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(敬請壁報發表作者於 8:00-9:00 張貼壁報, 15:40-16:10 將壁報取下)

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# 四環素與非離子型界面活性劑改質膨潤土的交互作用

# Interaction of Tetracyclines with non-ionic surfactant modified Bentonite.

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#### 摘要

本研究以非離子型界面活性劑來改質膨潤土,瞭解有機膨潤土與四環黴素(TC) 的交互作用。結果顯示,pH3的條件下,TC與帶負電的膨潤土表面以靜電力吸引, 故不論有無經過Brij30改質皆有最高TC吸附量;相較pH5的結果,pH8的條件 下有助改質膨潤土對TC的吸附,此可能是TC間疏水性作用結合,進入層間的分 子較大並與非離子型界面活性劑的疏水端作用,而促進改質膨潤土對TC的吸附。 關鍵字:四環素、非離子型界面活性劑、膨潤土

#### 前言

四環素類抗生素為廣效性的常用抗生素之一,其價格低廉且抑制細菌之活性 高。根據國貿局統計在2005-2006年間台灣共進口約56,6103公斤的四環素(林, 2008),除盤尼西林外,其為使用量第二多的抗生素。因四環素取得便利,易被農 民濫用,過度使用抗生素造成未代謝或部分代謝之抗生素再次進入環境中。許多 前人研究顯示,在地表水、地下水、湖泊、土壤及廢水甚至飲用水中都觀測到微 量四環類抗生素的存在(Ji et al., 2009; Homen and Santos, 2011)。

黏土礦物有粒徑小、比表面積大的優勢,對抗生素有非常高吸附力,其中膨 潤土(Bentonite)較常為學者利用。膨潤土主要由具高陽離子交換容量、高比表面積 且有膨脹性的蒙特石組成。蒙特石對陽離子及部份極性分子有良好的吸附力 (Ikhtiyarova et al., 2012),但蒙特石的親水性表面不利於非極性分子的吸附,故前 人研究中會加入陽離子型界面活性劑使蒙特石形成有機膨潤土(organobentonite) (Zhu et al., 1998; Shen et al., 2007; Park et al., 2011; Ikhtiyarova et al., 2012),促進 其對非極性分子的吸附力。但陽離子型界面活性劑通常對生物的毒性較高,若用 於動物用飼料,雖可減少未代謝之抗生素排出及逸散,也可能造成動物本身的毒 害。因此,本研究以非離子型界面活性劑來改質膨潤土,瞭解此類型界面活性劑 與黏土礦物的交互作用,並進一步探討其對四環黴素的吸附反應,以期其做為動 物用抗生素添加與飼料中載體(carrier)的角色評估,進而減少抗生素的快速釋出。

# 材料與方法

A. 不同 pH 下 TC 標準曲線製作

精秤 0.1 g TC,以去離子水定量至 200 mL,此時 TC 母液濃度為 500 mg L<sup>-1</sup>。
取 TC 母液以 0.01 M NaCl 稀釋配製成濃度分別為 5、10、25、40、50 mg L<sup>-1</sup>
的 50 mL TC 溶液,並調整 pH 至 3-9,再以分光光度計分析 TC 濃度。
B. 膨潤土樣品在不同 pH 下對 TC 的等溫吸附實驗

秤取 0.1 g 膨潤土樣品(包括原始未插層及改質膨潤土)置入 50 mL 離心管, 加入適量 0.01 M NaCl 背景液,震盪 1 小時確保膨潤土均勻分布於溶液中,再 加入不同初始濃度(50、200、400、600、800 及 1000 mg L<sup>-1</sup>)的 TC 溶液,並 調整 pH 至 3、5 以及 8,最後總體積為 25 mL。實驗中均以鋁箔紙包裹離心管 放入恆溫震盪箱,以 150 rpm 震盪反應 6 小時,過程中每隔 1.5-2 小時調整 pH,使 pH 維持於實驗 pH±0.1。再以 9055 g 離心 10 分鐘,吸取 10 mL 上清 液過濾,以分光光度計分析剩餘 TC 濃度。TC 的吸附量以初始濃度減去剩餘 濃度而得。等溫吸附實驗數據以 Freundlich 與 Langmuir 等溫吸附模式來進行 模擬。

#### 結果與討論

在不同 pH 條件下,膨潤土對 TC 的吸附皆較符合 Langmuir 等溫吸附模式。TC 在 pH 3 條件下以帶正電荷形態存在,故此時吸附機制以陽離子交換為主(Figueroa et al., 2004)。在 pH 3、5、8 條件下膨潤土對 TC 的最大吸附量分別為 290、268 及 203 mg/g。在 pH 5 的環境下,TC 以電荷平衡的電中性形態存在,此時與膨潤土的吸引僅憑陽離子橋、非靜電力、凡得瓦力與氫鍵,或是錯合(complexation)的方式與膨潤土結合(Figueroa et al., 2004),因此吸附量不如 pH 3。pH 8 時 TC 雖主要為帶 負電荷的型態,但 TC 上帶正電荷的二甲胺官能基之存在,使 TC 能以陽離子交換的方式吸附在膨潤土上,此外膨潤土表面存在的離子可能中和部份負電荷,使帶 負電為主的 TC 有機會擴散到膨潤土表面或層間,增加其以正電荷位置吸附於膨潤



#### 參考文獻

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# 構之影響

# Dynamics of microbial community structure during composting and vermicomposting of the mixture of pig manure and mushroom waste

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#### 摘要

農業有機廢棄物可利用傳統的一般堆肥化 (composting process)以及蚓糞堆 肥化(vermicomposting process)方式堆肥腐熟,推肥過程中微生物扮演重要角色, 過去研究鮮少有對兩種堆肥化處理後之微生物群落比較。本研究即在探討物豬糞 與養菇廢棄包混合物,經此兩種堆肥方法對微生物群落結構之影響。利用培養法 與未經培養之基因選殖法,評估各堆肥階段中的微生物群落結構的變化以及差異。 相較於培養法,未經培養之基因選殖法可分析出更多之微生物種類,結果顯示蚓 糞堆肥化處理會增加細菌種類多樣性。本研究成功建立一般堆肥與蚓糞堆肥廢棄 物中細菌群落結構之分析方法。

關鍵字:一般堆肥化、蚓糞堆肥化、微生物群落結構、選殖株基因庫構築

#### 前言

廢棄物未適當處理將導致土壤、空氣與水質的汙染(林,2012; Domínguez et al., 2012; 詹,2014)。若透過掩埋與焚化則需耗費大量人力與金錢,因此可利用為 堆肥化在利用(農委會,2008)。一般傳統堆肥化過程中有高溫階段,可殺死病原 菌,改變微生物族群結構(Tang et al., 2004;Tiquia 2005)。蚓糞堆肥化透過蚯蚓與微 生物共同作用以達到堆肥腐熟(Lazcano et al., 2008; Fornes et al., 2012)。不同的堆 肥化過程致使其中的微生物族群不同影響。本研究監測微生物群落結構之變化。



(二) 微生物群落結構分析試驗

以培養方式與分子生物技術測定在上述樣品中的微生物群落結構。

(1) 平板法與菌種純化 (Platind and pure culturing)

(2) 聚合酶連鎖反應-變性膠體梯度電泳 (PCR-DGGE)

(3) 選殖株基因庫的構築 (clone libraries)

#### 結果與討論

表十九、豬糞與養菇廢棄包混合原料、預堆14 天堆積物與各堆肥化處理下微生物 族群菌門分布百分比

Phylum	OW	PCW	BK55	COM55	VM55
			%		
Acidobacteria	9.1	4.2	17	18	7.7
Actinobacteria	3.8	-	-	-	7.7
Bacteroidetes	31	4.2	13	9.1	3.8
Ca. Saccharibacteria	-	4.2	-	4.5	-
Firmicutes	31	83	-	18	3.8
Gemmatimonadetes	-	-	4.2	-	-
α -	-	-	17	14	23
β-	-	-	17	-	27
γ -	23	-	13	23	7.7
δ-	-	-	4.2	4.5	7.7
Unclassified-	-	-	4.2	-	3.9
Planctomycetes	-	-	4.2	-	-
Verrucomicrobia	-	-	4.2	-	3.8
unclassified bacteria	3.8-	4.2	4.2	9.1	3.8

a.由於四捨五入的結果,每個樣的總克隆百分率可能不等於100%。

結果顯示一般堆肥中革蘭氏陽性菌群比例較高, 蚓糞堆肥中革蘭氏陰性菌比 例較高。由各樣品中分析測得之菌門數量,可推知相同原料經由蚓糞堆肥化後其 中細菌多樣性增加。本研究成功建立堆肥過程中細菌群落結構之分析方法,並探 討種不同堆肥化處理之細菌群落結構變化。

# 参考文獻

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# 利用宜蘭農地土壤的形態特徵推估平地造林的可行性

# Using rural soil morphological characteristics to estimate the feasibility of plain afforestation in Ilan

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#### 摘要

本研究將宜蘭縣 79 個土系,依其土壤母質、質地、斑紋、pH 值、有機質等 特性,歸併彙整為 10 個土區。主要分類方式是依該土系斑紋分布及質地來分析土 壤通氣排水能力,以此判斷未來若進行平地造林之可能性及所需之通氣排水改良 程度與方式,並將通氣排水改良程度較接近之土系歸併為同一土區,以作為土壤 管理上之參考。研究結果指出,宜蘭縣較適合改為平地造林之土地面積僅佔 4.8%。 **關鍵詞**:宜蘭縣土系、斑紋、通氣排水能力、平地造林

#### 前言

宜蘭縣共有79個土系,在土壤圖上的分布過於繁多,不論是在土地利用規劃, 抑或是土壤肥力管理上均相當不便;故為了管理上之方便,以及希望可以推廣給 宜蘭的農會與農民了解,我們依據土壤的母質、質地、斑紋、pH值、有機質等特 性來分析此79個土系,並假設未來農地可能部分進行平地造林,依林木生長所需 之通氣排水改良程度,將之彙整成相似的土壤 管理單位(土區)。

#### 材料與方法

本研究的資料來源為台北-宜蘭縣土壤調查報告(台灣省農業試驗所報告第三 十五號)。依照土壤之形態特徵(包括土壤顏色之色彩、色值與色度)、土壤質地、 土壤母質、斑紋、pH值、有機質等將土壤之特徵列出來,再透過 Excel 之篩選功 能將資料分類與篩選,進行許多種不同順序之篩選,以方便管理為目標,找出最 簡單明瞭之分類方式,之後,羅列出分類後各土區之特性,及其改良通氣排水所 需之程度。最後所選擇之方式,乃依其母質及 0-20 公分土層之質地,先粗略分為 4 區,之後再依其 0-60 公分土層之斑紋排列方式,輔以質地、pH值、有機質之特 性,分析其通氣排水能力及土壤肥力等能力,共分為 10 種土區。

#### 結果與討論

本研究分析時是假設將農地改為平地造林,而土壤中各項因子對植物影響最

巨者即為通氣排水之功能,因此分析時只看 0-60 公分之斑紋來分析其通氣排水能 力,而未看 60-120 公分,乃因為樹木長大後,其根系深入土壤,60 公分以下之水 分會藉由生長良好樹木的根系吸收與經樹葉蒸散,降低地下水位,所以即使原本 60 公分以下之通氣排水不好亦會被改善。土壤顏色最後未被列入分類之依據乃因 大多數宜蘭之土系,母質為黏板岩,而母質為黏板岩之土壤顏色均為灰黑色,所 以難以從其顏色深淺看出其肥力,且若表層有耕種,則顏色會改變,並較均質化。 除了因資料不足(無剖面資料或缺少部分數據)而無法羅列歸納之土系,本研究共歸 納出十個土區合計 45 個土系。各土區的特色如表一所示。

	母質	0-20公分之質地	改良強度	面積所占百分比
第一區	片岩	粗質地	弱(停止人工灌水)	0.43%
第二區	片岩	中粗質地、中質地	0-60均容易浸水 →不容易改 善,不適合造林	1.19%
<mark>第三區</mark>	片岩	中質地	只有表層土壤,無法造林	0.33%
第四區	片岩	中質地	進行中度改良(例:常耕犁,使 水蒸發)	0.26%
第五區	黏板岩	中粗質地、中質地	通氣排水佳,不須改良	0.28%
第六區	黏板岩	中粗質地、中質地、中細質地	0-60均容易浸水 →不容易改 善,不適合造林	18.90%
第七區	黏板岩	中質地	弱(停止人工灌水)、排水良 好、表層養分均充足,其中 ,有些土系中下層土壤需適 量施肥	2.60%
第八區	黏板岩	中質地、中細質地	只有表層土壤,無法造林	17.19%
第九區	黏板岩	中質地、中細質地	進行中度改良(例:停止人工灌 水,輔以耕犁,使水蒸發)、 排水良好、表層養分充足, 其下需適量施肥	1.23%
第十區	黏板岩	細質地	0-60均容易浸水→不容易改 善,不適合造林、排水不 良、需適量施肥	1.67%

