

Effects of an oil spill on the leaf anatomical characteristics of a beach plant (*Terminalia catappa* L.)

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Abstract This study investigated the short-term impacts of an oil spill on the leaf anatomical structures of *Terminalia catappa* L. from crude oil leakage in Rayong province, Thailand, in 2013. Approximately 3 weeks after the oil spill, leaves of *T. catappa* were collected along the coastline of Rayong from one affected site, five adjacent sites, and a control site. Slides of the leaf epidermis were prepared by the peeling method, while leaf and petiole transverse sections were prepared by paraffin embedding. Cell walls of adaxial epidermal cell on leaves in the affected site were straight instead of the jigsaw shape found in leaves from the adjacent and control sites. In addition, the stomatal index of the abaxial leaf surface was significantly lower in the affected site. Leaf and petiole transverse sections collected from the affected site showed increased cuticle thickness, epidermal cell diameter on both sides, and palisade mesophyll thickness; in contrast, vessel diameter and spongy mesophyll thickness were reduced. These significant changes in the leaf anatomy of *T. catappa* correspond with previous research and demonstrate the negative effects of oil spill pollution on plants. The anatomical changes of *T. catappa* in response to crude oil

pollution are discussed as a possible indicator of pollution and may be used in monitoring crude oil pollution.

Keywords Crude oil pollution · Epidermal cell wall · Histology · Leaf anatomy · Oil leakage · Thailand

Introduction

Oil spills have negative impacts on the coastal environment, as well as entire ecosystems, and are considered to be a significant problem throughout the world, including in Thailand (Singkran 2013). Thailand has increasingly faced oil spill incidents occurring in coastal areas over the last 30 years as a result of increased oil production and transportation due to the rising global demand for petroleum fuels and products (Singkran 2013, 2014). Such incidents have also had an effect on coastal livelihoods (Singkran 2013, 2014). In 2013, an estimated 50 tons of crude oil leaked into the Gulf of Thailand near the eastern coast of Thailand (Rayong province). The site of the oil slick was estimated at approximately 20 km off mainland and 35 km off Samet Island. The effects of this 2013 oil spill have raised concern in terms of both the ecological and economic impacts on Rayong's coastal communities. However, the negative effects of the oil spill have only been studied in Samet Island, where the oil slick had hit one of its bays (Ao Phrao) (Casarotto et al. 2014). Although Rayong province has been classified as a high-risk area for oil spills in Thailand (Singkran 2013), there is no report or study of oil spill impacts on mainland Rayong's coasts. These beach communities, including beach plants, are therefore likely to experience a higher risk of oil pollution.

Petroleum or crude oil is a mixture of many different hydrocarbons (alkanes, cycloalkanes, and various aromatic hydrocarbons), together with other organic compounds of sulfur,

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nitrogen, and oxygen, plus low concentrations of iron (Fe), nickel (Ni), copper (Cu), and vanadium (V) (Baker 1970; Maranhão et al. 2006). Oil pollution generally impacts beach vegetation by contaminating internal plant tissues (Anderson and Hess 2012) and by coating plant leaves (Pezeshki and De Laune 1993). Several cases have documented the response of plant growth and physiology to oil pollutants, spanning diverse plant species. Penetration of crude oil into the vascular system reduces the capacity for transportation of nutrients, water, or other essential solutions, which can decrease photosynthetic efficiency (Baker 1970; Pezeshki et al. 2000; Kasim 2005). The penetration depends on the type of oil, as well as the part of the plant oiled (leaves or roots), the cutin layer thickness, and the stomatal index (Baker 1970). For instance, the stomatal index (the number of stomata per unit area of leaf) of *Chromolaena odorata* was reduced and disrupted by crude oil (Gill et al. 1992). Omosun et al. (2009) also reported a decrease in the number of stomata associated with crude oil pollution, and further found that the cortical cell shape and size of plants growing in crude oil-polluted conditions differed from those grown in unpolluted sites (Omosun et al. 2008). Moreover, oil coating is known to block stomata and intercellular space, leading to stress conditions and resulting in reduced transpiration rates (Baker 1970) and gas exchange (Pezeshki et al. 2000). In terms of the cuticle layer, thicker cuticles along leaf blades and petioles can protect leaves exposed to oil (Knight et al. 1929; Baker 1970). Thicker and larger leaf epidermal cells were also observed in polluted plants, which is believed to protect against the translocation of metal ions from crude oil to vascular or photosynthetic tissues (Osuji and Onojake 2004; Gomes et al. 2011). As plants need effective photosynthesis, larger and more abundant chlorophyll are found in the palisade parenchyma during stress conditions, such as oil pollution (Fahn 1990; Klich 2000). Furthermore, the ability of plants to recover from oil pollution depends on the duration of oil exposure and the type of plant (Baker 1970; Pezeshki and De Laune 1993).

