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Initial Assessment of Fungal Population at Various Sites in Guimaras after the Solar I Oil Spill.

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Abstract. Many tropical environments areas are being heavily disturbed by human activity such as the marine environment. They have been a subject to contamination by organic pollutants from a variety of sources including accidental oil spills. On August 11, 2006, M/T Solar I chartered by Petron sunk off the coast of Southern Guimaras released more than two million liters of Bunker C oil into the sea and affected diverse coastal habitats and ecosystems with considerable environmental damage. There is little information on the effect of disturbance on fungi in tropical coastal ecosystems such as sandy beaches and mangroves which are habitats distinctive to the tropics. This study therefore, examined the effects of the oil spill on the fungal diversity and density among the contaminated sites in the southern part of the island of Guimaras, Philippines within two months after the spill. The initial assessment was conducted on October 11, 2006 at eight sites were selected composed of six oil-contaminated sites and two from un-contaminated areas that served as control. Samples obtained were composed of one beach water sample and three soil samples. Yeasts and filamentous fungi were examined by macroscopic and microscopic observation of colonies. Identification was based on available keys and monographs. Majority of species isolated were mitosporic and typical soil fungi which are terrestrial in origin but are considered as facultative marine fungi. There are more fungal isolates at oil-contaminated sites than un-contaminated sites at 23 and 13 species, respectively as well as a higher Shannon Index of Diversity (H') at 1.248 and 1.039, respectively. Among the filamentous fungi, nine genera were represented by *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Memnoniella*, *Monilia*, *Mucor*, *Penicillium*, *Pestalotia* and *Sporothrix*. There was a low index of similarity of species composition between oil-contaminated and un-contaminated sites as shown by Jaccard's coefficient of similarity (J = 0.40) and Sorensen coefficient of similarity: C = 0.57. All samples from oil-contaminated sites yielded a higher diversity and evenness of fungi compared with un-contaminated sites based on the Index of Diversity consistent with the overall trend of fungal occurrences. It appears that most of the oil-contaminated sites particularly beach and mangrove surface soil samples at Taklong Island exhibited a much higher fungal load compared with the un-contaminated sites that could possibly be attributed to the presence of oil in areas that favored their growth. This study provided some evidences on the short term impacts in terms of change in fungal assemblages and density. Although the report is not conclusive considering the absence of pre-spill data and variability between months or year, the evidence indicates that there was disturbance brought about by the oil. Thus, the need for a long-term monitoring program to examine the effects of oil in these areas to determine recovery and re-instatement of normal fungal flora.

Keywords:
Oil spill, marine fungi, bioremediation

Introduction

Fungi are achlorophyllous, heterotrophic organisms with absorptive or osmotrophic mode of nutrition (Alexopoulos, Mims, and Blackwell, 1996). Their cells secrete extracellular enzymes which break down potential food sources, which are then absorbed back into the fungal colony (Bennette, Wunch, and Faison, 2002). Such a mode of nutrition allows them to utilize almost an unlimited diversity of nutritional microniches, thus making them ubiquitous (Kendrick, 2001). They are important components of ecosystems as they are cosmopolitan and isolated from a variety of tropical, subtropical and temperate habitats (Smith

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and Berry, 1975). They are also considered to be the most active microorganisms in the decomposition of organic compounds both in sand and water (Moore-Landecker, 1996). They are abundant in soil, on vegetation and in both freshwater and marine waters. While most fungi recorded from marine sediments were collected from coastal regions and are typical soil fungi which are terrestrial in origin, they can be defined as facultative marine fungi in accordance with the classification of Kohlmeyer and Kohlmeyer (1979). However, overall fungal diversity is also greatly affected by the nature and availability of substratum (Jones, 2002).

