viable microorganisms (Atlas 1981). El-Tarabily (2002) reported that activities of aerobic and anaerobic bacteria, streptomycetes and non-streptomycete actinomycetes, filamentous fungi and yeasts were lower in oil-polluted mangrove sediment than in the unpolluted sites, but the total aerobic and anaerobic hydrocarbon-utilizing bacterial mass was higher in polluted sediment. Furthermore, an increase in the concentration of oil can have adverse effects on fungal diversity but enhance the community of hydrocarbon-utilizing fungi (Obire and Anyanwu 2009), observations similar to those of Salvo et al. (2005) who reported a lower diversity of fungi and density (Salvo and Fabiano 2006) in PAH-impacted sediments in Genoa-Voltri Harbour (NW Mediterranean, Italy). Sites impacted by polycyclic aromatic hydrocarbons (PAHs) are dominated by several species of *Penicillum* together with *Papulospora halima* Anast. *Cladosporium*, and *Aspergillus*, all of which are associated with PAHs. However, fungal community in petroleum contaminated land was demonstrated to have greater fungal diversity based on terminal restriction fragment patterns (Palmer 2000). This increased fungal diversity was probably due to the tolerance of mycoflora to total petroleum hydrocarbon (TPH) in the soil; impacted soil bacterial communities decreased as the fungi grew and util-

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ized TPHs. Interestingly, Pinholt et al. (1979) reported that the length of mycelium increased in oily soil, while the number of fungal colony forming units (CFU) was highest in control or unoiled soil. Krivobok et al. (1998) demonstrated that PAH concentration in soil can inhibit the growth of spore-bearing fungi and yet stimulate the growth of mycelia sterilia. In other words, oil can reduce fungal diversity, but allows the growth of hydrocarbon-utilizing fungi.

In 2006, the M/T Solar I owned by Petron Corporation sank off the coast of southern Guimaras, Philippines and discharged more than 2×10^6 l of Bunker C oil that affected sensitive coastal habitats. A rapid assessment of the impacts on fungal population was made by Sadaba et al. (2009). There was some disturbance of fungal density and species composition in oiled mangrove sediments and beach/surface water. However, there was little or minimal information on how fungal communities respond over the long-term to an initial exposure to oil. Thus, the present study was conducted to determine, whether effects were discernable after three years and whether any changes detected would indicate recovery of normal mycoflora in the oil-affected areas.

Materials and methods

Sites description

Samples were collected on March 18, 2009 from the locations at which initial assessment was conducted on October 11, 2006. There were eight sampling sites: six oil-contaminated Barangays and two reference sites. The six oil-contaminated Barangays are Brgy. Tando (beach: 10°28′20.0″ N, 122°29′33.8″ E, mangrove: 10°28′20.0″ N, 122°29′33.8″ E), Taklong Island (beach: 10°24′13″ N, 122°30′41.4″ E, mangrove: 10°25′38.8″ N, 122°30′30.75″ E), Cabalagnan (beach: 10°24′59.3″ N, 122°33′01.4″ E, mangrove: 10°24′54.2″ N, 122°32′58.6 ″E), Panobolon Island (beach: 10°24′29.4″ N, 122°33′33.5″ E, mangrove: 10°24′29.5″ N, 122°33′34.5″ E) in the municipality of Nueva Valencia, Inampulogan Island (beach: 10°26′43.3″ N, 122°40′59.9″ E, mangrove: 10°26′43.4″ N, 122°40′59.9″ E) and Brgy. Sabang (beach: 10°28′46.8″ N, 122°39′49.3″ E, mangrove: 10°28′48.1″ N, 122°39′49.2″ E) in the municipality of Sibunag. The two reference sites were Brgy. Lawi, Jordan (beach: 10°32′50.5″ N, 122°32′13.8″ E, mangrove: 10°32′50.9″ N, 122°32′13.1″ E) and Brgy. Getulio, Buenavista, Guimaras, Philippines (beach: 10°45′02.1″ N, 122°39′54.4″ E, mangrove: 10°44′54.2″ N, 122°40′04.5″ E).

Sampling, isolation, incubation and observation

Samples consisted of one surface or beach water sample and three soil samples: a) surface or beach soil taken within 2 m of the shoreline, b) surface mangrove soil and c) subsurface mangrove soil were taken within a 5-cm depth of the surface. This gave a total of four samples from each site. Soil was collected with the aid of a sterile cork borer (10 mm in diameter) fitted with a modified ejector. The sampler and ejector were washed with 70% ethyl alcohol and flamed before each sampling.