Although petroleum hydrocarbons or crude oil are widespread environmental pollutants, information about their toxicity to plants is limited, particularly the changes in plant anatomical characters. From prior studies, it can clearly be seen that the leaf anatomy of plants is sensitive to oil pollution. The anatomy of plants in response to oil pollution can therefore be used as a simple method for monitoring environmental pollutants and can be effective bio-indicators. In this study, the short-term effects of the 2013 Rayong oil spill on beach plants were investigated along the coastline of Rayong province, Thailand. The Indian almond or tropical almond tree, *Terminalia catappa* L. (Combretaceae), was selected as a representative of woody beach plants to study leaf anatomical structure (stomatal index, cuticle thickness, epidermal cell, and palisade mesophyll) and to investigate its potential in monitoring crude oil pollution. It was hypothesized that oil-

affected plants would have reduced synthetic efficiency; therefore, these plants would be associated with lower stomatal density (index), less sinuous anticlinal epidermal cell walls, higher epidermal cell height, thicker cuticle, and a well-developed palisade mesophyll. Our objective was therefore to determine how *T. catappa* would respond to oil exposure in the field.

Materials and methods

Study sites

Our study sites were located along mainland Rayong's coastline (eastern Thailand), 20 km away from where the oil spill incident occurred, and the offshore coast of Samet Island where the west coast was heavily impacted by the oil slick. Vegetation in the study sites was composed of beach plant communities, and most of it was planted by humans and cleared for tourism and accommodation. The seven sites included one affected site (6: Ao Phrao, Samet Island), five adjacent sites (1: Ban Chang, 2: Suchada Estuary, 3: Maha-Surasinghanat Military Camp, 4: Lan Hin Kao, 5: Suan Son) (Fig. 1), and one control site at the Faculty of Science, Mahidol University, Bangkok, Thailand.

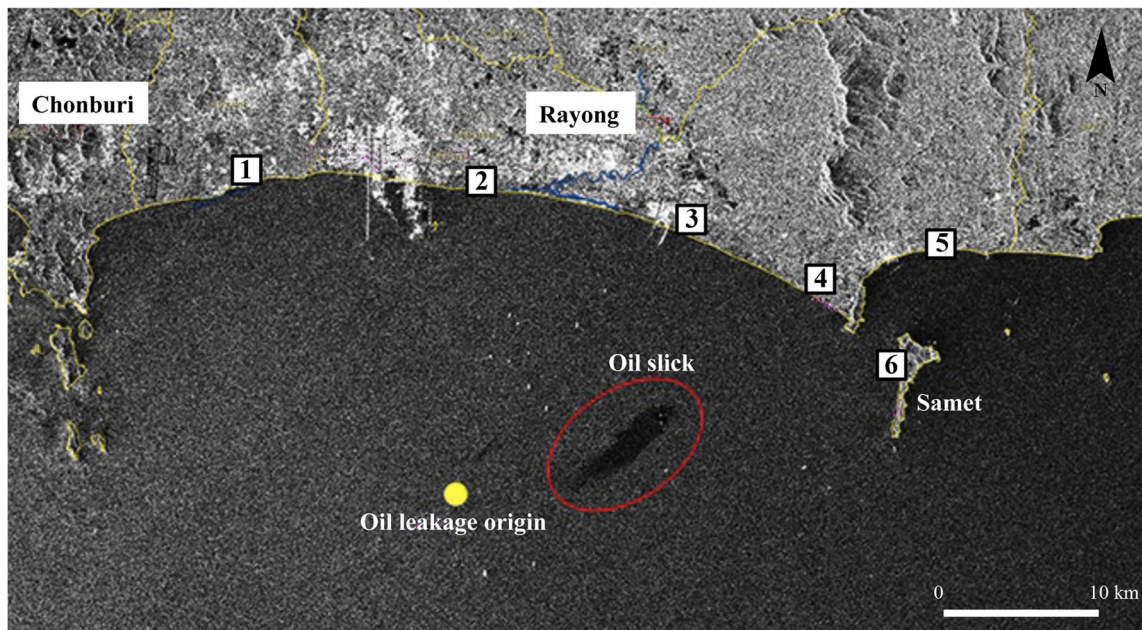
Plant samples

Three *T. catappa* trees >20 cm in diameter at breast height (DBH) were selected from each coastal study site as shown in Fig. 1, 3 weeks after the oil spill (August 2013), and the fifth leaf from the tip of the lowest branch was collected from each study tree. All plants in all six study sites were found in open areas along the beach. Leaves from each location were preserved in 70% ethanol. Leaves were also sampled following the same criteria as mentioned above from our control site (Mahidol University, Bangkok) and preserved in 70% ethanol.

Leaf anatomical study

The leaf epidermis of each sample was prepared by scraping away the undesired tissue with a razor blade; the surfaces were then stained for 10 min with 1% Safranin-O in water. The samples were then dehydrated by passing them through an ethanol series and cleared with xylene. The epidermal surfaces were mounted in DePeX mounting media (Traiperm et al. 2017).