The understanding and recognition of the effects of human disturbance on fungal diversity in the tropics is of great importance if renewable resources are to be conserved effectively (Tsui *et al*., 1998) and a healthy environment maintained. Disturbance can occur at all levels of ecological organization and at different temporal and spatial scales (Zak, 1992). However, such disturbances are actually common features in most ecosystems either due to anthropogenic or natural actions. Studies on natural disturbance include influence of seasonal changes in rainfall, treefall, and hurricane damage showed population shifts and changes to communities of fungi (Lodge and Cantrell, 1995). There are a number of reviews on the effects of human disturbance on fungal communities (Zak, 1992; Miller and Lodge, 1997), but most were restricted to the effects of disturbance on soil or mycorrhizal communities in temperate regions**.** There is little information on the effect of disturbance on fungi in tropical coastal ecosystems such as sandy beaches and mangroves which are habitats distinctive of the tropics (Tsui *et al*., 1998).

Many tropical environment areas are being heavily-disturbed by human activity. For example, the marine environment has been subjected to contamination by organic pollutants from a variety of sources such as uncontrolled releases from manufacturing and refining installations, spillages from oil tankers during transportation, direct discharges from effluent treatment plants, and run-off from terrestrial sources (Tsui *et al*., 1998). Among these sources, contamination resulting from oil spills from tanker accidents have attracted public attention to the fate and effects of petroleum hydrocarbons in marine environments (Huijer, 2005). It is estimated that world annual oil spills into the ocean amounts to about 1.7-8.8 million metric tons, approximately equivalent to about 0.1 to 0.2% of the world annual petroleum production (Harayama *et al*, 1999).

On August 11, 2006, M/T Solar I chartered by Petron sunk off the coast of Southern Guimaras released more than two million liters of Bunker C oil into the sea and affected diverse coastal habitats and ecosystems with considerable environmental damage. The contamination of environmentally-critical habitats had been noticed on larger biota, *i.e.* mangroves, sea grasses, faunal assemblages; while impacts on microbial population was not. Petroleum spillage is known for its environmental problems on water and soil. In addition, it also reduces microbial diversity through the phenomenon of selectivity where microorganisms capable of surviving in such a polluted environment are those that develop specific enzymatic and physiological responses that allow them to use the hydrocarbon compounds as substrates (Atlas, 1995, 1991; Atlas *et al*, 1991). So far very little published information is available for the effects of human disturbance such as oil spills on fungal diversity in the tropical marine environments (Hyde, 1989). The purpose of this study is, therefore, to examine, the effects of oil spill on fungal diversity and density among the contaminated sites in southern part of the island of Guimaras, Philippines within two months after the spill.

Materials and Methods

Sites and Sample Collection

The initial assessment was conducted on October 11, 2006. Eight sites were selected composed of six oil-contaminated, and two un-contaminated areas served as control. The un-contaminated sites were Barangays Lawi, Jordan and Brgy. Getulio, Buenavista; the oil-contaminated sites were Brgy.Tando, Taklong Island, Cabalagnan, Panobolon, Sabang, and Inampulogan. In each instance, obtained were one beach water sample, and three soil samples. The beach soils were taken thus: within 2 m from shoreline, surface mangrove soil, and subsurface mangrove soil taken from within 5 cm depth from surface. This gave four samples from each site (Figure 1).

Incubation Procedure

The spread plate method was used and serial dilutions of samples were made using sterile 2.5 % NaCl solution as diluent and plated on potato dextrose agar (PDA) supplemented with 2.5% NaCl (PDA) and antibiotic (Pen G, Berlin Manufacturing Corp.). Each sample made up of 10 g soil and 10 ml water, was diluted in 90 mL of sterile 1.5% NaCl solution and were subsequently serially diluted following Pepper and Gerba (2004) . Duplicate plates were made per sample type per site and were subsequently incubated for seven days at room temperature (26.0–28.1ºC, 43.4–53.2 % RH). Plates were examined for appearance of fungal colonies and counted daily for seven days; and morphologically-distinct colonies