Soil samples from each plot were placed in clean polyethylene plastic bags, labeled, thoroughly sealed, kept cool on ice inside an insulated box and immediately transported within 24 h to the laboratory for processing and examination of fungal communities.

The spread plate method was used, and serial dilutions of samples were made using sterile 1.5% NaCl solution as diluent, and plated onto potato dextrose agar plate supplemented with a 1.5% NaCl, penicillin G and streptomycin combination. A control plate was included by exposing a blank PDA plate in the middle of the work area for 15 min. Processing of samples was done at the Mycology Laboratory of the Oil Spill Response Program, Freshwater Aquaculture Station, UP Visayas, Miag-ao, Philippines within 24 h of field collection. The plates were subsequently incubated at 27±2°C and examined daily for the appearance of fungal colonies through three weeks, depending on the growth of species. Yeast and filamentous fungi isolated were expressed as colony-forming units per ml (CFU ml^{-1}) for water and CFU g^{-1} for soil samples. Fungal colonies were then isolated and purified for detailed examination and identification. Microscopic examination of isolates followed the slide culture technique of Riddell (1950), and descriptions of microscopic features such as spore size, color, shape, wall ornamentation; conidiophore size, phialides and conidial pattern were based on the workbook of Quimio (2001). Macroscopic descriptions included colony characteristics such as color (reverse and obverse), elevation, texture, margin and characteristics of aerial hyphae.

Identification of isolates was based on keys in Gilman (1957), Barnett and Hunter (1972), Onions et al. (1981), Watanabe (2002), Domsch et al. (2007) in addition to other available keys and monographs.

Data analysis

Frequency of occurrence of species (%) of fungi per site and sample type was computed as follows:

$$\text{Frequency of occurrence of species A (\%)} = \frac{\text{No. of collections of species A}}{\text{Number of samples examined}} \times 100$$

(Adapted from Hyde 1989, Sarma and Hyde 2001).

Frequency of occurrence was categorized into classes: very frequent ≥10%; frequent =5–10%; infrequent =1–5%; rare ≤1%.

The diversity of fungi in four samples from each of eight sites was assessed by the following diversity indices (Magurran 1988):

Shannon-Weaver index (H')=-Σ (*pi ln pi*); where pi is the proportion of individual that species *i* contributes to the total number of individuals:

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Table 1 Comparison of the 2006 (October)[1] and 2009 (March) comprehensive lists of fungi isolated from various types of samples at oil-contaminated and uncontaminated sites in Guimaras, Philippines. (x: indicates presence.)

No.	Species	Oil-contaminated		Uncontaminated	
		2006	2009	2006	2009
1	*Aspergillus* cf. *candidus* Link ex Fries	x		x	
2	*Aspergillus flavus*	x	x		x
3	*Aspergillus fumigatus*	x	x	x	x
4	*Aspergillus* cf. *glaucus*		x		
5	*Aspergillus japonicus*		x		x
6	*Aspergillus niger*	x	x	x	x
7	*Aspergillus ochraceous*		x		x
8	*Aspergillus* cf. *repens*		x		x
9	*Aspergillus* cf. *terreus* Thom	x		x	
10	*Aspergillus* cf. *terricola*		x		x
11	*Aspergillus* cf. *oryzae*		x		x
12	*Aspergillus* cf. *parasiticus*		x		
13	*Aureobasidium* cf. *pullulans* (de Bary) Arnaud	x			
14	*Aureobasidium* sp. 1 Viala & Boyer	x			
15	*Botrytis pyramidalis*		x		x
16	*Cladosporium cladosporioides*	x	x		x
17	*Cladosporium herbarum*		x		x
18	*Fusarium moniliforme*		x		x
19	*Fusarium oxysporum*		x		x
20	*Geotrichum candidum*		x		
21	Hyaline septate mycelia		x		x
22	*Memnoniella* sp. 1 von Hohnel	x			
23	*Memnoniella* sp. 2	x		x	
24	*Monilia sitophila*		x		x
25	*Monilia* sp. 1 Persoon ex Fries			x	
26	*Monilia* sp. 2	x			
27	*Mucor* sp. 1 Micheli	x			
28	Mycelia sterilia	x	x		x
29	*Olpitrichum* cf. *macrosporum*		x		
30	*Penicillium* cf. *brevicompactum* Dierckx	x			
31	*Penicillium* cf. *corylophilum*		x		x
32	*Penicillium* cf. *frequentans*		x		x
33	*Penicillium* cf. *funiculosum* Thom	x		x	
34	*Penicillium* cf. *verrucosum* Peyronel	x		x	
35	*Penicillium* sp. 4 Link	x		x	
36	*Pestalotia* sp. de Not.	x			
37	*Rhizopus microsporus*				x
38	*Sporothrix* sp. 1 Hektoen & Perkins			x	
39	*Sporothrix* sp. 2	x		x	
40	Thick brown septate mycelia		x		
41	*Sporobolomyces* sp. Kluyver & Van Niel	x	x	x	x
42	*Torulopsis* sp. Berlese	x	x		x
43	*Candida* sp. Berkh	x	x	x	x
44	Yeast 4	x			
	Summary				
	Total no. of species	23	26	13	22
	Shannon index of diversity: H'	1.248	1.25	1.039	1.25
	Shannon index of evenness: J'	0.916	0.89	0.963	0.93
	Simpson index of dominance: D'	0.642	0.64	0.926	0.64
	Jaccard's coefficient of similarity: J	0.20		0.13	
	Jaccard's coefficient of similarity: J (2006 oil-contaminated vs. uncontaminated) (Sadaba et al. 2009)			0.44	
	Jaccard's coefficient of similarity: J (2009 oil-contaminated vs. uncontaminated)			0.78	