表一、	10	個	土	品	え	特	
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#### 結論

本研究將宜蘭縣眾多之土系,以平地造林為目標,依其通氣排水能力及土壤 肥力等特性,統整歸併為十個土區,並註明各土區所需之通氣排水改良程度與方 式,以利管理。依據歸納的結果,宜蘭縣較適合改為平地造林之土地面積僅佔4.8%, 且其中只有約0.3%的土地(第五土區)不須進行通氣排水之改良,其他4.5%的土地 需進行弱度或中度通氣排水之改良。

# 雞糞生物炭對苗木生長與土壤性質的影響

# Effects of poultry litter biochar on planting stock growth and soil characteristics

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#### 摘要

本研究將一年生烏心石苗木種植在添加不同比例(0%、2.5%及5%)蛋雞糞生物 炭(C)的酸性森林土壤中,以添加1%市售豬糞堆肥(SMC)作為苗木的養分,於溫室 中培養19周,每周兩次調整土壤水分與每周一次調查生長性狀(株高與胸徑)。19 周後將植體小心取出,烘乾後將根、莖與葉部分別取下,磨碎分析植體中養分元 素的含量。另外將土壤風乾、磨碎與過篩後,分析土壤性質的變化。研究結果指 出:

- (1)到第6週,添加2.5%處理的苗木之苗高生長較有潛勢,其中又以550℃處理的 生長較快;250℃及350℃之2.5%添加量處理組的生長較慢。第6週之後,可能 受到天氣開始漸變炎熱,苗木的生長出現變化。19週後僅有 Control 以及 Control+SMC 兩個處理之植體仍然存活。苗徑的相對變化與苗高的變化有些不 同,到第6週時,550及350℃製備之5%添加量處理有明顯的苗徑生長,但處 理之間的苗徑生長差異量很小。
- (2)土壤的部分,添加生物炭使得酸性苗圃土壤的 pH 值由 pH 4.3 提高到 pH 5.5, EC 值由 0.11 dS/m 提高 1.2 dS/m。添加生物炭的土壤 pH 與 EC 值明顯較未添加 來的高,且 5%添加量又顯著比 2.5%添加量高。添加生物炭使得酸性苗圃土壤 的產生相當大的鹽害效應。另外,研究所使用的溫室並避沒有完善的溫適度控 制,適逢季節交替時節,溫室無法有效散熱,造成土壤蒸發與植物蒸散太快, 土壤中過多的鹽分無法有效淋洗至盆栽下層,也是造成苗木進一步因鹽害而枯 萎的原因。
- (3)添加 5%之 550℃蛋雞糞生物炭顯著提高土壤的有機碳含量(9.32%),高溫製備的 生物炭中的碳多以芳香環的形式存在,不易被分解成二氧化碳,因此穩定存在 於土壤中;低溫製備的生物炭中的碳有部份仍是屬於直鏈型的碳,容易被微生 物分解與利用,因此 C250-2.5 及 C250-5 處理組的有機碳含量與對照組沒有顯著 差異。添加生物炭顯著提高土壤中有效磷的含量,以 C350-5 處理組含量最高(207 mg/kg),其次為 C250-5 處理組(177 mg/kg),其他依次為 C350-2.5、C250-2.5、

C550-5、C550-2.5、Control、Control+SMC。重金屬銅及鋅有效性濃度(0.1N HCl 抽出)的變化,鋅的濃度(7~20 mg/kg)顯著高於銅(1.3~1.8 mg/kg);蛋雞糞生物炭 的添加顯著提高土壤中鋅的有效性濃度,C350-5 > C250-5 > C550-2.5≒C550-5 > C350-2.5 > C250-5。有效性鋅的濃度變化與有效性磷相當類似。

- (4)添加生物炭顯著降低植體根N的含量,5%添加量處理之植體N含量較高於2.5% 的添加處理。C550-5處理組之植體N含量最高,推測可能是土壤pH值的改變, 土壤中的硝化作用可能增加,苗木植體根吸收到較多的含氮養分。生物炭添加 顯著提高苗木植體根的磷含量,此與土壤中的有效性磷含量的變化類似。生物 炭添加顯著提高苗木植體根的鉀、鈣與鎂含量,5%處理組顯著高於2.5%處理組; 包括蛋雞糞生物炭本身與豬糞堆肥中都有豐富量的鉀、鈣與鎂,兩者的協同作 用下,植體根多表現鉀、鈣與鎂的高吸收量。
- (5)生物炭添加處理顯著降低植體根的鋅含量;雖然蛋雞糞生物炭的添加顯著提高 土壤中鋅的有效性濃度,但植體中並沒有顯著累積高濃度的鋅。我們推測這些 土壤中有效性的鋅離子可能與生物炭分子間產生鏈結,無法穿過根部的細胞膜, 因此根部不會累積大量的鋅離子。銅的情況與鋅大不相同,添加生物炭顯著提 高植體根部的銅含量,5%添加處理又明顯高於2.5%的添加處理;土壤中有效性 銅的含量亦因生物炭的添加有顯著的提高(圖 6),但是植體的吸收量顯著低於鋅。 植體根中鋅的含量(50~110 mg/kg)顯著高於銅(10~20 mg/kg)。
- (6)添加生物炭顯著增加植體莖部N、K、Ca、Cu、Zn的含量,5%添加量處理之植 體莖部含量較高於2.5%的添加處理。苗木植體莖部的N含量在生物炭添加處理 組中略高於根部。與根部類似,生物炭添加顯著提高苗木植體莖部的磷含量; 除了C350-5之外,5%的添加處理有顯著低於2.5%添加處理的趨勢。此與根部 的變化相反,表示根部的磷並沒有轉移到莖部。莖部的磷含量略低於根部。莖 部鉀的變化趨勢與根部類似,只是含量顯著較低。植體莖部鈣與鎂含量明顯高 於根部。植體莖部的銅與鋅含量大都較低於根部,此表示銅與鋅離子並沒有大 量往地上部輸送。
- (7)由於生物炭處理組的植體都已枯萎與落葉,並無植體葉片可供分析; Control 組 的葉片量稀少,僅供1次分析使用,無法重複,因次無標準偏差值(SD)。Control 組的葉片中,包括N、P、K、Cu與Zn都高於Control+SMC處理組,但Ca與 Mg則較低。
- (8)本研究中生物炭的添加有提高苗木生長的潛勢,增加植體養分的吸收,確有正面的效果。然而因外在生長條件的人為干擾與氣候季節性的遷變,影響苗木的正常生長。建議未來在研究或施用時,應將雞糞生物炭添加量下調至<2%,並盡量減少土壤水分過度的蒸散,以減少植體因添加生物炭所可能帶來的高鹽類的傷害。</p>

# 黏土礦物層面電荷在對苯二酚電化學行為之影響

# Effects of Layer Charge of Clay Minerals on Electrochemical Behavior of Hydroquinone

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#### 摘要

苯二酚是土壤中常見的酚類化合物,土壤中的黏土礦物對酚類化合物的催化 聚合與轉化反應常是土壤腐植質先驅物形成的關鍵。本研究之目的以不同黏土礦 物修飾網版印刷碳電極 (SPCE) 配合電化學分析法進行對苯二酚電化學分析以了 解黏土礦物層面電荷在對苯二酚電化學行為之影響。試驗結果指出,六種黏粒修 飾 SPCE 對對苯二酚均能測到兩個還原波峰(R<sub>1</sub>、R<sub>2</sub>) 與兩個氧化波峰(O<sub>1</sub>、O<sub>2</sub>),且 其中以 2:1 型膨潤石類 (SHCa-1、SWy-1 及 SWa-1) 測到的 R<sub>1</sub> 波峰電流量較大。 六種黏粒修飾預氧化網版印刷碳電極 (SPCE\*) 對對苯二酚測到的氧化還原波峰 電流量均有明顯增大的現象,且其中以 R<sub>2</sub>和 O<sub>2</sub>氧化還原波峰對增大及波峰電位偏 移的情形較為明顯,顯示黏土礦物層面電荷對對苯二酚之影響導致在偵測到的循 環伏安圖與波峰電流量會不同。

#### 前言

對苯二酚常被用於還原劑、抗氧化劑、醌染料與偶氮染料,也常是很多藥物 與農產品中的成分,若吸取過量對人體的神經系統及腎臟功能會產生傷害(Zhao, 2006)。酚酸類化合物以鄰苯三酚、鄰苯二酚與對苯二酚為土壤中最普遍的酚酸物 種,為土壤腐植物質先驅物中很重要的成分(Wang and Huang, 1989),而黏土礦物 對酚類化合物的催化轉化的腐植化作用是整個土壤化學反應的重要關鍵過程。近 年來有許多關於黏粒修飾電極的研究,受到關注的主要原因是黏土礦物為便宜又 穩定的材料,且具有預濃縮分析物的能力,在分析上可以有更高靈敏度與更低偵 測極限,可用於有機化合物、重金屬及農藥的偵測,且由於黏粒層中所具有的電 子傳導特性也適用於電化學分析、光催化性、感測器和生化感測器等(Fitch, 2011)。

#### 材料與方法

一、黏粒修飾網版印刷碳電極的製備

分別取標準黏土礦物 KGa-1、PFI-1、SHCa-1、SWy-1、SWa-1 及 VTx-1 之懸 浮液各 30 μL,滴在網版印刷碳電極表面後,使其自然風乾,重複此修飾電極的動 作三次即完成試驗所用之黏粒修飾電極的製備。 二、黏粒修飾電極對對苯二酚的電化學分析

以網版印刷碳電極與黏粒修飾網版印刷碳電極為工作電極、Ag/AgCl為參考電 極及白金絲為輔助電極,將三電極系統放入2mM對苯二酚溶液中,利用電化學分 析儀之循環伏安法在掃描速率為50mVs<sup>-1</sup>及掃描範圍由1.0V到 -1.0V下進行五 圈循環掃描,藉此穩定工作電極,記錄其循環伏安圖、陰極波峰電流量與陽極波 峰電流量。同上步驟,經預氧化處理後的網版印刷碳電極及黏粒修飾預氧化網版 印刷碳電極來偵測。

#### 結果與討論

一、 黏粒修飾電極在對苯二酚的電化學分析

SPCE 對對苯二酚偵測可得兩個還原波峰及兩個氧化波峰,其中以 R<sub>1</sub> 與 O<sub>2</sub> 氧化還原波峰對的可逆性較好,而 SPCE\*增加了 R<sub>2</sub>和 O<sub>2</sub>波峰對的氧化還原反應, 使 R<sub>2</sub>波峰電位往更正電位方向偏移,而 O<sub>2</sub>波峰電位往更負電位方向偏移,使其較 容易還原及氧化(圖 1)。



圖 1 以 SPCE 與 SPCE\*對 2 mM 對苯二酚所偵測之循環伏安圖

圖 2 不同黏粒修飾 SPCE 與 SPCE\*對 2 mM 對苯二酚所偵測之循環伏安圖

六種黏土礦物修飾 SPCE\*對對苯二酚所測得之循環伏安圖與波峰電流量明顯 與六種黏土礦物修飾在 SPCE 上的情形不同。SPCE\*上的黏粒修飾電極可偵測到更 大的氧化還原波峰電流量(圖 2)。由於 SPCE\*表面的氧化層膜有羥基和羰基,與黏 土礦物表面間產生的氫鍵會影響黏土礦物表面電荷的電場效應,進而影響電極表 面對對苯二酚偵測到的波峰電流量,及對苯二酚氧化還原波峰電位的偏移(圖 2)。

#### 結論

六種黏粒修飾 SPCE 對對苯二酚均能測到兩個還原波峰(R<sub>1</sub>、R<sub>2</sub>)與兩個氧化波 峰(O<sub>1</sub>、O<sub>2</sub>),且以 2:1 型膨潤石類(SHCa-1、SWy-1 及 SWa-1)測到的 R<sub>1</sub> 波峰電流量 較大。六種黏粒修飾 SPCE\*對對苯二酚測到的氧化還原波峰電流量均有增大的現 象,以 R<sub>2</sub>和 O<sub>2</sub>氧化還原波峰對增大及波峰電位偏移的情形較為明顯,顯示黏土礦 物層面電荷對對苯二酚之影響導致在偵測到的循環伏安圖與波峰電流量會不同。

## 文獻

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# 由鎘的次細胞分布與化學型態探討非洲鳳仙之的扦插育苗條件

# From the subcellular distribution and chemical forms of cadmium in the cuttings of *Impatiens walleriana* to select suitable rooting conditions