Preparations for examining petiole and leaf transverse sections were carried out using the modified paraffin method (Johansen 1940). The wax-embedded samples were cut with a Leica SM 2000R sliding microtome into 10–20- μ m-thick sections. Sections were then fixed onto slides using 0.5% gelatin. The sections were stained with Safranin-O and



Site 1 Ban Chang: 12°40'31.5"N, 101°05'04.1"E

Site 3 Maha-Surasinghanat Military Camp 12°37'57.2"N, 101°20'24.6"E

Site 5 Suan Son: 12°37'58.7"N, 101°27'56.8"E

Site 2 Suchada Estuary: 12°40'07.4"N, 101°12'08.1"E

Site 4 Lan Hin Kao: 12°35'55.8"N, 101°24'22.5"E

Site 6 Aou Phrao: 12°34'14.8"N, 101°26'56.9"E

Fig. 1 Oil slick captured by the satellite RADARSAT-2 on 27 July 2013 at 18:31 h (modified after GISTDA 2014)

counterstained with Fast Green FCF, then permanently mounted in DePeX mounting media (Johansen 1940).

Slides were examined using an Olympus CX3 Light Microscope. Photographs were taken by an Olympus BX43 compound microscope with an attached Olympus DP11 digital camera.

Statistical analysis

The Stomatal Index (SI) was calculated following Salisbury (1927). Averages and standard deviations (SD) for all quantitative characters such as cell size and cutin layer thickness were calculated from three replications (three trees) at each site. Each leaf was measured five spots, and these values were averaged to calculate a single value per site (15 measurements per site). A one-way analysis of variance (ANOVA within software SPSS version 18) was performed to test for significant differences across study sites. A post hoc analysis (Fisher's least significant difference, LSD, test) was also conducted if significant differences were found across sites.

Leaf development observation

To investigate the morphological development of *T. catappa* leaves from young to fully mature, young shoots at the Faculty of Science, Mahidol University, Bangkok, Thailand, were observed.

Results

Leaf epidermal characters

Epidermal cells on both leaf surfaces of *T. catappa* collected from adjacent sites (sites 1–5) and the control site were jigsaw in shape with deeply lobed anticlinal cell walls (Fig. 2: 1A–5A, 7A, 1B–5B and 7B), while those of *T. catappa* collected from the affected site (site 6) were irregular in shape with slightly undulate walls on the adaxial leaf surface (Fig. 2: 6A; Table 1). The highest stomatal index (23.27 ± 2.42) was found on the abaxial epidermis of *T. catappa* at site 3. Similar stomatal indices of *T. catappa* were observed at sites 1 (21.09 ± 1.68), 2 (21.45 ± 2.12), 4 (21.52 ± 2.94), 5 (22.52 ± 1.33), and the control site (22.19 ± 0.45). In contrast, the lowest stomatal index was found at the affected site (15.53 ± 3.09) (Fig. 3a). The stomatal index of site 6 (affected site) was significantly lower than from sites 1–5 (adjacent sites) (ANOVA, $p = 0.05$).

Leaf transverse section characters

Petiole transverse sections from Ao Phrao, the affected site (site 6), showed an ovate vascular ring (distinct from the typical cordate shape) (Table 1), and the cutin layer ($2.582 \pm 0.30 \mu\text{m}$; Fig. 3b) was significantly thicker than the cutin layers at adjacent and control sites (ANOVA, $p = 0.05$) (Fig. 4: 6A and 6B). Moreover, the average height of epidermal cells from Ao Phrao ($17.37 \pm 1.15 \mu\text{m}$; Figs. 3b; 4: 6B) was significantly greater than those of the adjacent and control sites (Fig. 4: 1B–5B and 7B).