[1]Adopted from Sadaba et al. 2009.

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Table 2 Over-all frequency of occurrence (%) of fungi collected in 2006[1] and 2009 from various types of samples at oil-contaminated sites in southern Guimaras, Philippines.

No.	Species	Frequency of occurrency (%)	
		2006	2009
Very frequent species (≥10%)			
1	*Aspergillus japonicus*		43.8
2	*Aspergillus ochraceous*		43.8
3	*Aspergillus flavus*		41.7
4	*Aspergillus niger*		37.5
5	*Fusarium oxysporum*		31.3
6	*Penicillium* cf. *frequentans*		25
7	*Aspergillus fumigatus*	10.4	20.8
8	*Cladosporium cladosporioides*		18.8
9	*Fusarium moniliforme*		18.8
10	Mycelia sterilia	14.6	18.8
11	*Aspergillus* cf. *terricola*		12.5
12	*Torulopsis* sp. (Yeast 2 in 2006 data)		12.5
13	*Aspergillus* cf. *oryzae*		10.4
14	Yeast 4	10.4	
Frequent species (5–10%)			
1	*Aspergillus* cf. *repens*		8.3
2	*Penicillium* cf. *corylophilum*		8.3
3	Hyaline septate mycelia		6.3
4	*Monilia sitophila*		6.3
5	Thick brown septate mycelia		6.3
6	*Aspergillus* cf. *candidus*	8.3	
7	*Aspergillus* cf. *terreus*	8.3	
8	*Candida* sp. (Yeast 3 in 2006 data)	6.3	
9	*Penicillium* cf. *verrucosum*	6.3	
10	*Sporothrix* sp. 2	6.3	
11	*Sporobolomyces* sp. (Yeast 1 in 2006 data)	6.3	
Infrequent species (1–5%)			
1	*Botrytis pyramidalis*		4.2
2	*Geotrichum candidum*		4.2
3	*Sporobolomyces* sp.		4.2
4	*Candida* sp.		4.2
5	*Aspergillus* cf. *glaucus*		2.1
6	*Aspergillus* cf. *parasiticus*		2.1
7	*Cladosporium herbarum*		2.1
8	*Olpitrichum* cf. *macrosporum*		2.1
9	*Aureobasidium* sp. 1	4.2	
10	*Aureobasidium* cf. *pullulans*	4.2	
11	*Memnoniella* sp. 1	4.2	
12	*Mucor* sp. 1	4.2	
13	*Penicillium* cf. *funiculosum*	4.2	
14	*Aspergillus niger*	2.1	
15	*Aspergillus flavus*	2.1	
16	*Cladosporium cladosporioides*	2.1	
17	*Memnoniella* sp. 2	2.1	
18	*Monilia* sp. 2	2.1	
19	*Penicillium* cf. *brevicompactum*	2.1	
20	*Penicillium* sp. 4	2.1	
21	*Pestalotia* sp.	2.1	
22	*Torulopsis* sp. (Yeast 2 in 2006 data)	2.1	
Summary	Total no. of species	23	26
	Shannon index of diversity: H'	1.248	1.25
	Shannon index of evenness: J'	0.916	0.89
	Simpson index of dominance: D'	0.642	0.64

[1]Adopted from Sadaba et al. 2009.