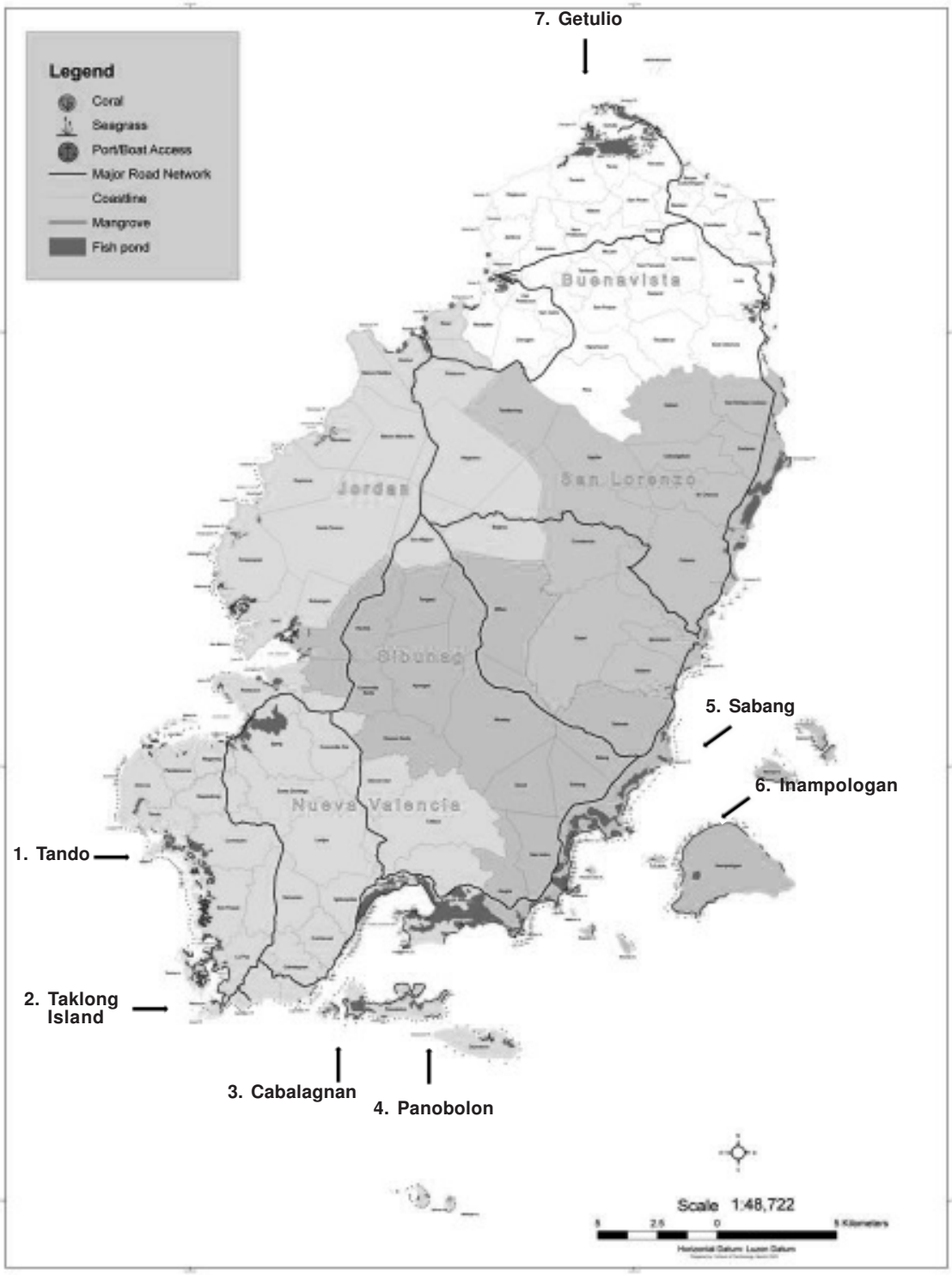

Figure 1. Sampling sites in Guimaras. 1-6 Oil-contaminated and 7-8 uncontaminated sites.

were isolated further and purified, and stored in test tubes overlaid with 5ml mineral oil. A control plate was included by exposing a blank PDA plate in the middle of the work area for 15 minutes. Isolation was done at the Fish Health Laboratory of SEAFDEC AQD, Tigbauan, Iloilo within six hours of field collection; while examination of fungal isolates was done at DOST VI Regional Calibration and Testing Center, La Paz, Iloilo City.