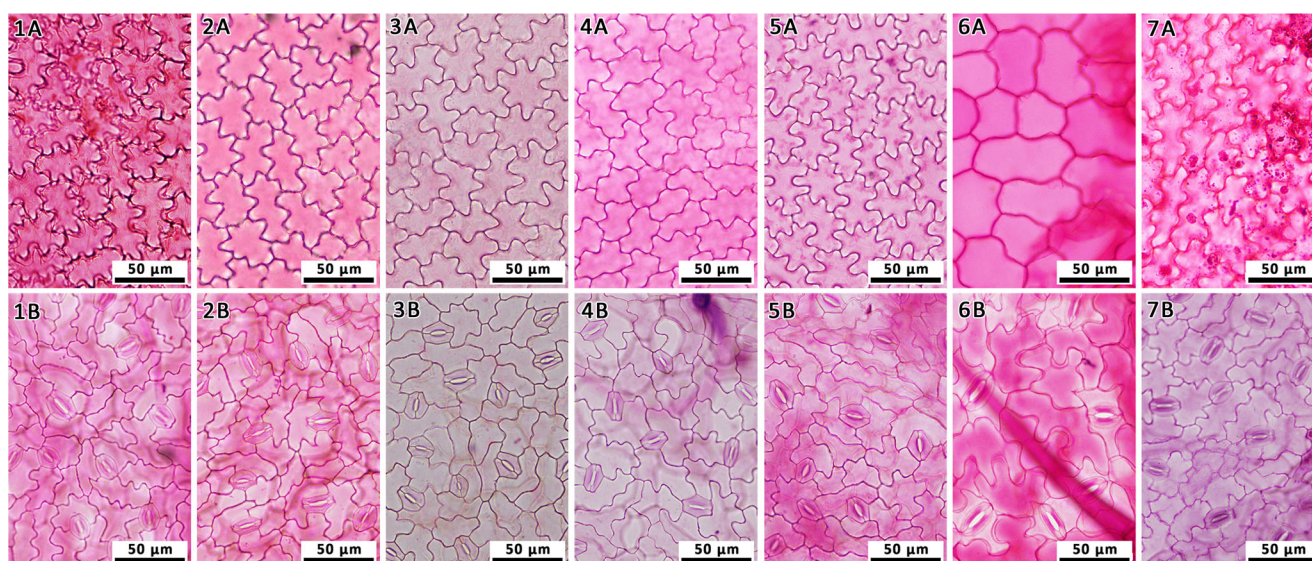


Fig. 2 Leaf epidermal cells of *Terminalia catappa* (40×). Adaxial surface (A), Abaxial surface (B). Numbers 1–6 represent the leaf epidermal surfaces from study sites 1–6, respectively. Number 7 shows leaf epidermal surfaces from the control site

The average vessel diameter from Ao Phrao ($41.04 \pm 1.66 \mu\text{m}$; Figs. 3b; 4: 6C) was significantly lower than all other sites except sites 2 and 3 (ANOVA, $p = 0.05$).

Midrib transverse sections from Ao Phrao had an ovate outline (instead of the usual cupuliform outline) (Table 1), significantly thicker cutin layers on both surfaces (Figs. 3c; 5), significantly greater epidermal cell heights (Fig. 5: 6B and 6C) (ANOVA, $p = 0.05$), and smaller vessel diameters (Fig. 3c) compared to those characters from the other adjacent sites and control site.

Both sides of the leaf blades from Ao Phrao (Fig. 5: 6E) had significantly larger epidermal cell heights and significantly thicker cutin layers (Fig. 5: 6E) compared to the other adjacent sites and control site (Fig. 3d). Palisade mesophyll of leaves from Ao Phrao was composed of two rows of elongated cells; the upper layer had larger cells than the lower layer (Fig. 5: 6E). In addition, leaves from Ao Phrao had significantly thicker palisade layers, and significantly thinner spongy layers, than all other sites (Fig. 3d).

Detailed quantitative results of leaf epidermal and transverse section characters are shown in Supplementary Table 1.

Leaf development observation

Leaf blade development was observed for 3 weeks, as the leaf is fully expanded and mature by the end of the third week. Leaf length was $14.17 \pm 0.263 \text{ cm}$ during the first week, $22.78 \pm 0.458 \text{ cm}$ during the second week, and $24.23 \pm 0.037 \text{ cm}$ during the third week.

Discussions

This study on the leaves of *T. catappa* collected along the coastline of Rayong province, Thailand, demonstrates how leaf anatomical structures responded to the Rayong oil spill in 2013. Short-term effects of the oil spill included physically impacting plants by coating plant leaf and soil surfaces. In this work, we collected *T. catappa* as a representative beach plant species about 3 weeks after the oil spill. We sampled fully expanded, mature leaves from each site and assumed that all mature leaves were incompletely developed 2 weeks before the oil spill occurred. This hypothesis is confirmed by our observations on leaf

Table 1 Qualitative leaf anatomical characters of *Terminalia catappa* from oil spill affected (site 6) and adjacent (sites 1–5) sites in comparison with the control site

<i>Terminalia catappa</i> from different locations	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Control
Leaf epidermal characters							
- epidermal cell shape and anticlinal cell wall on adaxial surface	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Irregular with slightly undulate	Jigsaw with deeply lobe
- epidermal cell shape and anticlinal cell wall on abaxial surface	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe	Jigsaw with deeply lobe
Leaf transverse section characters							
Petiole (μm)[*]							
- shape of vascular ring on petiole	Cordate	Cordate	Cordate	Cordate	Cordate	Ovate	Cordate
Midrib (μm)[*]							
- midrib outline	Cupuliform	Cupuliform	Cupuliform	Cupuliform	Cupuliform	Ovate	Cupuliform