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#### 摘要

非洲鳳仙可以累積高濃度的鎘,也可以利用扦插的方式育苗降低購苗成本, 但扦插育苗時間之長短可能影響植株特性,進而影響其對於鎘的累積能力。本研 究將非洲鳳仙扦插苗種植於不同鎘濃度的養液中 10~60 天,以探討鎘濃度及種植 時間長短對於其累積鎘行為之影響。試驗結果發現,在鎘濃度 5~10 µM 養液中種 植 30 天以上的非洲鳳仙扦插苗可累積高濃度的鎘,與其他種植天數比較,種植 30 或 40 天的非洲鳳仙扦插苗發育出較佳的耐受及解毒機制。

**關鍵詞**: 鎘(cadmium)、化學型態(chemical form)、非洲鳳仙(*Impatiens walleriana*)、 植生萃取(phytoextraction)、次細胞分布(subcellular distribution)

#### 前言

種植在鎘污染土壤中的非洲鳳仙可以累積高濃度的鎘,具有植生萃取之潛能, 其對鎘的耐受與解毒能力也被證實與鎘的次細胞分布及化學型態有關(Lai, 2015), 除了直接以種子育苗之外,非洲鳳仙可以利用扦插之方式育苗降低購苗之成本, 但扦插育苗時間之長短可能影響植株之生理特性,進而影響植生萃取之效果。本 研究將非洲鳳仙扦插苗種植於鎘濃度 1~10 μM (Cd<sub>1</sub>~Cd<sub>10</sub>)的養液中 10~60 天 (D<sub>10</sub>~D<sub>60</sub>),目的為了解上述處理因子對於累積鎘濃度及次細胞分布與化學型態之 影響,並藉由上述結果篩選出適合之扦插育苗條件。

#### 材料與方法

將非洲鳳仙扦插苗種植於鎘濃度分別為  $1 \cdot 2.5 \cdot 5 \ B \ 10 \ \mu M$  的養液中(pH 5.5), 將盆栽移入人工氣候室(26.2±1.5℃, RH = 80.7±10.1%, 120±6  $\mu$ mol sec<sup>-1</sup> m<sup>-2</sup> 照射 12 小時),每3 天定期更換養液,並於移植後第 10、20、30、40、50 及 60 天進行 採樣,上述每種處理進行三重複。採收後的根先浸泡於 20 mM 的 Na<sub>2</sub>-EDTA 溶液 中 15 分鐘,再將植物體區分成根、莖及葉三部份,取適量之新鮮植物體進行次細 胞分布及化學型態分析(He et al., 2013; Su et al., 2014),其餘植物體則經清洗、烘乾、 磨碎及分解之後,測定鎘之全量濃度。次細胞分布將細胞中的鎘區分成:(i)細胞 壁型態(F<sub>cw</sub>),(ii)可溶型態(F<sub>s</sub>)及(iii)細胞器型態(F<sub>co</sub>),化學型態則分成:(i)無機型 態(F<sub>E</sub>),(ii)水溶型態(F<sub>W</sub>),(iii)果膠酸/蛋白質隔離型態(F<sub>NaCl</sub>),(iv)不可溶磷酸鹽錯 合型態(F<sub>HAc</sub>)、(v)草酸錯合型態(F<sub>HCl</sub>)及(vi)殘餘型態(F<sub>R</sub>)。

#### 結果與討論

試驗結果發現,非洲鳳仙累積的鎘濃度隨養液中鎘濃度之增加而增加,在Cd5 及Cd<sub>10</sub>下累積之濃度較高,D<sub>30</sub>~D<sub>60</sub>根部累積之濃度約為550~1950 mg/kg,D<sub>20</sub>~D<sub>60</sub> 地上部累積濃度約為170~400 mg/kg。次細胞分布分析結果顯示,不論種植時間及 養液鎘濃度為何,根部中累積的鎘約有83~100%屬於移動性較高的 $F_s$ ,D<sub>20</sub>、D<sub>50</sub> 及D<sub>60</sub>地上部累積的鎘主要分布於 $F_{cw}$ 或是 $F_s$ ,D<sub>30</sub>及D<sub>40</sub>則主要分布於 $F_{cw}$ ,可以 藉此降低鎘對植物的毒害(Qiu et al., 2011)。化學型態分析結果顯示,D<sub>20</sub>~D<sub>60</sub>根部 累積的鎘主要屬於 $F_E$ ,而地上部中鎘的化學型態則受到種植時間之影響,D<sub>20</sub>及 D<sub>50</sub>葉部中及D<sub>50</sub>及D<sub>60</sub>莖中鎘的化學型態較為複雜,主要可能為 $F_E$ 、Fw或是 $F_{NaCl}$ , D<sub>30</sub>及D<sub>40</sub>葉部中的鎘主要為 $F_W$ 及 $F_{NaCl}$ ,而 $D_{20}$ ~D<sub>40</sub>莖中累積的鎘主要為 $F_{NaCl}$ ,  $F_{NaCl}$ 也被認為是植物對鎘的解毒機制(Lai, 2015)。綜合以上試驗之結果,在Cd<sub>5</sub>及 Cd<sub>10</sub>下種植D<sub>30</sub>以上非洲鳳仙扦插苗可累積高濃度鎬,且在Cd<sub>5</sub>~Cd<sub>10</sub>下D<sub>30</sub>~D<sub>40</sub> 的非洲鳳仙扦插苗已發育出對鎬的耐受及解毒機制,因此建議之扦插育苗條件為 在Cd<sub>5</sub>~Cd<sub>10</sub>濃度下育苗30~40天。

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編號 7

# Sorption equilibrium modeling of organic contaminants in vegetables

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## Abstract

The sorption equilibrium and uptake kinetics of toluene, p-xylene, and bisphenol A, naphthalene, and 4-bromo-diphenyl ether (BDE-3) in Chinese cabbage, Lettuce, Leafy rape, Chinese mustard, Scallion, Peanut, White radish, and Taro were examined. The kinetic experiments revealed that high uptake rates were observed for these plants with high lipid contents and for organic chemicals with high log  $K_{ow}$ . With respect to the organic chemicals, there was no reasonable trend for the values of  $K_{pl}$  within these plants. Moreover, regression correlation showed that log  $K_{lip}$ , the lipid-water partition coefficient, and log  $K_{ow}$  for these organic chemicals were strongly linear-related as following equation: log  $K_{lip} = 1.127 \log K_{ow} - 1.0837$  (n = 17, R<sup>2</sup> = 0.9279). The correlation allows to predict the sorption capacity of plant species for an organic compound when the plant composition and the log  $K_{ow}$  of the chemical are determined.

# Introduction

Industrial wastewater and fuel contaminants cause a serious problem to farms. Emerging organic pollutants contaminate soils by discharging wastewater from factories or leaking from underground storage tanks. The agricultural crops from the contaminated farms pose a serious risk to the health of human without awareness. Therefore, the mechanism related to plant uptake of organic pollutants is necessary to be studied. For facilitating the understanding about organic pollutant uptake in plants, a simple and predictable model should be developed to estimate the amounts of plant uptake. However, the physico-chemical properties of organic pollutants are complicated and the information for plant uptake is still limited. Consequently, this study is aimed to discuss the relationship between plant-derived log  $K_{\rm lip}$  (lipid-content related) and log  $K_{\rm ow}$  of organic pollutants, and to establish a correlative equation based on a series of observation from plant uptake kinetic experiments and sorption isotherms of various organic compounds.

#### Materials and methods

Toluene, *p*-xylene, bisphenol A, naphthalene and mono-BDE were used as target organic pollutants. Several kinds of common vegetables (chinese cabbage, lettuce, leafy rape, chinese mustard, scallion, peanut, white radish, and taro) were used for experiments of uptake kinetics. After the equilibrium time that is estimated by the kinetic experiments, sorption isotherm experiments were performed. Composition of all the plants was gravimetrically determined following the extraction procedure by sonication. The sorption equation used in this study was  $Q_{eq} = C_w K_{pl}$ , where  $Q_{eq}$  is the mass of the solute per unit wet mass of the plant,  $C_w$  is the aqueous concentration at equilibrium, and  $K_{pl}$  is the plant-water partition coefficient. Plant composition was decomposed into three major categories: lipids, carbohydrates, and plant water, and the equation was  $Q_{eq} = C_{pw} (f_{lip}K_{lip} + f_{ch}K_{ch} + f_{pw})$ , considering that only these components contribute to the sorption of organic pollutants.  $K_{lip}$  is the plant, and  $f_{lip}$ ,  $f_{ch}$ , and  $f_{pw}$  are the weight fractions of the lipids, carbohydrates, and water in the plant, respectively (Chiou *et al.* 2001).

#### **Results and discussion**

In order to precisely estimate the organic compound partitioning in plants, the plant compositions are essential to the establishment of estimating equations. The values of  $K_{\rm pl}$  were calculated from the linear regression of the sorption data. With respect to the organic chemicals, there was no reasonable trend for the values of  $K_{pl}$  within these plants. For instance, the partition between naphthalene and scallion revealed extremely high value of  $K_{pl}$  but the partition between naphthalene and cabbage dramatically reduced. However, excepting the special cases, the sequence of  $K_{pl}$  increase was consistent with the sequence of their log  $K_{ow}$ . From the regression analysis for the examined organic compounds, the log  $K_{lip}$  and log  $K_{ow}$  showed a highly linear correlation with a correlation coefficient  $R^2$  in 0.9279. Linear regression yielded a correlation equation as log  $K_{lip} = 1.127 \log K_{ow} - 1.084$ . Consequently, the sorption capacity of plants toward a certain organic chemical may be estimated by our established correlation equation based on plant compositions and the log  $K_{ow}$  of the organic chemicals. This result provides the further information about relationship between plant uptake and organic compound as well as the assessment of their fate in the environment.

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# Microbial degradation of two dndocrine disruptors, bisphenol A and tetrabromobisphenol A, by soil microbes

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# Abstract

Bisphenol A (BPA) is a widely used chemical in the production of plastics and resins. Tetrabromo-BPA (TBBPA), the BPA derivatives with bromide atoms, is one of the most commonly used brominated flame retardants. These compounds belong to estrogenic endocrine-disrupting chemicals (EDCs), and cause toxicity in human and other biota. The broad distribution of BPA-like EDCs in the environment raises great concern. Developing cheap and low environmental impact remediation methods for BPA-like EDCs is emergent. Therefore, the biodegradation abilities of diverse microorganisms toward BPA and TBBPA were investigated. The microcosm from acclimated sediment culture was used to evaluate the biodegradation ability of diverse microorganisms and isolate pure degraders. The microcosm completely removed BPA (10 mg L<sup>-1</sup>) within 28 h and transformed the contaminant into several metabolic intermediates. Four strains with BPA-degrading ability were isolated from the microcosm. Besides, from the TBBPA-acclimated sediment culture, one novel degrader was isolated. It degraded 70 % of the pollutant (4 mg  $L^{-1}$ ) within 5 days and transformed the pollutant to one degrading by-product. Our results are helpful to understand the natural attenuation processes of BPA-like EDCs, as well as to remediate those structural similar pollutants.

# Introduction

Bisphenol A (4,4'-isopropylidenediphenol, BPA) is widely used in the manufacturing of polycarbonate plastics and epoxy resins, the end-products of which include food containers, thermal papers, lacquers, and dental fillings. Globally, more than 2.2 million tons of BPA is consumed annually in which approximately 25% is estimated into the environment. Exposure to BPA through diet or skin contact results in various biological side effects. Tetrabromo-BPA (TBBPA) is one of the widely used brominated flame retardants. Its global usage ranges from 120,000 to 150,000 tons per year. It is considered to be important pollutant because TBBPA results in immunotoxicity, neurotoxicity, and high toxicity to aquatic life. The wide distribution and increasing concentration of BPA-like endocrine-disrupting chemicals (EDCs) in the environment threaten the health of human and other biota. The role of the specific degrader in the entire microcosm and the interaction between microorganisms remain unclear. The aim

of this study is to gain a greater insight into biodegradation of BPA-like EDCs.

# **Materials and Methods**

Sediment (0-10 cm depth) was collected from the river bank of Hsin-Dian Creek, a tributary of the Dan-Shui River, Taipei, Taiwan. The acclimated bacterial consortium collected from river sediment was washed once in the BSM medium and then cultured in 50 mL of the working medium. At selected time intervals, the concentration of target compounds was determined using a HPLC. The identification of intermediates was performed using LC/MS.

#### **Results and Discussion**

# BPA biodegradation by the aerobic microcosm

The acclimated bacterial consortium collected from river sediment exhibited considerable BPA degradation ability. Sixty percent of the compound was eliminated by the microcosms within 7 hr and the entire compound was depleted after 28 hr of incubation. The BPA concentration in the non-inoculated blank was constant throughout the experimental duration. The BPA concentration in sterilized control was reduced by 16% after 2 days of incubation.

10 colonies with distinct morphologies were selected from the BPA-containing selection agar plates. Among them, only four exhibited BPA degradation abilities. The RB-G strain was the most efficient degrader, requiring 7 days to remove all of the BPA. The other three strains took 9 days to deplete an identical amount of BPA.