Yeasts and filamentous fungal counts were expressed as colony-forming units per ml (CFU/ml) for water and colony-forming units per gram (CFU/g) for soil samples as done by Pepper and Gerba (2004). Fungal colonies were then isolated and purified.

Fungi were examined by macroscopic and microscopic observation of colonies using the slide-culture technique by Riddell (1950). Description of various species were based on colony characteristics and microscopic features such as such spore morphology, sizes, conidia formation and other relevant characters for identification based on keys by Barnett and Hunter (1987), Ellis (1971), Domsch, Gams and Anderson (1980) and Kohlmeyer and Kohlmeyer (1979).

Data Analyses

Fungal Diversity

Frequency of occurrence (%) of fungi on each sample type and site were calculated as follows based on Hyde (1989) and Sarma and Hyde (2001):

(1) Frequency of occurrence of species A (%)=

$$\frac{\text{No. of collections of Species A}}{\text{Number of samples examined}} \times 100$$

Fungi were grouped further based on their frequency of occurrence following Sarma and Hyde (2001): Very frequent $\geq$" 10%; Frequent = 5 to 10%; Infrequent =1-5%; Rare <" 1%.

The diversity of fungi on four sample types from eight sites was assessed based on the diversity indices (Magurran, 1988):

(2) Species diversity (Shannon Index (H')

Shannon Index (H') = - " (*pi ln pi*) ; where *pi* is the proportion of individual that the species *i* contribute to the total number of individuals as shown in the formula below

$$\frac{n_i}{N}$$

where:
N = total number of individuals (records)
n*i* = number of individuals *i*1, *i*2,*i*3,i4,…*i*x

(3) The Shannon evenness, *J'*, was expressed by:

Simpson Index D'= $\dfrac{1}{\sum P_i^2}$

$$J' = \frac{H'}{H'_{max}}$$

where, *H'max* is the maximum value of diversity for the number of species present (Pielou, 1975).

(4) Species dominance was computed based on the following formula:

(**5) Jaccard's index of similarity (*JI*)** was computed among the oil-contaminated and un-contaminated sites from various sample types based on the presence

$$C = \frac{2c}{2c + a + b}$$

or absence of each fungal species (Kenkel and Booth, 1992) as follows:

$$JI = c/(a+b+c),$$

where, *c* is the number of fungal species occurring in both hosts, *a* is the number of fungal species unique to the oil-contaminated sites; and, *b* is the number of fungal species unique to the un-contaminated sites

(6) Sorensen coefficient:
where:

a = number of species occurring in 'a' alone
b = number of species occurring in 'b' alone
c = number of co-occurrence of species

Fungal Density

Two-way ANOVA was employed to determine the differences in the mean fungal load of fungi on each sample type and site.

Results and Discussion

Impact on Fungal diversity

Overall Frequency of occurrence between oil-contaminated and un-contaminated sites

Majority of the species isolated were mitosporic and typical soil fungi which are considered facultative marine fungi in accordance with the classification of Kohlmeyer and Kohlmeyer (1979). There were more fungal isolates from oil-contaminated sites than from un-contaminated sites with (23 vs. 13 species respectively). A higher Shannon Index of Diversity (H') at 1.248 was likewise noted in the oil-contaminated compared with 1.039 in un-contaminated sites (Table 1). Only five genera of filamentous fungi (*Aspergillus, Memnoniella, Monilia*,

Table 1. Overall list of fungi isolated in oil-contaminated and uncontaminated samples from various sites in Guimaras. October 11, 2006. X indicates presence.