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Table 3 Frequency of occurrence of fungi collected in 2006[1] and 2009 according to type of sample examined at oil-contaminated sites in Guimaras, Philippines.

Species	Type of samples															
	Beach water (%)				Beach soil (%)				Mangrove surface soil (%)				Mangrove sub-surface soil (%)			
	Very frequent[2]		Frequent[3]		Very frequent		Frequent		Very frequent		Frequent		Very frequent		Frequent	
	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009
Aspergillus flavus		41.7	8.3			50				33.3				41.7		
Aspergillus fumigatus		25				16.7				33.3			41.7			8.3
Aspergillus japonicus		33.3				50				50				41.7		
Aspergillus niger		50	8.3			33.3				33.3				33.3		
Aspergillus ochraceous		50				41.7				41.7				41.7		
Aspergillus cf. *candidus*					33.3											
Aspergillus cf. *glaucus*				8.3												
Aspergillus cf. *oryzae*				8.3				8.3				8.3		16.7		
Aspergillus cf. *parasiticus*				8.3												
Aspergillus cf. *repens*								8.3		16.7						8.3
Aspergillus cf. *terreus*									33.3							
Aspergillus cf. *terricola*				8.3				8.3		16.7				16.7		
Aureobasidium cf. *pullulans*							8.3								8.3	
Aureobasidium sp. 1									25							
Botrytis pyramidalis								8.3				8.3				
Candida sp. (Yeast 3 in 2006 data)									33.3			8.3				8.3
Cladosporium cladosporioides		25				16.7	8.3			25						8.3
Cladosporium herbarum																8.3
Fusarium moniliforme		25						8.3		25				16.7		
Fusarium oxysporum		33.3				33.3				25				33.3		
Geotrichum candidum								8.3				8.3				
Hyaline septate mycelia				8.3				8.3								8.3
Memnoniella sp. 1							8.3				8.3					
Memnoniella sp. 2							8.3									
Monilia sitophila												8.3		16.7		
Monilia sp. 2							8.3									
Mucor sp. 1											8.3				8.3	
Mycelia sterilia		25						8.3	16.7	25			41.7	16.7		
Olpitrichum cf. *macrosporum*								8.3								
Penicillium cf. *verrucosum*			8.3				8.3								8.3	
Penicillium cf. *brevicompactum*							8.3									
Penicillium cf. *corylophilum*				8.3				8.3				8.3				8.3
Penicillium cf. *frequentans*		33.3				33.3						8.3		25		
Penicillium cf. *funiculosum*											8.3					
Penicillium sp. 4											8.3					
Pestalotia sp.											8.3					
Sporobolomyces sp. (Yeast 1 in 2006 data)	25	25		8.3												8.3
Sporothrix sp. 2									25							

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(Table 3 continued)

Species	Type of samples											
	Beach water (%)				Beach soil (%)				Mangrove surface soil (%)			
	Very frequent[2]		Frequent[3]		Very frequent		Frequent		Very frequent		Frequent	
	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009	2006	2009
Thick brown septate mycelia						16.7						
Torulopsis sp. (Yeast 2 in 2006 data)		16.7				16.7		8.3				8.3
Yeast 4										41.7		
Summary												
Total no. of species (2006)				4				9				6
Total no. of species (2009)				19				20				21
Shannon index of diversity: H' (2006)				0.145				0.359				0.341
Shannon index of diversity: H' (2009)				1.17				1.19				1.2
Shannon index of evenness: J' (2006)				0.097				0.241				0.228
Shannon index of evenness: J' (2009)				0.93				0.92				0.91

[1]Adopted from Sadaba et al. 2009.
[2]Very frequent species (≥10% frequency of occurrence).
[3]Frequent species (5–10% frequency of occurrence).

$$pi = \frac{ni}{N}$$

where

N=total number of individuals (records)

n*i*=number of individuals *i*1, *i*2, *i*3, *i*4,…*i*x

Species dominance was computed by using the Simpson index (D') as shown in the formula:

$$D' = \frac{1}{\sum Pi^2}$$

$$\text{Shannon evenness (J')} = \frac{H'}{H'max}$$

where, *H'max* is the maximum value of diversity for the number of species present (Pielou 1977).