Differences in shape are highlighted in gray

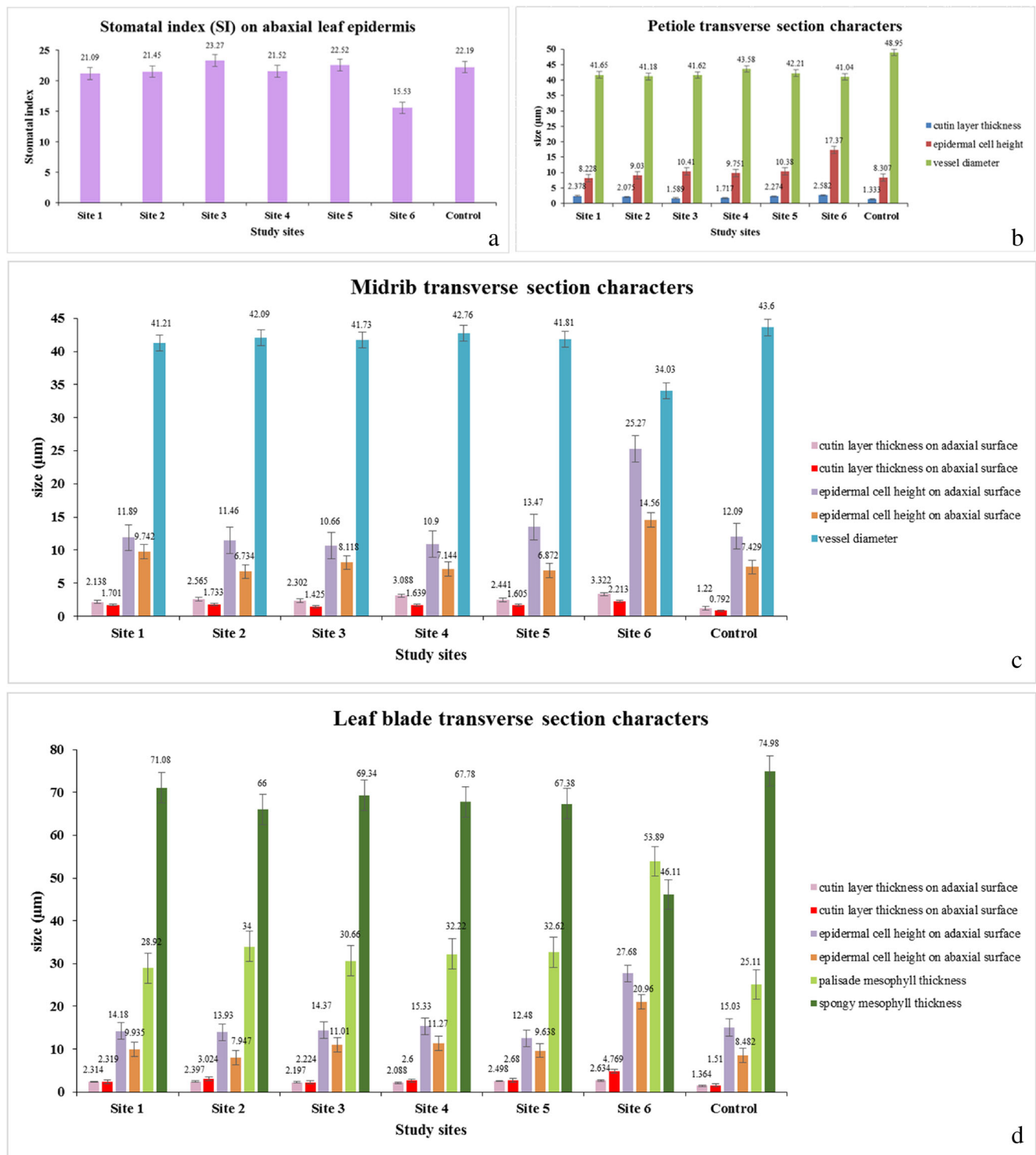


Fig. 3 Bar graph showing anatomical leaf characters: stomatal index (a), transverse section characters of petiole (b), midrib (c), and leaf blades (d). Error bars depict standard deviation from the average

development, which found that leaves take about 2–3 weeks to fully mature. In addition, young leaves are still undergoing cell division and differentiation (Evert 2006), which suggests that cells in *T. catappa* leaves were adapting to environmental changes during the oil spill (Klich 2000).

The decrease of average stomatal frequency in relation to increasing pollution has been widely reported in many studies (Gill et al. 1992; Omosun et al. 2008, 2009) and is in concordance with this study showing the lowest stomatal frequency in leaves from polluted Ao Phrao (Fig. 3a). The reduction in