Penicillium, Sporothrix) were noted in the un-contaminated sites compared to the nine in the contaminated areas. The five genera are also represented in the oil-contaminated sites which had in addition, *Aureobasidium*, *Cladosporium*, *Mucor* and *Pestalotia*. Two species (*Monilia* sp. 1 and *Sporothrix* sp. 1) were recorded only in the un-contaminated sites compared with 11 species (*Aspergillus flavus*, *Aureobasidium cf. pullulans*, *Aspergillus* sp. 2, *C. cladosporioides*, *Memnoniella* sp 1, *Monilia* sp. 2, *Mucor* sp. 2, Mycelia sterilia, *Penicillium cf. brevicompactum*, *P. cf. funiculosum*, *Pestalotia* sp.) in the contaminated sites (Table 1). Furthermore, twelve species were counted only at oil-contaminated sites while two species were isolated only at un-contaminated sites (Table 1). In addition, there is a low index of similarity of species composition between the two sites as shown by Jaccard's coefficient of similarity (J=0.44) and Sorensen coefficient of similarity C = 0.61. Among the four species of yeasts collected,

Table 2. Overall frequency of occurrence (%) of fungi from various samples at oil-contaminated sites in Guimaras, Philippines. October 11, 2006.

No.	Species	Total collections	Freq. of occ. (%)*
	Very frequent species (= 10%)		
1	*Aspergillus cf. fumigatus*	8	16.7
2	Yeast 4	5	14.6
3	Mycelia sterilia	7	10.4
	Frequent species (5-10%)		
4	*Aspergillus cf. candidus*	4	8.3
5	*Aspergillus cf. terreus*	4	8.3
6	Yeast 3	3	6.3
7	*Penicillium cf. verrucosum*	3	6.3
8	*Sporothrix* sp 2	3	6.3
9	Yeast1	3	6.3
	Infrequent (1-5%)		
10	*Aureobasidium* sp 1	2	4.2
11	*Aureobasidium cf. pullulans*	2	4.2
12	*Memnoniella* sp 1	2	4.2
13	*Mucor* sp 1	2	4.2
14	*Penicillium* cf. *funiculosum*	2	4.2
15	*Aspergillus niger*	1	2.1
16	*Aspergillus flavus*	1	2.1
17	*Cladosporium cladosporioides*	1	2.1
18	*Memnoniella* sp 2	1	2.1
19	*Monilla* sp 2	1	2.1
20	*Penicillium cf. brevicompactum*	1	2.1
21	*Penicillium* sp 4	1	2.1
22	*Pestalotia* sp	1	2.1
23	Yeast 2	1	2.1
	Total Samples Examined	48	
	Summary		
	Total no. of species	**23**	
	Shannon Index of diversity: H'	**1.248**	
	Shannon Index of eveness: J'	**0.916**	
	Simpson Index of dominance: D'	**0.642**	

only Yeast 2 and Yeast 4 were isolated from oiled sites.

A comparison of very frequent species showed that *Aspergillus cf. fumigatus*, Yeast 4 and Mycelia sterilia had more than 10% occurrence in oil-contaminated sites. The same is true for *Penicillium cf. verrucosum, Aspergillus cf. terreus, Penicillium cf. funiculosum* (Tables 2 and 4). These genera were also isolated from other un-contaminated sites such as mangrove rhizophere (Tariq *et al.,* (2008), coastal sediments (Manzoor *et al.* 2004), and mangrove seedlings (Mehdi and Saifullah (2000). However, a closer examination of the results of the present study showed that most of the genera represented here such as *Aspergillus* (Austin *et al.*, 1977; Davies and Westlake, 1979; U. S. Congress Office of Technology Assessment, 1991; Okerentugba and Ezeronye, 2003; Mancera-López, *et al.,* 2007); *Cladosporium* (Walker *et al.*, 1973; U.S. Congress Office of Technology Assessment, 1991); *Mucor* (Adekunle and Adebambo, 2007); *Penicillium* (Austin *et al.*, 1977; Davies and Westlake, 1979; Llanos and Kjoller, 1976; U. S. Congress Office of Technology Assessment, 1991; Okerentugba and Ezeronye,

Table 3. Frequency of occurrence of fungi from various samples at oil-contaminated sites in Guimaras, Philippines. October 11, 2006.