Percentage similarity (Kenkel and Booth 1992, Jones 2000): Jaccard's coefficient was computed among the oil-contaminated and uncontaminated sites from various sample types based on the presence or absence of each fungal species, where *c* is the number of fungal species occurring in both types of sites, *a* is the number of fungal species unique to the oil-contaminated sites and *b* is the number of fungal species unique to the uncontaminated sites:

$$J = \frac{c}{a+b+c}$$

Statistical analysis

The results were evaluated statistically through t-tests at a significance level of $p<0.05$ using Statistica® v.8 software.

Results

Over-all fungal population from oil-contaminated and uncontaminated samples from various sites in Guimaras, Philippines

Twelve fungal genera, i.e., *Aspergillus, Botrytis, Cladosporium, Fusarium, Geotrichum, Monilia, Olpitrichum, Penicillium, Rhizopus, Sporobolomyces, Torulopsis, Candida*, a Mycelia sterilia and two unidentified species (hyaline septate mycelia and the thick brown septate mycelia) were isolated in the oil-contaminated and uncontaminated samples from various sites in Guimaras in 2009. The collections were mostly dominated by hyphomycetes represented by Moniliales in the genus *Aspergillus. A* total of 26 fungal species was found in the oil-contaminated sites, with a Shannon index of diversity (H') of 1.24, Shannon index of evenness (J') of 0.89 and Simpson index of dominance (D') of 0.64. A total of 22 fungal species was found in the uncontaminated sites, with the Shannon index of diversity (H') of 1.25, Shannon index of evenness (J') of 0.93 and Simpson index of dominance (D') of 0.65. The Jaccard's coefficient of simi-

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Table 4 Over-all frequency of occurrence (%) of fungi collected in 2006[1] and 2009 from various types of samples at uncontaminated sites in southern Guimaras, Philippines.

No.	Species	Frequency of occurrence (%)	
		2006	2009
Very frequent species (≥10%)			
1	*Aspergillus flavus*		50
2	*Aspergillus japonicus*		43.8
3	*Aspergillus niger*		37.5
4	*Aspergillus fumigatus*		31.3
5	*Aspergillus ochraceous*		31.3
6	*Fusarium moniliforme*		31.3
7	*Fusarium oxysporum*		31.3
8	Mycelia sterilia		25
9	*Aspergillus* cf. *oryzae*		18.8
10	*Cladosporium cladosporioides*		18.8
11	Hyaline septate mycelia		18.8
12	*Penicillium* cf. *corylophilum*		12.5
13	*Penicillium* cf. *frequentans*		12.5
14	*Rhizopus microsporus*		12.5
15	*Torulopsis* sp. (Yeast 2 in 2006 data)		12.5
16	*Penicillium* cf. *verrucosum*	18.75	
17	*Aspergillus* cf. *terreus*	12.5	
18	*Penicillium* cf. *funiculosum*	12.5	
Frequent species (5–10%)			
1	*Aspergillus* cf. *repens*		6.25
2	*Aspergillus* cf. *terricola*		6.25
3	*Botrytis pyramidalis*		6.25
4	*Cladosporium herbarum*		6.25
5	*Monilia sitophila*		6.25
6	*Sporobolomyces* sp. (Yeast 1 in 2006 data)	6.25	6.25
7	*Candida* sp. (Yeast 3 in 2006 data)	6.25	6.25
8	*Penicillium* sp. 4	6.25	
9	*Aspergillus niger*	6.25	
10	*Aspergillus* cf. *candidus*	6.25	
11	*Aspergillus* cf. *fumigatus*	6.25	
12	*Memnoniella* sp. 2	6.25	
13	*Monilia* sp. 1	6.25	
14	*Sporothrix* sp. 1	6.25	
15	*Sporothri*x sp. 2	6.25	
	Summary		
	Total no. of species	13	22
	Shannon index of diversity: H'	1.04	1.24
	Shannon index of evenness: J'	0.96	0.93
	Simpson index of dominance: D'	0.93	0.65

[1]Adopted from Sadaba et al. (2009).

larity was high at 0.78. Finally, *Aspergillus* cf. *glaucus* Link, *A.* cf. *parasiticus* Speare, *Geotrichum candidum* Link: Fr., *Olpitrichum* cf. *macrosporum* (Farl. *ex* Sacc.) Sumst. and thick brown septate mycelia were isolated only from oil-contaminated sites, while *Rhizopus microsporus* Tiegh. was collected only from uncontaminated sites (Table 1).