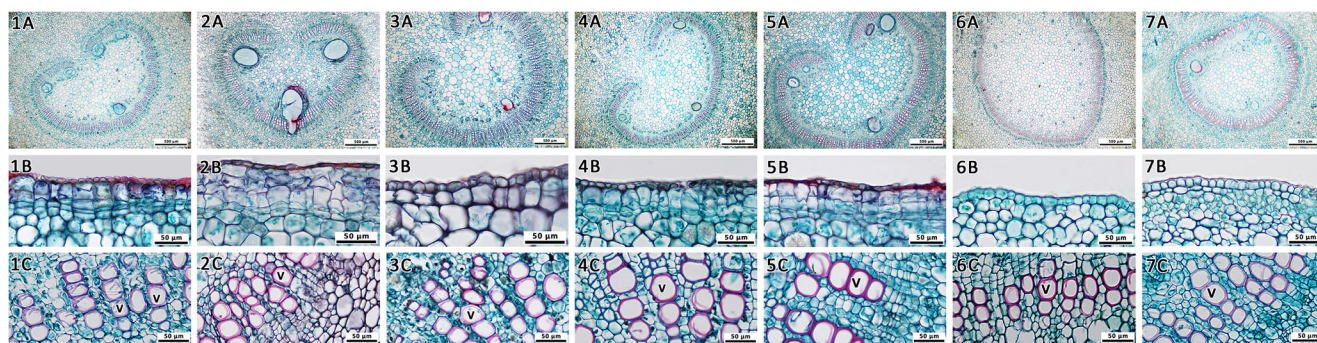


Fig. 4 Petiole transverse sections of *Terminalia catappa*: transverse section of the petiole showing a ring of vascular tissue (4 $\times$) (A), cutin layer and epidermal cells (40 $\times$) (B), and xylem showing vessel elements

(40 $\times$) (C). Numbers 1–6 represent the petiole transverse sections from study sites 1–6, respectively. Number 7 shows the petiole transverse section from the control site. Abbreviation: v, vessel element

stomatal frequency could be a result of oil contamination in the soil, which may create conditions of drought stress in plants (Omosun et al. 2008, 2009). Reducing the stomatal index is probably a mechanism to reduce and/or inhibit water loss during transpiration (Maranho et al. 2006), which can lead to reduced transpiration (Baker 1970), gas exchange (Pezeshki and De Laune 1993; Pezeshki et al. 2000), and photosynthesis (Baker 1970; Anderson and Hess 2012). However, stomatal density is also related to other environmental and physiological conditions (Klich 2000), such as humidity, the concentration of pollutants (Woodward 1987; Musselman and Minnick 2000), temperature (Momen et al. 1999), and carbon dioxide concentration (Ferdinand et al. 2000). Moreover, oil that coats plant leaves can increase leaf temperature, block light, and obstruct stomata, leading to less efficient gas exchange (Pezeshki and De Laune 1993;

Pezeshki et al. 2000) and photosynthesis (Baker 1970; Anderson and Hess 2012).

The thickness of the cutin layer normally indicates a leaf's ability to avoid excessive water evaporation (Evert 2006; Maranho et al. 2006). The induction of waxy cuticle formation under conditions of stress, such as high light intensity, high temperature, low humidity, and high amounts of crude oil (Christodoulakis and Fasseas 1990; Maranho et al. 2006), accords with the recognized importance of water conservation by plants. Our cuticle results showed that the oil spill probably affected leaf anatomical structures, as seen in the Evert petiole and leaf blade transverse sections. Both sides of the epidermis (from both petioles and leaf blades) collected in the affected site were cutinized and thicker than those collected in the adjacent and control sites. Baker (1970) reported that plants with thick cuticles and fewer stomata inhibit oil penetration.