Sample type	No.	Species	Total collections	Freq. of occ. (%)*
Surface Water	Very frequent species (= 10%)			
	1	Yeast 1	3	25
	Frequent (5-10%)			
	2	*Aspergillus niger*	1	8.3
	3	*Aspergillus flavus*	1	8.3
	4	*Penicillium cf. verrucosum*	1	8.3
		Shannon Index of diversity: H'	0.145	
		Shannon Index of evenness: J'	0.097	
Beach Soil	Very frequent species (= 10%)			
	1	*Aspergillus cf. candidus*	4	33.3
	2	*Aspergillus cf. fumigatus*4	3	25
	Frequent species (5-10%)			
	3	*Penicillium cf. verrucosum*	1	8.3
	4	*Penicillium cf. brevicompactum*	1	8.3
	5	*Monilia* sp 2	1	8.3
	6	*Memnoniella* sp 1	1	8.3
	7	*Memnoniella* sp 2	1	8.3
	8	*Aureobasidium cf. pullulans*	1	8.3
	9	*Cladosporium cladosporioides*	1	8.3
	10	Yeast 2	1	8.3
		Shannon Index of diversity: H'	0.359	
		Shannon Index of evenness: J'	0.241	
Mangrove Soil: surface	Very frequent species (= 10%)			
	1	*Aspergillus cf. terreus*	4	33.3
	2	Yeast 3	4	33.3
	3	*Aureobasidium* sp 1	3	25
	4	*Sporothrix* sp 2	3	25
	5	Mycelia sterilia	2	16.7
	Frequent species (5-10%)			
	6	*Penicillium* sp 4	1	8.3
	7	*Penicillium cf. funiculosum*	1	8.3
	8	*Mucor* sp 1	1	8.3
	9	*Memnoniella* sp 1	1	8.3
	10	*Pestalotia* sp	1	8.3
		Shannon Index of diversity: H'	0.458	
		Shannon Index of evenness: J'	0.307	
Sample type			Total collections	Freq. of occ. (%)*
Mangrove Soil: Sub-Surface	Very frequent species (= 10%)			
	1	Yeast 4	5	41.7
	2	*Aspergillus cf. fumigatus*	5	41.7
	3	Mycelia sterilia	5	41.7
	Frequent species (5-10%)			
	4	*Aureobasidium cf. pullulans*	1	8.3
	5	*Penicillium cf. verrucosum* -	1	8.3
	6	*Mucor* sp 1	1	8.3
		Shannon Index of diversity: H'	0.341	
		Shannon Index of evenness: J'	0.228	

Table 4. Overall Frequency of occurrence of fungi from various samples at un-contaminated sites in Guimaras, Philippines. October 11, 2006.

No.	Species	Total collections	Freq. of occ. (%)*
	Very frequent species (= 10%)		
1	*Penicillium cf. verrucosum*	3	18.75
2	*Aspergillus cf. terreus*	2	12.5
3	*Penicillium cf. funiculosum*	2	12.5
	Frequent species (5-10%)		
4	*Penicillium* sp. 4	1	6.25
5	*Aspergillus niger*	1	6.25
6	*Aspergillus cf. candidus*	1	6.25
7	*Aspergillus cf. fumigatus*	1	6.25
8	*Memnoniella* sp2	1	6.25
9	*Monilia* sp1	1	6.25
10	*Sporothrix* sp1	1	6.25
11	*Sporothrix* sp2	1	6.25
12	Yeast1	1	6.25
13	Yeast3	1	6.25
	Summary		
	Total no. of species	**13**	
	Shannon Index of diversity: H'	**1.039**	
	Shannon Index of evenness: J'	**0.963**	
	Simpson Index of dominance: D'	**0.926**	

2003; Salvo *et al*, 2005; Mancera-López *et al.,* 2007); *Pestalotia* (Cerniglia and Sutherland, 2001) are non-lignolytic have also been reported in other oil-contaminated sites. The occurrence of mycelia sterilia only at oil-contaminated mangrove sub-surface site in Taklong Island could be a result of the high concentrations of oil. This is similar to the report by Krivobok *et al*. (1998) when they reported that high polycyclic aromatic hydrocarbon (PAH) concentrations inhibited the growth of sensitive fungal strains and stimulated the emergence of sterile mycelia. Other PAH-metabolizing non-lignolytic species have been listed in Cerniglia and Sutherland (2001).