The effect of a mixed culture on BPA degradation and their interactions were investigated according to degradation kinetics. The most efficient BPA degrading strain, RB-G, was selected and mixed with RB-I, the most divergent strain based on the 16S rRNA gene sequence. The degradation rate of these strains was faster than that of the single strain culture that it took 6 days to degrade all of the BPA. When all four of the isolated strains were mixed together, the BPA degradation rate was faster than that of the single strain culture.

# **TBBPA** biodegradation by the isolated pure strains

A collection of 12 colonies with distinct morphologies was selected. Within 6-days of incubation, only one microbe removed about 71% of TBBPA. All obtained mass spectra of these possible intermediates were compared to the standard spectra of NIST Mass Spectral Library. Only one compound, 2,6-dibromo-4-(2-ydroxypropan-2-yl)phenol, was tentatively identified as the intermediate of TBBPA biodegradation. Therefore, TBBPA transformation in the bacterial consortium collected from river sediment was depended more than one degrader.

# The Synthesis of Carboxymethyl Cellulose Stabilized Fe Nanoparticles and its Removal of Decabrominated Diphenylether

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# Abstract

Polybrominated diphenyl ethers (PBDEs) belong to the large family of brominated flame retardants. A stabilizer, sodium carboxymethyl cellulose (CMC), was applied to modify NZVI to be a well–suspended nanoparticle (NP). The synthesized CMC stabilized Fe NPs contained metallic Fe<sup>0</sup> and partial oxidation with reduced Ni<sup>0</sup>. CMC stabilized Fe NPs have an average size of 86.7 nm due to the brush structures of CMC, which proved electrostatic repulsion force. The catalyst Ni loading enhances the removal and degradation efficiency. The rate-limiting step in the removal of DBDE by CMC–Ni/Fe NPs could be a surface chemical reaction due to the effect of a CMC layer on the Fe NPs. The colloidal stability was also decreased with an increase in pH value which accompanied the change of removal rate constant. This study provided information to understand the influence of a CMC layer on the behaviour of Fe NPs, which could further assess the potential of the application of CMC–Ni/Fe NPs on environmental in-situ remediation.

# Introduction

Polybrominated diphenyl ethers (PBDEs), the classic brominated flame retardants, are widely used as additives in many household and commercial products to prevent fire. Deca–BDE (DBDE) is expected to sorb onto soil or sediment particles owing to its hydrophobicity, causing an increased concern due to its occurrence in wildlife and humans during the past years. Since stabilized Fe NPs have been increasingly used to treat the remediation of environmental pollutants, it is critical to understand how stabilized Fe NPs behave and interact with pollutants when they are introduced into the environment. The objective of this study was to investigate the effect of carboxymethyl cellulose (CMC) on the reactivity of Fe NPs toward DBDE and propose its possible reaction mechanisms.

## **Materials and Methods**

Stabilized NZVI was synthesized by modifying the borohydride reduction approach with adding CMC. 2 mL of prepared CMC–Ni/Fe NPs were added to an amber vial that

contained 2 mL of 20 mg L<sup>-1</sup> DBDE solution. At predetermined time intervals, samples were extracted with EA and analyzed using GC/ $\mu$ –ECD. In addition, the samples were analyzed to determine the concentration of DBDE that was adsorbed on the Fe surface and remained as a residue in the solutions.

# **Results and Discussion**

# Characteristics of synthesized nanoscale iron particles

The morphology of CMC stabilized Fe was determined by TEM, in which the individual NP was spherical with an average particle size of 86.7 nm. There were many small unshaped NPs packed together on the Fe surface with a size of about 5–10 nm. The packing could be the self-assembled of amorphous primary Fe into spherical secondary Fe due to the excess CMC and Ostwald ripening. The metal surfaces also reflected a CMC-coating artifact instead of amorphous Fe. The Fe surface was negatively charged for pH 3.4-10. The attached CMC stabilizer molecule not only provides a strong inter–particle electrostatic force and/or steric repulsion force to overcome the attractive van der Waals and magnetic forces between particles, but also maintains a great specific surface area of finer NPs.

# Removal of DBDE by CMC-Ni/Fe NPs

The removal of DBDE by both CMC–Fe<sup>0</sup> and CMC–Ni/Fe NPs was observed in the beginning for approximately 60 min. DBDE removal efficiency was only 20% through an adsorption process on the CMC–Fe<sup>0</sup> surface. The removal rate constant *k* of DBDE increased from 0.137 to 0.216 min<sup>-1</sup> with an increase in Ni load from 0.5 to 5.0 wt%. The DBDE degradation was also increased with an increased Ni load.

# Effect of temperature on the behavior of CMC-Ni/Fe NPs

The reaction temperature had a positive effect on the removal of DBDE. The  $E_a$  obtained was 37.7 kJ mol<sup>-1</sup>, suggesting that a surface chemical reaction rather than a diffusion-mediated reaction was the rate-limiting step in the removal of DBDE.

# Effect of pH value on the behavior of CMC-Ni/Fe NPs

The removal efficiency and removal rate increased with an increase in pH value. In the meanwhile, the particle size of CMC–Ni/Fe NPs was reduced from 175 nm to 85 nm with an increase in pH from pH 5 to pH 10. The pH<sub>zpc</sub> of CMC is approximately 2.0 and the negative surface charge of CMC–Ni/Fe increased when pH increased. The conceptual scheme of CMC–Fe NPs pointed out that CMC was coated on the Fe surface with brush structures which can provide steric stabilization to prevent aggregates. The carboxyl groups of CMC are protonated under acidic conditions, and reduce the colloidal stability. The pH dependence of CMC could induce the tendency of DBDE removal rates at various pH values.

# Degradation of one emerging contaminant, hexabromocyclododecane, by one enzyme, haloalkane dehalogenase

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# Abstract

The enzyme, haloalkane dehalogenase (HLD) Lin B, derived from one soil microbe, Bradyrhizobium japonicum. Hexabromocyclododecane (HBCD) is one of the most widely used brominated flame retardants (BFRs) for producing common construction materials such as expanded polystyrene and extracted polystyrene. Due to its characteristics of long-term persistence, high bioaccumulability, and potential toxicity, developing remediation methods toward this emerging contaminant is urgent. The aim of this study is to evaluate the transformation ability of HLD toward HBCD. After 24 hours of reaction, 49.3%, 60.9%, and 92.8% of HBCD was degraded by 100, 200, and 500 mg/L of HLD proteins, respectively. Furthermore, 4.05, 9.64, and 16.7 µM of bromide ions were released in the above conditions. The degradation and debromination of HBCD were both dose-dependent. Michaelis-Menten parameters  $K_{M}$  = 12.32  $\mu M$ and  $v_{max} = 0.16 \ \mu mol \ L^{-1} \ min^{-1}$  were deduced from exposure data with multiple enzyme/substrate ratios. HLD-catalytic conditions were conducted in multiple temperature and pH value. After 60 min incubation, the HBCD concentrations decreased to 78.2%, 69.7% and 59.4% at 25 °C, 37 °C and 50 °C, respectively. The HBCD concentrations decreased to 67.7%, 60.3%, 71.9% and 82% at pH 5.9, 6.9, 8.6 and 10.2, respectively. Maximal activity was detected at 50 °C and at pH 6.9. The degradation and debromination ability of our HLD toward HBCD were analyzed in this study. Our results demonstrated that the haloalkane dehalogenase transformed HBCD and released bromide ions through hydrolytic reactions. The novel degradation ability of HLD provided effective and alternative remediation strategy for removal of HBCD and facilitate the fate prediction of BFRs in the environment.

# Introduction

Brominated flame retardants (BFRs) are organobromide compounds that have an inhibitory effect on the ignition of combustible plastics and textile applications, e.g. electronics, clothes and furniture. Hexabromocyclododecane (HBCD) is one of the most extensively used BFRs with a global market value of 12,500 metric tons in 2011. It is mainly applied to expanded and extracted polystyrene, which are common materials for foam packing, household furniture, and appliances. Haloalkane dehalogenases (HLDs) are  $\alpha/\beta$ -hydrolases which convert halogenated compounds to corresponding alcohols,

halides and protons. Its property for HBCD degradation is limited and waited to be investigated.

# **Materials and Methods**

Expression of LinB was induced with 0.5 mM isopropyl  $\beta$ -D-thiogalactopyranoside when the culture reached an OD<sub>600</sub> of 0.4-0.6. The cells were disrupted by sonication. Cell debris and larger particles were removed by centrifugation at 15,000 rpm for 30 min. The supernatant obtained was used for purification. Two sets of LinB treatment experiments toward HBCD and other brominated compounds were performed under specific concentration of enzyme and substrate, pH value, temperature, and reaction time. The concentration of HBCD was then determined by GC/µECD after extraction.

# **Results and Discussion**

# HBCD degradation under different LinB dosage

In this study, HBCD is one kind of halogenated and cyclic aliphatic hydrocarbon. Structurally, it is larger than those substrates which are found can be transformed by LinB before, such as 2-bromobutane, 2-bromopentane, methyl 2-bromopropionate, or ethyl 2-bromobutyrate. HBCD (5.3  $\mu$ M) was obviously degraded by LinB with a variety of enzyme dosages at 37 °C for 24 hr. The degradation activity was entirely eliminated with heat-treated LinB that was inactivated at 100 °C for 10 min. In addition, the heat-inactivated LinB experiment can also demonstrate that our HBCD determination would not be overestimated by protein adsorption.

The HBCD transformation rate increased with the LinB dosing concentrations from 100, 200, to 500 mg/L. Meanwhile, more bromide ions were released in the above conditions, suggesting the transformation and debromination of HBCD catalyzed by LinB were both dose-dependent. With respect to the bromide ions releasing under the high dosage of LinB (200 and 500 mg/L), the concentration was far exceeding than that of theoretically debrominating reaction where one molecular bromide ion was fully converted and released from the HBCD. The amount of bromide ion release was near 3-fold of the molar ratio to the amount of HBCD transformation, elucidating that the further by-product after HBCD transformation can be subsequently debrominated by LinB.

# The degradation activity of LinB toward HBCD

Michaelis-Menten kinetic modeling was used to determine kinetic constant of LinB. HBCD mixtures without stereochemical separation at initial concentrations of 0.08, 0.16, 0.27, 0.56, and 2.14  $\mu$ M were individually mixed with 10 mg L<sup>-1</sup> of LinB. The fitting results elucidated that the V<sub>max</sub> was 0.47  $\mu$ mole L<sup>-1</sup> h<sup>-1</sup> and the *K<sub>m</sub>* was 0.75  $\mu$ M.

# 穿透式 X 光顯微鏡觀測鉀型態及黏土礦物絮聚結構

# Potassium speciation and clay mineral flocculation structure by Transmission X-Ray Microscopy

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### 1. Abstract

The estimation of available K is one of the most common practices in the agriculture. This study investigated 1) the comparison of the amount of K speciation by traditional method (TM) and sequential extraction process (SEP) 2) the clay particle flocculation structure after suspension with KCl solution by Transmission X-Ray Microscopy (TXM) technique. The results showed that the amount of K speciations were higher in forms unavailable for plant-uptake than in available forms. Most of K speciations were associated with residual, organic matter, Fe or Mn oxide and exchangeable. In farming long term applied K fertilizer and red soil were higher exchangeable K than farming long term without K fertilizer and forest soil. This indicated that K fertilizer increased various K concentration. The TXM image of clay particles showed the porous cellular structure of soil aggregates in KCl solution had large and very large empty voids. TXM nanotomography is a new technique that can be useful in understanding the clay mineral micro-structure.

2. Key words: Potassium; Sequential extraction process; Clay mineral; TXM

## 3. Introduction

The sequential extraction process (SEP) is a step in analyzing the partitioning of metals affected by environmental conditions. While, traditional method (TM) use for potash fertilizer recommendation. To unusual behavior of clay minerals when swelling or shrinking is desiderative of high technology of Transmission X-Ray Microscopy (TXM). This technique is a soft X-ray and is suited to studying nanoparticles. The objectives of this research were to 1) compare the K speciation extracted by TM and SEP and 2) determined the clay particle flocculation structure by TXM technique.

## 4. Materials and methods

4.1 Soil sampling

Four soil samples as farming long term without K fertilizer (10 years) farming

long term applied K fertilizer (more than 10 years 168-240 kg  $K_2O$  ha<sup>-1</sup>year<sup>-1</sup>), forest soil and farming red soil (450-500 kg  $K_2O$  ha<sup>-1</sup>year<sup>-1</sup>) were selected from area in Taiwan. All soil samples were collected at the depth of 0-30 cm, dried and ground to pass a 2 mm sieve for physicochemical and potassium speciation analysis.

4.2 Physicochemical and mineralogical analysis

The soil physicochemical properties followed the method of soil analysis.

# 4.3 Potassium speciation by sequential extraction and traditional method

Three sequential extraction process methods were followed: Tesseir et al., (1979), Shuman (1983) and Krishhnamurti et al., (1995). Traditional method followed Helmke and Sparks (1996).