Overall frequency of occurrence in oil-contaminated sites

Thirteen species occurred very frequently in oil-contaminated sites, viz., *Aspergillus japonicus* Saito (43.8%), *A. ochraceous* Wilhelm (43. 8%), *A. flavus* Link: Fr. (41.7%), *A. niger* Tiegh. (37.5%), *Fusarium oxysporum* Schlechtdl. (31.3%), *Penicillium* cf. *frequentans* Westling (25%), *A. fumigatus* Fresen. (20.8%), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (18.8%), *Fusarium moniliforme* Sheld. (18.8%), Mycelia sterilia (18.8%), *A.* cf. *terricola* Marchal (12.5%), *Torulopsis* sp. (12.5%), and *A.* cf. *oryzae* (Ahlburg) Cohn (10.4%). There were only five species recorded as frequent: *A.* cf. *repens* (Corda) de Bary (8.3%), *Penicillium* cf. *corylophilum* Dierckx (8.3%), hyaline septate mycelia (6.3%), *Monilia sitophila* Pers. *ex* Fries. (6.3%), and thick brown septate mycelia (6.3%). Other species were recorded as infrequent. The Shannon index of diversity (H') was 1.25, the Shannon index of

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Table 5 Frequency of occurrence of fungi in 2006[1] and 2009 according to type of sample examined at uncontaminated sites in Guimaras, Philippines.

Species	Type of samples							
	Beach water (%)		Beach soil (%)		Mangrove surface soil (%)		Mangrove sub-surface soil (%)	
	2006	2009	2006	2009	2006	2009	2006	2009
Aspergillus flavus		50		50		50		50
Aspergillus fumigatus		50	25	25		25		25
Aspergillus japonicus		50		50		25		50
Aspergillus niger	25	50		50		25		25
Aspergillus ochraceous		50		25		25		25
Aspergillus cf. *candidus*			25					
Aspergillus cf. *oryzae*		25				25		25
Aspergillus cf. *repens*				25				
Aspergillus cf. *terreus*					50			
Aspergillus cf. *terricola*				25				
Botrytis pyramidalis								25
Candida sp. (Yeast 3 in 2006 data)					25			25
Cladosporium cladosporioides		25		25		25		
Cladosporium herbarum						25		
Fusarium moniliforme		25		50		25		25
Fusarium oxysporum		25		25		25		50
Hyaline septate mycelia		25				25		25
Memnoniella sp. 2							25	
Monilia sitophila						25		
Monilia sp. 1			25					
Mycelia sterilia		25				25		50
Penicillium cf. *verrucosum*			25				50	
Penicillium cf. *corylophilum*								50
Penicillium cf. *frequentans*				25				25
Penicillium cf. *funiculosum*					50			
Penicillium sp. 4					25			
Rhizopus microsporus				25		25		
Sporobolomyces sp. (as Yeast 1 in 2006)	25							25
Sporothrix sp. 1					25			
Sporothrix sp. 2					25			
Torulopsis sp. (as Yeast 2 in 2006)		25				25		
Summary								
Total no. of species (2006)	2		4		6		2	
Total no. of species (2009)	12		12		15		15	
Shannon index of diversity: H' (2006)	0.05		0.11		0.21		0.07	
Shannon index of diversity: H' (2009)	1.05		1.05		1.17		1.15	
Shannon index of evenness: J' (2006)	0.04		0.08		0.14		0.05	
Shannon index of evenness: J' (2009)	0.98		0.98		0.99		0.98	

Note: All species collected occurred as very frequent ($\geq$10% frequency of occurrence).
[1]Adopted from Sadaba et al. 2009.

evenness (J') was 0.89, while the Simpson index of dominance (D') was 0.64 (Tables 2 and 3).