The presence of more species in oil-contaminated sites could possibly be due to the influence of petroleum spillage that did not necessarily reduce microbial diversity through the phenomenon of selectivity. The oil could have possibly caused a shift in the population of fungi towards those capable of surviving such polluted environment, that is, those that develop specific enzymatic and physiological responses that allow them to use the hydrocarbon compounds as substrates (Atlas, 1985, 1991; Atlas *et al*, 1991). Reduction in fungal diversity was observed by Salvo *et al* (2005) among PAH-impacted sediments of Genoa-Voltri Harbour (NW Mediterranean, Italy) but most of the genera identified such as *Mucor*, *Chaetomium*, *Trichoderma* and *Cladosporium* are typical of soil ecosystems. While individuals were evenly distributed among the species in both oil-contaminated and un-contaminated sites, the Simpson's Index of dominance in oil-contaminated sites was lower when compared with un-contaminated sites at 0.642 and 0.926, respectively, suggesting that the dominance of *Penicillium* and *Aspergillus* resulted from the addition of oil in the area.

Microbial selectivity could also be gleaned from the report of Hyde (1989) in Brunei wherein the presence of hydrocarbons reduced the diversity and numbers of saprotrophic fungi on intertidal mangrove wood. He reported that species diversity at Seria mangrove was low (23 species) when compared to Kampong Serasa (45 species) and the number of identified species was also lower (92 vs. 132). It was not established how the hydrocarbons entered the mangrove. It could have been due to spillage from oil pipelines, or to natural seepage but thick layers of

Table 5. Frequency of occurrence (%) of fungi from various samples at un-contaminated sites in Guimaras, Philippines. October 11, 2006.

Sample type	No.	Species	Total collections	Freq. of occ. (%)*
		Very frequent species (= 10%)		
Water	1	*Aspergillus niger*	1	25
	2	Yeast 1	1	25
		Shannon Index of diversity: H'	0.056	
		Shannon Index of evenness: J'	0.037	
		Very frequent species (= 10%)		
Beach Soil	1	*Aspergillus cf. candidus*	1	25
	2	*Aspergillus* cf. *fumigatus*	1	25
	3	*Penicillium cf. verrucosum*	1	25
	4	*Monilia* sp 1	1	25
		Shannon Index of diversity: H'	0.112	
		Shannon Index of evenness: J'	0.075	
		Very frequent species (= 10%)		
Mangrove Soil: surface	1	*Aspergillus cf. terreus*	2	50
	2	*Penicillium cf. funiculosom*	2	50
	3	Yeast 3	1	25
	4	*Sporothrix* sp 1	1	25
	5	*Sporothrix* sp 2	1	25
	6	*Penicillium* sp 4	1	25
		Shannon Index of diversity: H'	0.205	
		Shannon Index of evenness: J'	0.137	
		Very frequent species (= 10%)		
Mangrove Soil: sub-Surface	1	*Penicillium verrucosum*	2	50
	2	*Memnoniell*a sp 2	1	25
		Shannon Index of diversity: H'	0.074	
		Shannon Index of evenness: J'	0.050	

oily mud (3-7 mm) coated most of the lignicolous samples. The presence of hydrocarbons on the substratum and mangrove mud reduces aeration and slows down the activity of micro-organisms resulting from deficiency of nutrients essential for the growth of the micro-organisms via mineralization of organic matter (Scherrer and Miller, 1989). However, a study on a site contaminated with oil in Mauritius (Beau Champ mangrove), showed that the intertidal manglicolous fungi had a higher diversity (36 species) compared to a nearby undisturbed mangrove where only 30 species were collected (Poonyth, unpublished; also in Tsui *et al*., 1998.). This might be due to tolerable levels of contaminants that have not reached amounts deleterious to the mycota or, such condition might even be favorable for the fungi to flourish. However, the absence of baseline studies prior to contamination makes it difficult to confirm this observation (Tsui *et al*., 1998).