4.4 Clay suspension and Transmission X-Ray Microscopy

Clay mineral particle was separated from soil samples by technique Stokes' law. 0.1 g of clay particles were put into a 1.5 mL Eppendorf Tube along with 1 mL of DI water, 0.025 M, and 1 M KCl. Shake for 5 minutes and examine in the TXM at beamline BL01B of National Synchrotron Radiation Research Center (NSRRC), Taiwan.

#### 5. Results and discussion

The results showed that the amount of K speciation by TM were high in mineral K, HNO<sub>3</sub> K, Non-exchangeable K, NH<sub>4</sub>OAcK, exchangeable K and water soluble K respectively. SEP indicated that most of K speciations in soil were associated with residual, organic matter, Fe or Mn oxide and exchangeable fractions. In farming long term applied K fertilizer and red soil had higher an amount of exchangeable K than farming long term without K fertilizer and forest soil. This indicated that one way to increase the available K is to apply K fertilizer. The TXM image of clay particles suspension with K solution showed that the porous cellular structure of soil aggregates in KCl solution had more large and very larger empty voids than in deionized water.

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# *Rhizobium capsici* sp. nov., isolated from root tumor of a green bell pepper (*Capsicum annuum* var. *grossum*) plant

# 從青椒根瘤中分離出 Rhizobium capsici 菌種

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# Abstract

A novel, Gram-staining-negative, rodshaped, aerobic and motile bacterium, designated strain CC-SKC2<sup>T</sup>, was isolated from the root tumor of a green bell pepper (*Capsicum annuum* var. *grossum*) plant in Taiwan. Cells were positive for oxidase and catalase activities and exhibited growth at 25–37 C, pH 4.0–9.0 and tolerated NaCl concentrations up to 4.0 % (w/v).

# Introduction

*Rhizobium* species, affiliated to the family *Rhizobiaceae*, are traditionally considered as legume endosymbionts and have generally been isolated from nodules of leguminous plants (Amarger et al. 1997; de Lajudie et al. 1998; Lindstro m 1989; Squartini et al. 2002; Valverde et al. 2006; van Berkum et al. 1998; Wang et al. 1998;Wei et al. 2002). The members of the family *Rhizobiaceae* have been undergone several taxonomic reassessments. For example, Young et al. (2001) proposed the reclassification of *Allorhizobiumundicola* (de Lajudie et al. 1998) and species of *Agrobacterium* (Conn 1942) such as *Agrobacterium rhizogenes*, *Agrobacterium rubi*, *Agrobacterium tumefaciens* and *Agrobacterium vitis* under the genus *Rhizobium*.

Here, we investigate the taxonomic status of a novel bacterium, designated strain CC-SKC2<sup>T</sup>, isolated from the root tumors of a green bell pepper (Capsicum annuum var. grossum L.) plant in Taiwan using polyphasic approach.

# Materials and methods

- 1. Bacterial strains and growth conditions.
- 2. Morphological tests, biochemical characterization, determination of the predominant ubiquinone, polar lipids and DNA base composition, cellular polyamines and fatty acid methyl ester (FAME) analyses, nodulation capacity of strain CC-SKC2<sup>T</sup>, Nitrogen-fixing ability.
- 3. PCR amplification of 16S rRNA and other housekeeping genes, phylogenetic

analysis, DNA–DNA hybridization analysis.

## **Results and discussion**

Cells are Gram-stain-negative, rod-shaped, 2.8–3.0 lm in length and 0.9–1.2 lm in diameter. Colonies are circular, smooth and cream-colored after two days of incubation on yeast extract mannitol agar. The growth temperature ranges from 25 to 37 °C, pH 4.0-9.0 and tolerates 4 % (w/v) NaCl concentration. utilized in theBIOLOGGN-II system: dextrin,N-acetyl-Dgalactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, D-fructose, L-fucose, Dgalactose, gentiobiose, a-D-glucose, m-inositol, a-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, b-methyl-D-glucoside, D-psicose, D-raffinose, Lrhamnose, D-sorbitol, D-trehalose, turanose, xylitol, pyruvic acidmethyl ester. sucrose. succinic acidmono-methylester, acetic acid, cis-aconitic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, a-hydroxybutyric acid, bhydroxybutyric acid, a-keto butyric acid, D,L-lactic acid, propionic acid, quinic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, Lpyroglutamic acid, L-serine, L-threonine, c-amino butyric acid, urocanic acid, inosine, uridine, thymidine, 2-aminoethanol, glycerol, D,L-a-glycerol phosphate, a-D-glucose-1-phosphate and D-glucose-6-phosphate. Alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, a-galactosidase, b-galactosidase, a-glucosidase, b-glucosidase and N-acetyl-b-glucosaminidase are positive in the API-ZYM system. Oxidase and catalase are positive. The fatty acid profile consists of C16:0, C19:0 cyclo x8c, C14:0 3-OH and/or C16:1 iso I and C18:1 x7c and/or C18:1x6c. The polyamine pattern shows predominance of spermidine and moderate amounts of sym-homospermidine. The predominant quinone system is ubiquinone (Q- 10). The polar lipid profile is composed of major amounts of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, moderate amounts of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine and an unidentified aminolipid. The DNA G+C content of the type strain is  $60.5 \pm 1.8 \text{ mol}\%$ .

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# 放線菌拮抗土傳性病害之研究

# Study on actinomycetes antagonistic to soilborne pathogens

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#### 摘要

本研究自全台各地4 個土壤和1 個堆肥分離得177 株放線菌,與番茄萎凋病菌 (Fusarium oxysporum f. sp. lycopersici) 進行對峙培養篩選出抑制能力較佳的菌株A47、A177、和A297,測定供試放線菌產生幾丁質分解酵素、纖維素分解酵素、明膠分解酵素、蛋白質分解酵素、脂質分解酵素和澱粉分解酵素等胞外酵素,吲哚乙酸和載鐵物質等植物調節物質,結果顯示A47、A177 和A297產生胞外酵素和植物調節物質能力較強。

#### 前言

台灣氣候高溫多溼又缺乏冬季低溫,造成植物病原菌肆虐,農民大多使用農藥進行防治,雖然農藥多數能有效降低病害,但土傳性病害不易防治且土壤灌注 農藥容易有環境汙染的疑慮,再加上廣泛地使用農藥使得病蟲害開始出現抗藥性 (Palaniyandi et al., 2013),化學肥料過量或錯誤的使用亦造成環境危害。微生物製 劑取代農藥和化學肥料作為抑制病害和促進植物生長被認為是一個

可行的方法 (Xue et al., 2013) ,而放線菌則被認為極具潛力,因其可產生抗生素、 細胞壁分解酵素、超寄生、競爭養分和誘導植物系統性抗病。此外,放線菌亦可 產生植物賀爾蒙、載鐵物質、溶磷和促進固氮菌或叢枝菌根菌共生等。

## 材料與方法

接種試驗分為先接種萎凋病菌後接種放線菌和先接種放線菌後接種萎凋病菌,番茄種子育苗14-21 天至長出兩片真葉移植至含有250 克石英砂的三吋盆訂,一部份處理於第一天先接種放線菌第四天接種萎凋病,另一部份處理為第四天接種萎凋病第七天接種放線菌,共11 個處理,每處理5 重複。將盆栽置於生長箱中,溫度設定為28 ℃,日照12 小時。FOL146孢子懸浮液濃度為10<sup>4</sup> spores/g,放線菌孢子懸浮液為4×10<sup>5</sup> spores/g。

待五週後觀察番茄植株罹病度。罹病度(Disease severity) = 病班長 ×100

# 結果與討論

編號 13



不論是先接種放線菌還是先接種萎凋病菌,接種放線菌均降低了番茄的罹病 度。在先接種番茄萎凋病菌的情況下,僅有A177 和A297 和對照組相比達顯著差 異,以A297 防治效果最佳,罹病度為14.8%。在先接種放線菌的情況下,和對照 組相比罹病度均達顯著差異。雖然番茄先接種放線菌或萎凋病菌其罹病度並沒有 達顯著差異,但就趨勢而言,先接種萎凋病菌後接種放線菌的番茄罹病度較高, 菌可預防番茄萎凋病發生 接種番茄萎凋病菌的處理和對照組相比並沒有顯著差 異,但先接種A177 和A297 番茄萎凋病菌有減少的趨勢,但僅有接種A297處理和 對照組有顯著差異,因此先接種放線菌較能有效降低番茄萎凋病菌的數量。 結論

預先接種A297 孢子懸浮液可直接減少番茄萎凋病菌數量,降低番茄罹病度; A47 和A177 雖然並沒有顯著降低番茄萎凋病菌的數量,也能降低番茄罹病度, 推測可能是藉由誘導植物產生系統性抗病所致。

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# Assessing the authenticity of commercial deep-sea drinking water by chemical and isotopic approaches

以化學與同位素特徵驗證市售海洋深層水之來源標示

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#### 摘要

本研究以同位素及化學元素結合統計學中主成份分析方法(PCA),檢測台灣市售 海洋深層水之來源。由結果顯示,除了製造海洋深層水之水體來源不同,以不同 淡化方式及是否添加海水濃縮液,都將影響主成份分析中的表現。本研究利用穩 定氫、氧同位素與微量元素分析,並結合主成份分析之統計,成功驗證台灣市售 海洋深層水之來源標示。

關鍵字:來源標示、深層海水,氫氧同位素、微量元素、主成份分析

前言

地表約70%面積被海洋所覆蓋,大量的海水能透過海水淡化等技術加以利用。 隨著近年來海洋資源研究的投入,利用深層海水之產業蓬勃發展,其中最常見的 利用方式為以深層海水淡化製成之海洋深層水,因海洋深層水之售價要高於一般 市售瓶裝水許多。有鑑於商業應用與環境科學之結合,本研究利用海洋深層水中 的同位素及化學元素,結合主成份分析方法,鑑定其標示來源之真偽。

#### 材料與方法

水樣之氫、氧同位素組成以水同位素分析儀(LWIA)分析,其定義為:δ=(R<sub>sample</sub> /R<sub>SMOW</sub>-1)×1000,特徵元素分析是利用感應偶和電漿質譜儀(ICP-MS),分析樣品 之40種特徵元素濃度。主成份分析(PCA)是一種多變量統計技術,藉由多數聚整 合為單一變項之線性組合來解釋其供變數結構,以達到資料簡化的目標。將水樣 數據做為資料點,轉動資料點之軸心,有最大特徵值之軸為PC1,具有最大的解 釋變異能力,依此類推為PC2。

#### 結果與討論

主成份分析(PCA)中,利用氫、氧同位素、EC 值與 40 種微量元素進行整合並區

分目標,表1為各主成份(PC)組成及其可解釋的最大變量,透過PC1、PC2及PC3 可解釋達總變異59.5%的變異。在PC1及PC2的比較圖(圖1(a))中,TW 明顯偏離 於淡化海水樣品及FW,因此透過圖1(a)及圖1(b)能明顯區分出自來水(TW)與純 化淡水(ROW、DIW)之差異性。此外,圖1(a)與(b)均能區分出ROW、DIW 與DSDW 組之差異,因此發現以相同淡化方式處裡下,水體本質不同亦有明顯的差異。SDW 及SSDW 組於圖中之分布極為接近,這是因為其水源地背景極度相似所導致。另 外,由於MDS-18水樣主體為陸域淡水及海水濃縮礦物質液,因此於圖上的分布 介於純化之陸域淡水及淡化海水之間。



圖 1 (a) 樣品之 PC1 與 PC2 比較圖(b) 樣品 PC1 與 PC3 比較圖

#### 結論

本研究利用氫、氧同位素組成與多變量統計之主成份分析結果評估台灣市售海 洋深層水之標示來源,總結 PCA 分析之結果,市售商品常強調其產品相較一般瓶 裝水,具有多種對身體有益之營養元素,然而其營養成份並未於此次分析結果中 呈現出來,甚至發現自來水(TW)樣品之大部份元素含量竟比海水來源樣品之元素 濃度高。

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# 田菁根瘤菌耐鹽性研究

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#### 摘要

近年來因為人口不斷的增加及可耕地面積的減少,所以農人為了增加田地的單位 面積產量及收益行使了較為集約的耕作方法,農民會施入大量的化肥補充,而造 成鹽分的累積。隨著鹽害問題日益嚴重,前人的研究發現將在高 EC 值土壤中篩出 的根瘤菌接種於田菁根部,能有助於田菁對鹽害的耐受度增加,但此樣的結果在 高 EC 的環境下較容易發生,而在低 EC 環境中反而不容易發生,因此此次的研究 想探討這些在高 EC 環境下篩出之根瘤菌能否在接種於低 EC 時得到同樣的結果。 此次實驗選定 0、10、20、30、40 dS/m,作為實驗之 EC 值環境,接種十株根瘤菌 觀察其生長狀況。研究結果發現菌株 323、326 呈好低 EC 值,316、324、328 呈 好中 EC 值,317、321、327 呈好高 EC 值。