Overall frequency of occurrence in uncontaminated sites

Fifteen species were very frequent in the uncontaminated sites. These were: *Aspergillus flavus* (50%), *A. japonicus* (43.8%), *A. niger* (37.5%), *A. fumigatus* (31.3%), *A. ochraceous* (31.3%), *Fusarium moniliforme* (13.3%), *F. oxysporum* (31.3%), Mycelia sterilia (25%), *A.* cf. *oryzae* (18.8%), *Cladosporium cladosporioides* (18.8%), hyaline septated mycelia (12.5%), *Penicillium* cf. *corylophilum* (12.5%), *P.* cf. *frequentans* (12.5%), *Rhizopus microsporus* (12.5%) and *Torulopsis* sp. (12.5%). Frequently occurring species were: *Aspergillus* cf. *repens* (6.3%), *A.* cf. *terricola* (6.3%), *Botrytis pyramidalis* (Bonorden) Sacc. (6.3%), *Cladosporium herbarum* (Pers.: Fr.) Link (6.3%), *Monilia sitophila* (6.3%), *Sporobolomyces* sp. (6.3%) and *Candida* sp. (6.3%). Other species were infrequent. The Shannon index of diversity (H') was 1.24, the Shannon index of evenness (J') was 0.93, while the Simpson index of dominance (D') was 0.65 (Tables 4 and 5).

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Table 6 Comparison of 2006[1] and 2009 fungal density (CFU ml^{-1} or g^{-1} for water or soil, respectively) among various types of samples collected from oil-contaminated and uncontaminated sites in Guimaras, Philippines.

Site	Surface/Beach water		Beach soil		Mangrove soil, surface		Mangrove soil, subsurface	
	2006	2009	2006	2009	2006	2009	2006	2009
Tando	4.0×10^{1}	6.0×10^{3}	2.7×10^{4}	5.6×10^{5}	1.3×10^{4}	4.8×10^{6}	1.8×10^{4}	3.6×10^{4}
Taklong	6.4×10^{1}	8.4×10^{3}	4.3×10^{4}	5.6×10^{5}	9.0×10^{4}	7.2×10^{5}	1.7×10^{4}	2.6×10^{4}
Cabalagnan	5.5×10^{1}	8.0×10^{3}	7.2×10^{3}	1.2×10^{4}	2.6×10^{4}	4.6×10^{4}	4.2×10^{4}	1.1×10^{7}
Panobolon	5.5×10^{1}	2.4×10^{3}	1.1×10^{4}	6.9×10^{4}	1.8×10^{4}	1.8×10^{4}	9.0×10^{3}	4.0×10^{6}
Sabang	9.5×10^{1}	1.4×10^{4}	1.7×10^{4}	2.0×10^{4}	1.5×10^{4}	1.0×10^{4}	1.5×10^{4}	6.3×10^{6}
Inampulogan	6.4×10^{1}	2.4×10^{3}	4.1×10^{4}	3.3×10^{3}	1.4×10^{4}	1.3×10^{4}	8.6×10^{4}	2.0×10^{5}
Mean	6.2×10^{1}	6.9×10^{3}	2.4×10^{4}	2.0×10^{5}	2.93×10^{4}	9.3×10^{5}	3.11×10^{4}	3.5×10^{6}
Getulio[2]	8.2×10^{1}	8.0×10^{3}	3.3×10^{4}	5.0×10^{3}	3.4×10^{4}	2.5×10^{6}	1.9×10^{4}	4.5×10^{4}
Lawi[2]	7.5×10^{1}	8.0×10^{3}	5.0×10^{3}	5.1×10^{3}	4.2×10^{4}	2.3×10^{3}	2.0×10^{4}	2.1×10^{4}
Mean	7.85×10^{1}	8.0×10^{3}	1.9×10^{4}	5.0×10^{3}	3.8×10^{4}	1.2×10^{6}	1.95×10^{4}	3.3×10^{4}

[1]Adopted from Sadaba et al. 2009.
[2]Reference sites (uncontaminated).

Fungal load among various types of samples in oil-contaminated and uncontaminated sites in Guimaras, Philippines

The highest colony forming unit (CFU) count in beach water was from Sabang (1.4×10^{4}) and the lowest from Panobolon (2.4×10^{3}) and Inampulogan (2.4×10^{3}). For beach soil, the highest CFU was from Tando (5.6×10^{5}) and Taklong (5.6×10^{5}) and lowest in Inampulogan (3.3×10^{3}). In mangrove surface soil, the highest CFU was also from Tando (4.8×10^{6}) and lowest from Sabang (1.0×10^{4}). In mangrove subsurface soil, the highest CFU was from Cabalagnan (1.1×10^{7}) and lowest from Taklong Island (2.6×10^{4}) (Table 6). Among the oil-contaminated sites, Cabalagnan had the highest CFU density, while Inampulogan had the lowest value. Among the uncontaminated sites, Getulio had higher value compared than Lawi (Table 6).