All samples from oil-contaminated sites yielded higher diversity and evenness of values compared to un-contaminated sites (Tables 3 and 5).

Fungal Density

Fungal density was computed to compare the various samples collected from oil-contaminated and un-contaminated sites. There was no significant difference in fungal density among beach water samples. Beach soil samples on the other hand, at Taklong (4.3×10^4 (CFU g^{-1}) and Inampulugan (4.1×10^4 CFU g^{-1}) were higher than the un-contaminated sites at Getulio (3.3×10^4 (CFU g^{-1}) and Lawi (5×10^3 (CFU g^{-1}). Fungal density within the mangroves showed that surface soil samples recorded the highest at Taklong (9×10^4 CFU g^{-1}) than Getulio (3.4×10^4 (CFU g^{-1})) and Lawi (4.2×10^4 (CFU g^{-1}). All other oiled sites, however, showed lower values than the

Table 6. Comparison of fungal density of different types of samples collected from oil-contaminated and un-contaminated sites in Guimaras, Philippines. October 11, 2006.

SITE	Surface/Beach Water	Beach Soil	Mangrove soil, surface	Mangrove soil, subsurface
1. Tando	4.0×10^{1}	2.7×10^{4}	1.3×10^{4}	1.8×10^{4}
2. Taklong	6.4×10^{1}	4.3×10^{4}	9.0×10^{4}	1.7×10^{4}
3. Cabalagnan	5.5×10^{1}	7.2×10^{3}	2.6×10^{4}	4.2×10^{4}
4. Panobolon	5.5×10^{1}	1.1×10^{4}	1.8×10^{4}	9.0×10^{3}
5. Sabang	9.5×10^{1}	1.7×10^{4}	1.5×10^{4}	1.5×10^{4}
6. Inampulogan	6.4×10^{1}	4.1×10^{4}	1.4×10^{4}	8.6×10^{4}
Mean	6.2×10^{1}	2.4×10^{4}	2.93×10^{4}	3.11×10^{4}
7. Getulio*	8.2×10^{1}	3.3×10^{4}	3.4×10^{4}	1.9×10^{4}
8. Lawi*	7.5×10^{1}	5.0×10^{3}	4.2×10^{4}	2.0×10^{4}
Mean	7.85×10^{1}	1.9×10^{4}	3.8×10^{4}	1.95×10^{4}

un-contaminated sites at Lawi and Getulio. The oil-contaminated mangrove sub-surface soil samples yielded higher fungal density at Cabalagnan (4.2×10^{4} (CFU g^{-1})) and Inampulugan (8.6×10^{4} (CFU g^{-1})) compared with Lawi (1.9×10^{4} (CFU g^{-1}) and Getulio (2.0×10^{4} (CFU g^{-1}) (Table 6). It appears that most of the oil-contaminated sites particularly beach and mangrove surface soil samples at Taklong Island exhibited much higher fungal loads compared to the un-contaminated sites. This could possibly be attributed to the presence of oil in these areas that favored the appearance and proliferation of hydrocarbon degraders (Atlas, 1995, 1991; Atlas *et al*, 1991). This result is consistent with the high diversity of fungi collected at contaminated sites indicative of the impacts of oil as a form of disturbance on the fungal population in a tropical setting. This can however, only be established with further monitoring of fungal diversity and density in these areas.

Conclusion

There is a paucity of information available on the effects of oil spill on fungi in the tropics. This study provided some evidences on the short term impacts of change in fungal assemblages and density. Although there was no pre-spill data to show variability between and among months or year, the present evidence does indicates that there was a disturbance brought about by the oil. Thus, the need for further studies and a long-term monitoring to examine the effects of oil in these areas, to determine recovery and re-establishment of normal fungal flora.

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