關鍵字:集約耕作、根瘤菌、鹽害

## 前言

當今的人口數量不斷增加,而可耕地因為不同的開發而減少,且在有限的可耕地 面積下,農民為了使田地能有更好的產量及收益行使了集約的耕種方法,農民就 使用大量的化肥來增加地力,導致了鹽害土壤的產生。在鹽分高的環境下,由於 外界的水勢能降低,使得植物根系難以吸收水分,且濃度過高的鈣、鎂、鉀、鈉 離子也抑制了其他的陽離子吸收,因而造成作物生長出現障礙。一般的土壤飽和 抽出液,當 EC 值>4dS/m 時就可以被定義為鹽害土壤(海水的 EC 值大約為 45~50dS/m),大多數的作物生長受阻,僅有少部分對鹽害不敏感的作物可以生長, 如田菁及萬壽菊。而隨著鹽害問題日漸嚴重,前人的研究中(黃,2013)發現將在 高 EC 值土壤中篩出的根瘤菌接種於田菁根部,能有助於田菁對鹽害的耐受度增加, 且能藉由根瘤的產生行使固氮作用提供氮素,但此樣的結果在高 EC 的環境下較容 易發生,而在低 EC 環境中反而不容易發生,因此此次的研究想探討這些在高 EC 環境下篩出之根瘤菌能否在接種於低 EC 環境時也得到同樣的結果。

#### 目的

探討由高 EC 土壤中篩出之十株根瘤菌的耐鹽性。

#### 材料與儀器

高 EC 土壤篩出之根瘤菌(黃,2013)、NA 培養基、分光光度計(波長 600 nm) 方法

(1)將保甘油之有益微生物先接種於NA固態培養基上,培養3天後,再接入100 毫升滅菌過的1/2NB液態培養基中培養3天。

(2)配製 NaCl 檢量線,得知欲營造之 EC 值需要多少量的 NaCl。

(3)將培養好的菌液加入不同 EC 值的液態培養基中震盪培養 3 日,在以分光光度 計測其吸光值評斷其生長情形。

(4) 再以吸光值及 EC 值作長條圖呈現結果。

#### 結果與討論

喜好低度鹽分的菌株在 EC 值 0~20 dS/m 生長狀況較 EC 值=20~40 dS/m 為佳,且 其菌數對濃度的關係呈負相關,即隨著濃度增加,培養液中的菌數隨之減少,此 類的菌種編號為 323 和 326。喜好中度鹽分又可分為兩種,一種是在 EC 值=10~30 dS/m 生長狀況較佳,如 316。另一種為在 EC 值=20 dS/m 環境菌株的數量尤為突 出,與其他 EC 值具明顯差異,如 324 和 328。喜好高度鹽度的菌種在 EC=20~40 dS/m 下生長狀況較好,且隨著 EC 值得提高,菌的數量有上升之趨勢,也就是說鹽分濃 度和菌種的生長狀態呈正相關,此類之菌株有 317、321 和 327。最後一類無明顯 關係者,在不同 EC 下,其菌數沒有達到顯著差異或是濃度與菌數無相關性,可能 升減夾雜,此類的菌種代號為 320 和 322。以上是以菌種對鹽分的喜好分類,但是 並不代表菌種實際上對鹽分的耐性。若要以對鹽分的忍耐力來對 10 株菌株進行分 類,可以吸光值(ABS)=1.2 來做區分。將五種濃度的 ABS 值做平均,若其平均值 >1.2,將其歸類為對鹽分忍受力高者,相對的,ABS<1.2 只為對鹽分忍受力較差。 對鹽分忍受力高者,雖然不是在它喜好的 EC 值下,其菌的濃度依然大於忍受力較 差者,此類應用於接種於鹽害土壤幫助作物生長較具潛力,因為它具有較強的忍 受力,菌種在鹽害環境下具有濃度優勢,較能成為優勢菌種。

## 結論

菌株 323、326 呈好低 EC 值, 316、324、328 呈好中 EC 值, 317、321、327 呈好 高 EC 值。

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圖一.耐高鹽菌株生長狀況 圖二.耐低鹽菌株生長狀況

# 阿里山地區淋澱化作用伴隨黏粒洗入之土壤特性與化育作用

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### 摘要

黏粒洗入伴隨淋澱化作用或具淋澱化特徵,是阿里山地區可能發生的土壤生成 化育作用,雖然黏粒在土層間移動不是淋澱化作用之主要機制,但由砂岩或頁岩 所風化之細質地土壤卻可觀察到黏粒膜等黏粒洗入證據。本研究區採集三個土壤 剖面,有兩個剖面為黏粒聚積淋澱土(Ultic Haplorthod),而另一個為典型極育土 (Typic Hapludult);黏粒含量在淋澱土剖面 B 層內有顯著增加,而淋澱化作用造成 有機碳、鐵及鋁在淋澱層內累積。從微形態特徵觀察可發現,確實在淋澱層土壤 中發現有機物複合鐵鋁物質與黏粒包覆在粗石英顆粒表面。因此,在本研究區所 發現之淋澱土生成作用以淋澱化作用伴隨黏粒洗入作用。

關鍵字:淋澱土、淋澱化作用、黏粒洗入作用、化育作用

#### 前言

淋澱土一般生成於溫帶至寒帶高山地區,以冷涼潮濕的環境為主,地表植被以 針葉林,風化自透水性佳的粗質地酸性母質上。其土壤面積在全球佔4%,主要分 布於高緯度國家如加拿大、北歐和俄羅斯等。然而,在台灣亞熱帶地區亦可發現 其生成,主要分布於中高海拔地區,嘉義阿里山在早些年前於祝山和大塔山區域 發現淋澱土分布。但有一差異在於高山地區土壤母質多為泥岩、頁岩或板岩,此 經風化過程後黏粒含量較高,黏土礦物組成與土壤內鐵和鋁含量有關(Lin, 2002), 跟一般常見由粗質地母岩生成而來的淋澱土不同,因此推測台灣區域中高海拔山 區因土壤質地細緻,在化育過程中可能有淋澱化作用伴隨黏粒洗入的情形(邱, 2004)。本研究探討阿里山地區土壤樣體形態特徵、理化特性、礦物組成與土壤分 類,並針對樣體間淋澱化程度差異進行討論,以及影響該區域土壤生成化育差異 原因。

#### 材料與方法

 研究區域:樣點分布於阿里山區祝山林道、小笠原山及萬歲山,海拔高度從 2200 m 至 2500 m,年均溫為 10.8℃,年降雨量約為 4,000 公釐,土壤溫度屬於 mesic 境況,土壤水分境況則屬於 udic。母質以頁岩及砂岩為主,植被以檜木類為主。
 土壤樣體採集及分析:在三個地點各收集 1 個土壤剖面,依美國土壤調查手冊分 層採集與描述,以土環法測定土壤總體密度,分析質地(吸管法)、土壤 pH(土水 比 1:1)、土壤有機碳含量(濕式氧化法),無定形鐵、鋁(草酸銨萃取法)、游離鐵、 鋁(DCB 法)、有機形複合鐵、鋁(焦磷酸鈉萃取法)及 ODOE。另外製作土壤薄切 片利用偏光顯微鏡觀察土壤微形態特徵。

#### 結果與討論

1.土壤樣體均屬於高度淋洗之強酸性土壤、低鹽基(交換性鉀、鈉、鈣、鎂離子)含量與低鹽基飽和度。土壤樣體分成二類,pedon1502為第一類在化學性質雖然符合淋澱土的特性,但具黏量含量高使得土壤較亮,因此分類以Typic Hapludult。

另外 Bhs 層微形態特徵,可見大量有機物與鐵、鋁形成有機態錯合物已經包覆在 礦物粒子的表面或孔隙與孔洞中,以及孔隙邊緣與孔洞中有黏粒之定位排列,顯 示淋澱化作用與黏粒移動為該土壤樣體之主要土壤化育作用。第二類土壤 pedon1501 及 1503 符合淋澱土的特性,黏量含量較少為砂質壤土分類上為 Ultic Haplorthod。因此淋澱化作用為該土壤樣體之主要土壤化育作用。

- 2.有機碳、黏粒以及無定形、有機態與游離態鐵、鋁含量,由表層隨深度增加而減少,而在漂白層中含量均為最低,再隨深度增加而有累積之趨勢,在淋澱層或下層達到最大量,顯示有機質與黏粒以及鐵、鋁在土層中趨向同時一起移動。
- 3.Fep/Feo的比值可判斷土壤中無定形鐵、鋁存在形態的相對量,當比值越大,表示 鐵鋁以有機錯合物存在的型態居多,大部分土壤層間中 Fep/Feo均較 Feo/Fed 高顯 示游離態之鐵、鋁物質,其無定形之鐵、鋁以有機型態之含量多,因此推測鐵、 鋁在土壤中是以有機態鐵、鋁錯合物為主要移動形式。
- 4.土壤樣體微形態特徵觀察中發現漂白層之土壤基體,主要由大量粗顆粒之石英粒子緊密堆積而成,粒子間有明顯空隙存在,亦有大量孔洞。多數礦物粒子無表面 包覆,但依然有零星有機物質存在,土壤基質顏色較黑。黏粒洗出作用明顯,但 有機物未完全由漂白層中洗出。淋澱層微形態特徵可觀察到大量有機物、鐵、鋁 與黏粒混合形成深褐色的髓質物質綿密的分佈於孔隙以及孔洞邊緣,甚至包覆在 粗顆粒表面。髓質物質呈現鐵與有機物混合之暗紅色,亦有少量之黏粒存在而呈 現黃褐色或亮黃色。

#### 結論

本研究區淋澱化作用較易出現在地形平坦處,由於該區域母質屬於頁岩和泥岩, 黏粒洗入作用也在此盛行,所收集之土壤樣體分類主要為兩種,依據土壤黏粒含 量分為含量較高之典型極育土 Typic Hapludult,二為黏粒聚積淋澱土 Ultic Haplorthod。有機碳、黏粒,無定形、有機態以及游離態鐵、鋁含量在土層分佈狀 況,顯示在質地較粗的上層主要為有機物與鐵形成錯合物起向下移動。除此之外 黏粒含量在剖面間有逐漸累積現象,推測黏粒移動可能亦伴隨有機錯合之淋澱化 作用。微形態特徵上,發現顆粒表面或孔隙周圍被有機態錯合物質包覆,為有機 物、鐵及鋁洗入之特徵,此外也能判斷出黏粒洗入,更加確認淋澱化作用伴隨黏 粒洗入之化育作用。

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# 水稻內生細菌之分離與植物生長促進特性研究

# Isolation and plant growth promoting characterization of endophytic bacteria from rice cultivar

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# Abstract

Plant growth promoting rhizobacteria (PGPR) which can serve as biofertilizers or biopesticides attract extensive attention worldwide. Studies on PGPR associated with rice, a major grain crop in Asia seems important and promising. In the present study we made an attempt to isolate and characterize endophytic bacteria from the roots of rice cultivar. Phylogenetic analyses of these isolates were performed based on their 16S rDNA sequences. Besides, functional traits regarding plant growth promotion including free-living nitrogen fixation and mineral phosphate solubilization were also determined. Out of 68 isolates a total of 13 candidates were selected. Free-living nitrogen fixing activities of these superior isolates determined by acetylene reduction assay (ARA) ranged from  $1.2 \times 10^{-2}$  to  $2.8 \times 10^{-2}$  µmol/flask hr. All these isolates also showed solubilization of tricalcium phosphate as revealed by the appearance of clear zone around colonies. The 16S rDNA sequences of 13 isolates assigned them to the genus *Enterobacter* and *Kosakonia*. The clarification of distribution and potential of these endophytes may help in developing agriculture-used bioinoculants.

Keywords: Plant growth promotion, endophytic bacteria, rice

# Introduction

To fulfill agricultural production in a sustainable manner, the explorations of PGPR as bioinoculants used in various crops are still ongoing. The present studies were undertaken to perform the isolation of endophytic bacteria from roots of rice cultivar, and their plant growth promoting traits as biofertilizers were determined.

## Materials and methods

Indigenous isolates from interior of surface-sterilized roots were obtained on three

nitrogen-free selective media. Functional traits regarding plant growth promotion including free-living nitrogen fixation and mineral phosphate solubilization were determined. Besides, phylogenetic analyses of these isolates were performed based on their 16S rDNA sequences derived from genomic DNAs.