Discussion

Fungal species composition and density appear to have changed among the oil-contaminated sites along the coast of southern Guimaras, Philippines over the three-year period following the sinking of M/T Solar 1 in 2006. Considering species composition, only 15 fungal species were found in 2006 at oil-contaminated sites, with the dominance of *Aspergillus* spp., suggesting that these species might have the ability to secrete enzymes that degrade oil, allowing them to use hydrocarbons as an energy source (Atlas 1981, Salvo et al. 2005, Okereke et al. 2007, Obire and Anyanwu 2009) or tolerate higher oil concentration and toxicity, allowing them to thrive in freshly oiled sites. Eighteen species were collected only in 2009 and were also dominated by the genus *Aspergillus* but with different species. Their appearance may be attributed to the lowered concentration and toxicity of oil. In uncontaminated sites, nine and 18 fungal species were exclusively present only in 2006 and 2009, respectively (Table 1). Such change in species composition may be attributed to seasonal availability of fungal propagules, availability of substrates for colonization, nutrient supply, survival and dispersal of light propagules, collection site, time of the year relative to humidity, rainfall, wind speed and proximity to source where they were produced (Lee and Baker 1972, Follosco and Uyenco 1984).

More species were recorded as very frequent (more than 10% occurrence) in 2009 (17 spp.) than in 2006 (eight spp.) in oil-contaminated sites (Tables 2 and 3). However, the results of the t-test showed no significant difference ($p>0.05$) among the very frequent species, while there was a significant difference among the infrequent species ($p<0.05$) between year. Among the genera collected, *Aspergillus* was the best represented in overall frequency in the 2006 and 2009 collections, similar to previous reports (Zhu et al. 2001, Salvo et al. 2005, Okereke et al. 2007, Obire et al. 2008, Obire and Anyanwu 2009). In addition, it is interesting to note that there were more species in oil-contaminated sites in 2009 than in 2006 (18 species were exclusive to the 2009 collection) (Table 1), indicating that the reduction of oil in these areas might triggered a shift toward the normal mycoflora.

There was a noticeable increase in fungal species in the 2009 collections compared with those in 2006 (22 and 13 species, respectively) (Tables 4 and 5). However, a t-test showed no significant difference among the very frequent and frequent species among uncontaminated sites ($p>0.05$), while there was a significant difference in the infrequent species among uncontaminated sites ($p<0.05$). The genus *Aspergillus* (Lee and Baker 1972, Dunn and Baker 1984, Follosco and Uyenco 1984, Salvo and Fabiano 2006, Gomes et al. 2008) was most frequent in uncontaminated samples followed by *Fusarium* (Sarquis and Borba 2007, Singh and Sharma 2009) and by Mycelia sterilia (Dunn and Baker 1984, Salvo and Fabiano 2006) (Tables 4 and 5). The dominance of these mitosporic fungi, although not necessarily of the same species composition, in both collection periods at the same sites may be attributable to their ubiquity in nature (Onions et al. 1981), or they might be members of the orig-

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inal indigenous mycoflora. Other factors such as seasonal availability of fungal propagules, availability of substrates for colonization, nutrient supply, greater facility of formation, survival and dispersal of their light propagules, collection site, time of the year relative to humidity, rainfall, wind speed and proximity to source where they were produced (Lee and Baker 1972, Manzoor et al. 2004) may also be responsible for the dominance of the three types of fungi observation.

In this study, fungal density was computed to compare 2006 and 2009 data from various samples collected from oil-contaminated and uncontaminated sites. In general, there was a significantly higher fungal density in all samples collected from oil-contaminated sites in 2009 than in 2006 and from uncontaminated sites (except for beach soil in Getulio: reference site) (Table 6). We believe that the remarkable increase in the fungal community over the three-year period after the oil spill clearly indicates recovery and re-establishment of normal fungal flora among the oil-contaminated sites in Guimaras. This speculation is further strengthened by the apparent changes in fungal species composition especially noted at oil-contaminated sites. Unfortunately, there is little direct information available in the literature that could support the preceding speculation regarding the responses of mycoflora caused by oil spills in coastal habitats in the tropics. In conclusion, our present study provides some baseline information on how fungi respond over the long-term to oil spills in a tropical setting.

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