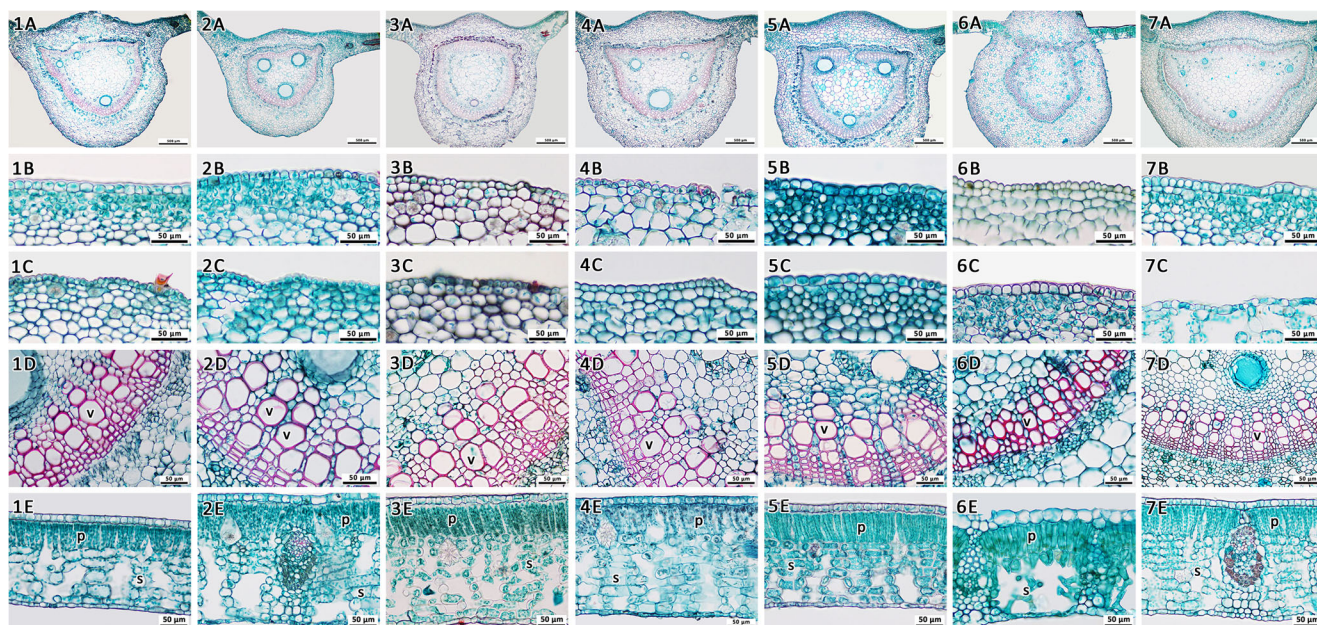


Fig. 5 Leaf transverse sections of *Terminalia catappa*: outline of the midrib (4 $\times$) (A), cutin layer and epidermal elements on the adaxial (B) and abaxial (C) surfaces, xylem showing vessel elements (40 $\times$) (D); leaf blade transverse section showing the cutin layer, epidermal cells, and the

palisade and spongy mesophylls (E). Numbers 1–6 represent leaves collected from study sites 1–6, respectively. Number 7 represents a leaf from the control site. Abbreviations: p, palisade mesophyll; s, spongy mesophyll; v, vessel element

This assertion was confirmed by an experiment testing kerosene application to xerophytes; *Sedum* showed no oil penetration after 8 h of exposure (Knight et al. 1929).

The epidermal cells of *T. catappa* collected from the polluted site were thicker and larger than those in the other sites (Fig. 3b–d). The increased epidermal cell diameters on both sides of petioles and leaves were probably caused by the absorption of heavy metals (Ni, V, Cu, Cd, and Pb), which are normal constituents of crude oil (Osuji and Onojake 2004). The absorption may start from the stomata into intercellular spaces, before crossing into vascular tissue (Baker 1970). Moreover, this absorption probably occurred via the root system from contaminated soil (Gomes et al. 2011). The thickened adaxial and abaxial epidermis of *Brachiaria decumbens* after the uptake and accumulation of heavy metals was proposed to be a strategy to reduce water loss by transpiration transport (Gomes et al. 2011). Gomes et al. (2011) also suggested that lower transpiration rates by plants under stress may reduce root absorption and limit acquisition of metal ions. Moreover, thicker abaxial and adaxial epidermises may be an avoidance mechanism against the inhibitory effects of a pollutant on physiological activities, or to prevent metal ion translocation into vascular or photosynthetic tissues (Gomes et al. 2011). However, some studies have reported that epidermal cell size generally decreases in leaves exposed to pollutants (Sridhar et al. 2005; Gostin 2009). Accordingly, either increased diameter or thickness of epidermal cells can be a mechanism against toxicity in different plant species.

Maranho et al. (2006) revealed that leaves exposed to crude oil pollution had an elongated palisade parenchyma with 2–3 layers within the mesophyll, while the rest was filled by spongy cells with intercellular spaces. Such results are in agreement with this study, which showed an increase in palisade mesophyll thickness and a reduction in spongy layer thickness for leaves collected from the polluted site. Plants with thick leaves which invest more in palisade parenchyma than in the spongy parenchyma can lead to increased photosynthetic efficiency, since the majority of chloroplasts are found in the palisade cells (Fahn 1990). This enhances photosynthesis per unit leaf area and enables greater efficiency in water use while plants are experiencing drought conditions (Klich 2000).

Apart from all the leaf anatomical characters, we suggest that variation in vessel diameter might be a useful bio-indicator for monitoring petroleum crude oil. When radially examining the diameter of transportation cells in the xylem, we detected reduced diameters in the crude oil polluted site compared to the adjacent and control sites, which is congruent with the study by Maranho et al. (2009). This finding is also supported by a decrease of tracheids along the stem of *Podocarpus lambertii* when exposed to petroleum pollution (Maranho et al. 2009) as a reduction in the size of vascular elements (particularly the diameter) tend to occur in drought conditions (Van den Oever et al. 1981).

Conclusion

To summarize the above results, leaf anatomical structures, including stomatal index, cuticle thickness, epidermal cell, and palisade mesophyll of *Terminalia catappa*, a tree beach plant, collected from the affected site 3 weeks after Rayong oil spill in 2013 were significantly different from the adjacent and control sites. These significant changes in the leaf anatomy of *T. catappa* correspond to previous research on the effect from oil spill pollution on plants, which indicate that oil spills have short-term impacts on beach plants from oil contamination inside the plant organs and coating of plant leaves. Thus, leaf anatomy may be used as a possible pollution indicator or in monitoring crude oil pollution.