## **Results and discussion**

A total of 68 isolates from interior of surface-sterilized roots were obtained on three nitrogen-free selective media. A higher proportion of bacteria isolated from organic farming soils showed promising nitrogen fixing and phosphate solubilizing activities, as compared with those from conventional farming soils. 13 superior candidates with potential in the promotion of plant growth were selected. Free-living nitrogen fixing activities of these isolates determined by ARA ranged from  $1.2 \times 10^{-2}$  to 2.8 x  $10^{-2}$  µmol/flask hr. All these isolates also showed solubilization of tricalcium phosphate as revealed by the appearance of clear zone around colonies. There were six genotypes obtained from 13 isolates based on their 16S rDNA sequences, which assigned them to the genus Enterobacter and Kosakonia. These bacterial lineages encompassed E. oryzendophyticus, K. oryzae, and K. sacchari, with sequence similarity ranged from 98.95% to 100% compared to type strains. Interestingly, 12 out of 13 isolates were obtained from rice roots grown in organic farming soils while only 1 from conventional farming soil. The clarification of distribution and potential of these endophytes may help in developing agriculture-used bioinoculants. All these isolates addressed here showed plant growth promoting activities but might be opportunistic pathogens to human beings since they were affiliated with bacterial family Enterobacteriaceae. Although some of these bacterial lineages have been demonstrated as inhabitants inside rice roots, it still raised an issue that biosafety of these bioinoculants should be taken into considerations when they are used in environments.

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# 台灣北部烘爐山森林土壤之特性、化育與分類

# Characteristic, pedogenesis, and classification of the forest soils in Honglu Mountain, northern Taiwan

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#### 摘要

烘爐山屬於大屯火山亞群,約在40萬年前停止噴發,為一座由多鋁型玄武岩/ 玄武岩質安山岩所構成的複合式火山,本研究在該地區山頂、背坡與趾坡之土壤 樣體,研究其土壤特性、化育作用與分類。研究區域屬副熱帶氣候,茂盛的植被 提供大量有機質來源,而大量的可交換性鋁增加了土壤酸度。選擇性化學抽出分 析指出,土壤中的活性鋁主要以鋁--有機質複合物為主,而鋁英石在酸性環境下不 易生成(pH < 5)。三個樣體因其 Feo/Fed 比值甚低(<0.4),且其他理化分析結果皆未 能完全符合美國土壤分類系統中火山灰土壤性質(andic soil properties)之定義,其亞 類為火山 灰特性低鹽基濕潤弱育土(Andic Dystrudepts),而不屬於灰燼土 (Andisols)。

關鍵詞:灰燼土、土壤化育、玄武岩/玄武岩質安山岩、地形土序

#### 前言

台灣位於環太平洋火山帶,早期火山活動平繁,而以北部大屯火山群為主。土 壞受化育母質的影響而大多成為灰燼土,由於大量的無定型物質,灰燼土具有低 總體密度、高磷酸根結持度及良好的水分結持力等特性。然而受到氣候及時間的 影響,前人研究大屯山發現在海拔 900 公尺以上主要為灰燼主而在 300 公尺以下 為弱育土,400-500 公尺的過度區域為火山灰土壤性質的分界線,為具有火山灰土 壤性質的弱育土(Tsai et al., 2010)。過去已有許多研究陽明山地區內的火山灰土壤, 但調查資料尚未齊全,而烘爐山為其中之一。因此,本研究目的以環境因子、野 外形態特徵、理化及礦物性質研究烘爐山地區土壤之特性、化育與分類。

#### 材料與方法

土壤 pH 以水、1 N KCl 及 1 N NaF 測定;交換性鋁以 1 N KCl 測定;土壤有 機碳以濕氧法測定;磷酸根結持度根據 Blakemore 的方法測定;以 0.2 M 草酸銨法 測定土壤無定型鐵鋁矽含量;以 0.1 M 焦磷酸鈉(pH 10)測定土壤有機鍵結型態鐵

#### 結果與討論

三個樣體的總體密度介於 0.5~1.0 Mg/m<sup>3</sup>之間,而背坡及趾坡樣體並不符合火 山灰土壤性質的定義;表層土壤受到植被的覆蓋,含有較多的有機質,而隨著深 度增加而減少;三個樣體中,除了趾坡樣體的 O/A 化育層之外,其他化育層的 pH 介於 4~5 之間, 且隨著深度增加有稍微上升的趨勢, 而在表層土壤的 pH-H2O 最 低。一般在灰燼土中,當 pH 小於 5 時,黏土礦物以 2:1 型矽酸鹽礦物為主,而土 壤受強烈淋洗下成強酸性時,土壤礦物以非鋁英石礦物為主,且會生成大量的鐵、 鋁有機質複合物; $K^+$ 能夠交換出 2:1 型矽酸鹽礦物交換位置上的  $Al^{3+}$ ,而  $Al^{3+}$ 水解 釋出  $\mathrm{H}^{\!+}$ ,因此以 KCl 所得的 pH 會比水測得的還低,而三個樣體  $\Delta \mathrm{pH}$  介於  $0.3{\sim}2$ 個單位,差值越大表示交換性鋁含量越多;以 NaF 所得的 pH 代表土壤中無定型 物質多寡的依據,當大於9.4時代表土壤中可能含有大量的無定型物質,除了山頂 及趾坡的 O/A 層外,其他的測值均大於 10,代表研究樣體中含有很多無定型物質; 當土壤無定型物質越多,對磷酸根的吸持會越多,因此背坡及趾坡樣體的磷酸根 結持度,並不符合火山灰土壤性質的標準(>85%);在選擇性化學抽出中,三個樣 體的 Fep/Feo及 Alp/Alo比值大於 0.5,表示主壤中的活性鐵鋁以有機質鍵結型態為 主;而 Feo/Fed 比值能夠表示土壤風化的程度,當比值小於 0.4 代表土壤風化程度 高,三個樣體的 Fe<sub>0</sub>/Fe<sub>d</sub> 均小於 0.4,火山灰土壤性質的標準中 Al<sub>0</sub>+1/2Fe<sub>0</sub> 含量必須 大於 2%, 而三個樣體 Alo+1/2Feo 含量小於 2%, 並不符合火山灰土壤性質, 以上 兩點可看出受到氣候及時間的影響,已逐漸喪失火山灰的性質而逐漸轉變為弱育 土;而土壤中鋁英石的含量並不高,在0.3~2%間,水鐵礦含量在1.5~3%間。

#### 結論

由於氣候及時間的影響,烘爐山三個土壤樣體,其總體密度、磷酸根結持度及 Al<sub>0</sub>+1/2Fe<sub>0</sub>含量並不能完全符合美國土壤分類系統所定義之火山灰土壤性質,因此 三個樣體均被分類為弱育土,其所分類至亞類的土壤名稱為低鹽基濕潤弱育土 (Andic Dystrudepts)。

#### 参考文獻

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# 比較不同栽培種玉米對土壤鎘的吸收與累積

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#### 摘要

本研究以鎘污染場址之土壤進行盆栽試驗,比較 15 種玉米栽培種間,植株生 長勢與鎘吸收累積之差異。鎘較易累積於玉米根部且不易轉移至地上部,利用玉 米高生物量之特性,對植體鎘濃度產生稀釋效應,可使得植體內鎘濃度降低並避 免鎘毒性的危害,並可藉此篩選出適合栽種於潛在污染風險之農地的低鎘吸收品 種。

#### 前言

鍋(Cadmium)為一種有毒的重金屬元素,環境中的鎘主要來自於工業廢棄物棄 置以及廢水不當排放,使農田受到汙染,由於自然環境中植物對鎘的毒性反應較 低,缺乏生物屏障效應,易累積於植物經由食物鏈進入人體,進而對人體產生危 害。根據過去的研究指出,可作食用或飼料用之玉米對於鎘有低累積性及高生物 量的特性,然而玉米在品種間植株型態有顯著差異,因此評估安全玉米品種與特 定環境的適栽性應有更詳細的探討,觀察不同品種玉米對鎘之耐受性與累積性之 差異進行評估,可作為未來選擇適當玉米親本以培育安全玉米品種的依據。本研 究使用鎘高汙染之農地,透過十五種品種之玉米(包括:食用及飼料用玉米)經土壤 盆栽試驗,比較不同品種間重金屬鎘的累積及初步篩選具較低鎘累積性的品種。

#### 材料與方法

本研究使用十五種玉米品種又以農藝分類為食用及飼料用玉米,食用玉米則 又分為超甜玉米(歸類為 Group 1),華珍、雪珍、夏蜜、SC-132、和台南 26 號,以 及糯性玉米玉美珍、黑美珍、台南 23 號、台南 25 號和白玉米台南 22 號(糯性和白 玉米同歸類為 Group 2);飼料用玉米(歸類為 Group 3)為台農 1 號、農興 688、明豐 3 號、台南 20 號和台南 24 號。以來自台中市龍井區受鎘污染之農地土壤(7.77 mg Cd kg<sup>-1</sup>)進行盆栽試驗,每盆分別盛裝 8 kg 土壤,每一盆種植 1 株,每品種設置三盆 為重複,於種植 12 週後(為玉米穀粒成熟期)採收,並進行農藝性狀調查與植體鎘 濃度的分析測定。

#### 結果與討論

本研究結果顯示,飼料玉米乾重顯著高於食用玉米(Table 1); 鎘主要累積於玉 米根部,不易轉移至地上部,而在地上部與果穗之鎘濃度,皆以超甜玉米顯著高 於其餘兩者,而地下部則無顯著差異(Table 2)。再者,若以每株玉米的鎘累積量比 較不同品種間的差異(Table 3),則超甜玉米與飼料玉米間則無顯著差異性,由此可 推論,飼料玉米的植株乾重顯著高於超甜玉米,對植體鎘濃度產生稀釋作用,尤 其台南 20 號的稀釋作用最明顯。

Table 1 Effect of Cadmium contaminated soft on dry weight of fifteen maize cultivars in the 12 <sup>m</sup> we	Table 1	1 Effect of	Cadmium	contaminated	soil on c	Iry weigh	t of fifteen	maize cu	ultivars in	the 12 <sup>th</sup>	week	s.
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		Shoot	Root	Corn
		g plant <sup>-1</sup>		
	Cultivars	5 plant		
Group 1	華珍	36.81(2.23) †	4.73(0.39)	15.70(1.64)
	雪珍	44.65(2.08)	5.53(0.64)	20.63(1.86)
	夏蜜	49.50(3.54)	7.78(0.88)	17.35(1.56)
	SC-132	38.10(3.85)	2.77(0.67)	18.73(3.03)
	台南26號	51.89(5.07)	7.23(0.85)	3.42(0.17)
Group 2	五美珍	45.69(4.92)	2.43(0.50)	24.83(2.98)
•	黑美珍	51.87(4.29)	5.94(0.87)	31.08(2.54)
	台南22號	48.04(2.88)	5.28(1.92)	22.27(1.04)
	台南23號	47.26(4.53)	4.42(0.34)	23.37(3.21)
	台南25號	58.92(5.92)	4.88(0.34)	24.54(6.25)
Group 3	明豐3號	62.13(5.26)	8.95(0.64)	13.99(5.57)
•	農興688	43.82(5.01)	7.56(1.77)	2.07(0.02)
	台農1號	56.46(2.78)	8.52(1.37)	23.26(0.89)
	台南20號	81.23(10.00)	7.06(0.42)	31.29(5.31)
	台南24號	69.81(6.83)	28.14(0.13)	18.01(5.44)
ANOVA for	significance of		a. a.	
Group		$(G3 > G1 = G2)^{\bullet \bullet \bullet}$	$(G3 > G1 = G2)^{\bullet \bullet}$	N. S.
Cultivar	GI	(夏=26>雪>132=举)**	ns	(举 - 雪 - 夏 - 132 > 26)***
	G2	(25 > <b>玉</b> = <b>ℝ</b> = 22 = 23)**	ns	( <b>𝔅</b> > 𝔹 = 22 = 23 = 25)***
	G3	(20 > 24 > 明 = 台 > 688)**	ns	(20> 台 > 24 > 明 > 688)***

*ns* not significant \*\*\* *p*<0.001 ; \*\* *p*<0.01

Table 2 Cadmium concentration in shoot, root and corn in fifteen maize cultivars in the 12<sup>th</sup> weeks.