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References

- Anderson CJ, Hess TA (2012) The effects of oil exposure and weathering on black-needle rush (*Juncus roemerianus*) marshes along the Gulf of Mexico. *Mar Pollut Bull* 64:2749–2755
- Baker JM (1970) The effects of oils on plants. *Environ Pollut* 1:27–44
- Casarotto A, Jiraphanvanich W, Kampa A, Limpiwattakee W, Luangaramvej P, Wallace C, Whyte J (2014) Documentation and analysis of the Rayong oil spill: characterizing the health, economic, and social impacts of the incident and response. Worcester Polytechnic Institute, Worcester
- Christodoulakis NS, Fasseas C (1990) Air pollution effects on the leaf structure of *Laurus nobilis*, an injury resistant species. *Bull Environ Contam Toxicol* 44:276–281
- Evert RF (2006) Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development, 3rd edn. John Wiley & Sons, New Jersey
- Fahn A (1990) Plant anatomy, 4th edn. Pergamon Press, Oxford
- Ferdinand JA, Fredericksen TS, Kouterick KB, Skelly JM (2000) Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. *Environ Pollut* 108: 297–302
- Gill LS, Nyawuame HGK, Ehikhametor AO (1992) Effect of crude oil on the growth and anatomical features of *Chromolaena odorata* L. *Newsletter* 5:46–50
- GISTDA (2014) Oil slick captured by the satellite RADARSAT-2 on 27 July 2013 at 6.31 p.m., Showed oil film around Map Ta Phut Industrial Estate, Rayong province, Thailand. Retrieved August 10, 2014, from http://www.gistda.or.th/gistda_n/dmdocuments/20130727-oilspill-rd-rayong/oilspill_rd_20130727_rayong.jpg
- Gomes MP, Marques TCLLD, e Melo S, MDOG N, EMD C, Soares ÂM (2011) Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. *Sci Agric* 68:566–573
- Gostin IN (2009) Air pollution effects on the leaf structure of some Fabaceae species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 37:57–63

- Johansen DA (1940) Plant microtechnique. Mc Graw Hill Book Company, Inc., New York
- Kasim WA (2005) The correlation between physiological and structural alterations induced by copper and cadmium stress in broad beans (*Vicia faba* L.) Egypt J Biol 7:20–32
- Klich MG (2000) Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. Environ Exp Bot 44:171–183
- Knight H, Chamberlin JC, Samuels CD (1929) Limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol 4:299–321
- Maranho LT, Galvão F, Preussler KH, de Muñiz GIB, Kuniyoshi YS (2006) Efeitos da poluição por petróleo na estrutura da folha de *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae. Acta Bot Bras 20:615–624
- Maranho LT, Dziedzic M, Muñiz GIB, Kuniyoshi YS, Galvão F (2009) Effects of the pollution by petroleum on the tracheids along the stem of *Podocarpus lambertii* Klotzsch ex Endl., Podocarpaceae. Braz J Biol 69:263–269
- Momen B, Anderson PD, Helms JA (1999) Temperature dependency of acid-rain effect on photosynthesis of *Pinus ponderosa*. Environ Pollut 113:223–230
- Musselman RC, Minnick TJ (2000) Nocturnal stomatal conductance and ambient air quality standards for ozone. Atmos Environ 34:719–733
- Omosun G, Markson AA, Mbanasor O (2008) Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. Am Eurasian J Sci Res 3:70–74
- Omosun G, Edeoga HO, Markson AA (2009) Anatomical changes due to crude oil pollution and its heavy metals component in three *Mucuna* species. Recent Res Sci Technol 1:264–269
- Osuji LC, Onojake CM (2004) Trace heavy metals associated with crude oil: a case study of Ebocha-8 oil spill polluted site in Niger Delta, Nigeria. Chem Biodivers 1(11):1708–1715
- Pezeshki SR, De Laune RD (1993) Effect of crude oil on gas exchange functions of *Juncus roemerianus* and *Spartina alterniflora*. Water Air Soil Pollut 68:461–468
- Pezeshki SR, Hester MW, Lin Q, Nyman JA (2000) The effects of oil spill and clean-up on dominant US gulf coast marsh macrophytes: a review. Environ Pollut 108:129–139
- Salisbury EJ (1927) On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Philos Trans R Soc Lond 216:1–65
- Singran N (2013) Classifying risk zones by the impacts of oil spills in the coastal waters of Thailand. Mar Pollut Bull 70:34–43
- Singran N (2014) Contingency plan improvement for managing oil spills in the coastal waters of Thailand. Mar Pollut Bull 89:149–159
- Sridhar BM, Diehl SV, Han FX, Monts DL, Su Y (2005) Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (*Brassica juncea*). Environ Exp Bot 54:131–141
- Traiperm P, Chow J, Nopun P, Staples G, Swangpol SC (2017) Identification among morphologically similar *Argyrea* (Convolvulaceae) based on leaf anatomy and phenetic analyses. Bot Stud 58(1):25
- Van den Oever L, Baas P, Zandee M (1981) Comparative wood anatomy of *Symplocos* and latitude and altitude of provenance. Int Ass Wood Anat Bull (NS) 2:3–24
- Woodward FI (1987) Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. Nature 327:617–618