		Shoot	Root	Corn
	Cultivars	mg kg <sup>-1</sup>		
Group 1	華珍	3.85(0.88) <sup>†</sup>	14.75(0.83)	0.89(0.14)
	雪珍	6.00(0.31)	25.50(8.36)	1.22(0.06)
	夏蜜	4.36(0.10)	45.72(20.90)	0.98(0.07)
	SC-132	5.05(0.08)	19.30(2.01)	0.97(0.14)
	台 南26號	3.62(0.12)	14.18(1.41)	1.50(0.30)
Group 2	玉美珍	3.07(0.36)	50.27(9.15)	0.40(0.02)
	黑美珍	1.34(0.45)	51.48(0.18)	0.27(0.07)
	台 南22號	5.03(0.41)	32.27(1.60)	0.61(0.05)
	台南23號	3.19(0.19)	35.48(2.04)	0.46(0.05)
	台南25號	1.72(0.12)	20.55(0.86)	0.19(0.001)
Group 3	明豐3號	3.57(0.39)	16.64(3.50)	0.66(0.004)
	農興688	2.35(0.23)	19.97(0.66)	0.46(0.06)
	台農1號	4.61(0.38)	57.49(5.39)	0.93(0.22)
	台 南20號	2.20(0.18)	23.38(3.30)	0.23(0.05)
	台南24號	3.76(0.39)	23.03(2.07)	0.32(0.10)
ANOVA for	significance of			
Group		$(G1 > G2 = G3)^{***}$	ns	(G1 > G2 = G3)***
Cultivar	G1	(雪>132>夏>華=26)***	(雪>132>夏>華=26)***	(26>雪>華=夏=132)**
	G2	(22 > 玉 = 23 > 25 > 黒)***	(黒>玉=22=23>25)***	(22 > 玉 = 23 > 黒 = 25)**
	G3	(台>明=24>688=20)***	(台 >明=20=24 >688)***	(台>明>688=24>20)**
† Values i	n the parenthese	s are standard erro $(n = 3)$ .		
ns not sig	nificant			
*** p<0.0	01; ** p<0.01			
-				

Table 3 Cadmium accumulation in shoot and root in fifteen maize cultivars in the 12<sup>th</sup> weeks. Shoot Root

	Cultivars	μg plant <sup>-1</sup>	
Group 1	華珍	140.75 (33.00) <sup>†</sup>	73.11 (4.13)
<u>^</u>	雪珍	266.89 (4.12)	232.64 (72.67)
	夏蜜	215.55 (12.42)	847.40 (33.76)
	SC-132	192.28 (18.18)	62.39 (19.65)
	台 南26號	189.02 (23.49)	85.98 (13.03)
Group 2	玉美珍	137.24 (7.47)	78.97 (21.39)
	黑美珍	69.54 (25.08)	307.38 (44.87)
	台南22號	242.32 (26.81)	165.01 (65.34)
	台南23號	149.90 (13.94)	146.90 (19.10)
	台南25號	99.69 (3.19)	106.57 (10.42)
Group 3	明 豐3號	217.64 (5.30)	211.80 (45.02)
	農興688	105.16 (22.29)	138.88 (28.83)
	台農1號	262.19 (33.74)	552.26 (48.67)
	台 南20號	175.69 (17.05)	203.57 (30.65)
	台南24號	266.30 (46.66)	280.19 (50.50)
ANOVA fo	r significance of		
Group		(G1 = G3 > G2)***	$(G1 = G3 > G2)^*$
Cultivar	G1	(雪>132=夏=26>華)***	(夏>雪>華=132=26)***
	G2	(22 > 5 = 23 > 92 = 25)***	(型> 22> チ = 23>25)***

G2	(22 > 玉 = 23 > 黒 = 25)***	(黒> 22 > 玉 = 23 > 25)***
G3	(24 = 台 > 明 > 20 > 688)***	(台 > 明 = 20 = 24 > 688)***

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# 土壤有效性鉛的空間分佈與土壤性質之關係

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#### 摘要

土壤中重金屬的空間分佈通常是不均匀,而且濃度常有很大的差異。然而在 稻田土壤中重金屬持續積累對水稻生產上的風險。研究田區位於台灣中部,面積 約0.2 公頃。共採集 24 個點的土壤樣本,測定土壤 pH 值、有機質、砂粒、黏粒、 有效性鉛和 DCB 法萃取的鉛、錳、鐵等理化性質。我們發現有效性鉛可能因為表 土和底土中不同的土壤性質造成鉛固定有些微的不同。

#### 前言

土壤污染物的空間分佈是界定與評估汙染範圍的重要依據。由於重金屬會受土 壤中黏土、有機物、氧化物等物質所吸附或螯合,因此重金屬在土壤中的移動是 有限的。而土壤中重金屬的空間分佈通常是不均匀的,而且濃度常有很大的差異。 然而在稻田土壤中重金屬持續積累對水稻生產上的風險已經日益受到關注。雖然 植物吸收的鉛(Pb)通常較低,但也發現植物體內鉛濃度會隨土壤鉛有效性的增加而 提升。因此,應考慮土壤鉛有效性,進而評估水稻種植的安全性。而先前研究顯 示土壤性質可能影響鉛有效性。本研究的目的是調查在污染土壤中有效性鉛的空 間變化與土壤性質間的相關性。

#### 材料與方法

研究區位於台灣中部,面積約 0.2 公頃。試驗區的鉛來源是經由化工廠所排放 所造成。在研究田區中共選 24 個點採集土壤樣本(Fig. 1),分別在 0-15 公分與 15-30 公分的深度採集表土和底土樣品。每個土壤樣品經乾燥、研磨並過 10 mesh 篩網。 接著對每個土壤樣本測定 pH 值、有機質 (Organic Matter, OM)、砂粒、黏粒、有 效性鉛 (0.1 N 鹽酸萃取)和次亞硫酸鈉-檸檬酸鈉-碳酸氫鈉法 (Sodium Dithionite-Citrate- Bicarbonate, 簡稱 DCB 法) 萃取的鉛、錳、鐵以評估其在田區 中的空間分佈的變化。

#### 結果討論

在 Fig. 1 中顯示有效性鉛在表土的空間分佈了極大的變化。鉛濃度從田區的西 南方急劇遞減到田區的東北方。經由統計分析表土中的有效性鉛與土壤性質我們 發現有效性鉛的變化(即 CV)比其它性質高得多。有效性鉛約從 75 至 1600 mg kg<sup>-1</sup>(Table 1)。因此,在該田區有些區域種植水稻是有鉛超標的風險。在 Table 2 顯 示底土的性質,發現表土有效性鉛遠大於底土。這表明,鉛從表土向下移動是會 受到限制。此外,我們發現有效性鉛分別與表土和底土中不同土壤性質有顯著相 關(Table 3)。這可能因為不同土壤性質造成鉛固定量有些微的不同。

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Fig. 1. Configuration of sampling locations spatial distribution of Pb availability (Pb<sub>avail</sub>) for topsoil in the study site.

Table 1. Summary statistics of pH, organic matter, sand, clay, available Pb, and DCB-extracted Pb, Mn, and Fe for the topsoil (0-15 cm) samples.

Table 2. Summary statistics of pH, organic matter, sand, clay, available Pb, and dithionite-citrate-bicarbonate extracted Pb, Mn,

and Fe for the subsoil (15-30 cm) samples.			
	and Fe for	the subsoil (15-30	cm) samples.

																-	
	pН	OM <sup>a</sup>	Sand	Clay	Pb <sub>avail</sub> b	DCB <sup>c</sup> Pb	DCB-Mn	DCB-Fe		pН	OM <sup>a</sup>	Sand	Clay	Pb <sub>avail</sub> b	DCB <sup>c</sup> Pb	DCB-Mn	DCB-Fe
			%			mg kg-1		g kg-1				%			mg kg-1		g kg-1
Mean	5.12	1.73	57.0	13.6	313.62	95.10	6.17	5.36	Mean	5.87	0.85	63.0	11.17	30.28	17.11	74.16	12.66
SD	0.30	0.30	5.90	5.21	339.42	61.56	3.79	1.58	SD	0.37	0.24	3.17	1.55	36.00	11.18	84.37	2.29
CV (%)	5.90	17.51	10.35	38.34	127.4	64.74	61.40	29.49	CV (%)	6.24	27.71	5.05	13.89	118.9	65.34	113.8	18.07
Minimum	4.61	1.38	44.0	4.0	74.89	36.52	2.40	2.19	Minimum	5.12	0.55	58.0	8.0	ND <sup>e</sup>	ND	9.07	8.62
Medium	5.04	1.65	56.0	14.0	141.08	65.37	4.99	4.89	Medium	5.99	0.78	64.0	12.0	22.05	16.98	35.30	12.49
Maximum	5.89	2.64	72.0	26.0	1600.2	247.08	15.45	10.00	Maximum	6.37	1.36	68.0	14.0	152.7	37.64	326.2	17.19
Skewness	0.68	2.10	0.43	0.12	2.56	1.01	1.51	1.24	Skewness	-0.65	0.74	-0.20	-0.30	2.30	-0.09	1.82	0.17
$(n=24)^{d}$									$(n = 24)^{d}$								
a Organic matter						a Organic ma b Available F	itter b extract	ed by 0.1 N	HCI								

b Available Pb extracted by 0.1 N HCl c Dithionite-citrate-bicarbonate extraction

d Sample size

Sample siz

c Dithionite-citrate-bicarbonate extraction

d Sample size e Lower than the detection limit (0.23 mg kg<sup>-1</sup>)

Table 3. Correlation	n coefficients of	of available Pb	between each	other properties	s selected for	the topsoi
and subsoil sample	s.					

	pН	OM <sup>a</sup>	Sand	Clay	DCB-Pb	DCB-Mn	DCB-Fe
Topsoil (0-15 cm)	0.37	-0.21	0.47	-0.37	0.48	0.47	0.52
Sbsoil (15-30 cm)	-0.37	0.42	-0.27	-0.18	0.64	-0.06	-0.22
$(n = 24)^{\circ}$							

a Organic matter

b Dithionite-citrate-bicarbonate extraction

c Sample size

# 水稻植株鉛分配與根圈氧化鐵相關性之探討

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#### 摘要

水稻根圈的氧化作用促使氧化鐵沉澱堆積於根表面,而形成鐵膜(iron plaque), 也可能成為水稻吸收土壤重金屬的屏障。本研究嘗試以根圈袋(rhizo-bag)設計之盆 栽試驗,探討水稻根圈氧化鐵堆積對植株鉛濃度分佈的影響。本研究選用栽培種 水稻「台農 71 (TNG71)」為供試品種,以取自台灣中部受鉛污染農地之土壤,經 鐵添加處理後進行盆栽試驗。結果顯示,鐵添加處理顯著提升氧化鐵在水稻根圈 的堆積量,而且顯著增加鉛的截留量,故植株鉛吸收量也因鐵添加處理而顯著降 低;同時,本研究結果也發現,鉛並非主要被截留於根表面的鐵膜,而是被截留 在根圈土壤表面沉澱堆積的氧化鐵。經本研究結果可推論得知,促進根圈氧化鐵 堆積以截留鉛將可阻隔其向根表面移動,達到降低水稻植株對土壤鉛吸收的作 用。

#### 前言

經前人研究確認,水稻根表上的鐵膜(iron plaque)能做為避免水稻吸收土壤中 重金屬的屏障(Zheng et al., 2012; Yamaguchi et al., 2014),隨著水田土壤受鉛污染的 狀況日益嚴重,水稻根系鐵膜對鉛吸附的影響需要深入探討(Liu et al., 2011), Li et al. (2016)也認為鐵膜對土壤鉛的固定作用同時受水稻品種及土壤性質的影響。本 研究目的試圖使用根圈袋的盆栽試驗,調查種植在鉛污染土壤中的水稻植株鉛分 佈,以及沉澱在根表鐵膜上或根圈中的氧化鐵濃度對鉛阻隔的影響。

#### 材料與方法

本研究以台灣育出的日本型水稻—台農 71(TNG71)做為盆栽實驗的材料,先 前研究中顯示,TNG71 根表有較多的鐵膜形成。土壤採自中台灣受鉛汙染的地區, 實驗處理組合分為二,原始鉛汙染土壤以 0 ppm、750 pm 的氯化鐵處理,另一組 合為額外添加 300 ppm 鉛至供試土壤後,分別加入 0 ppm、750 pm、1500 ppm 氯 化鐵。盆栽設計為以打洞後能使土壤溶液通過的 PE 塑膠杯,與裝有 500 克實驗土 壤的一公升 PVC 塑膠瓶的組合,杯中填入 400 克酸洗過篩(10 mesh)的石英砂後移 植三棵發芽水稻。水稻根會在 PE 杯盛裝的石英砂中生長以模擬根圈,而 PVC 塑 膠瓶中的土則為土體。種植 45 天後採收,水稻地上部及地下部以蒸餾水清洗後分 開,地下部另外以 DCB (dithionite-citrate-bicarbonate)洗去鐵膜。水稻植體以 65 度 烘 72 小時至乾,接著以混和酸(HNO<sub>3</sub>/HClO<sub>4</sub>, 4:1, v:v)分解。每盆的石英砂風乾取 樣,亦以 DCB 洗去氧化鐵及沉澱的鉛。

#### 結果與討論

水稻植體分解液及地下部和石英砂 DCB 萃取液,以 ICP-MS 或 FAAS 測定鐵、 鉛濃度。我們發現沉澱在石英砂上的鐵量(Table 1),會隨著鐵添加的增加而增加。 鉛 300 ppm 添加的土壤中,以 750 ppm 及 1500 ppm 鐵處理的石英砂,其表面的鐵 沉澱量高於根圈。此外,石英砂中的鉛量會隨著鐵添加而增加,但在鐵膜中有相 反的趨勢,表示土壤中鐵濃度的增加會使有效性鉛向根圈移動,並沉澱在水稻根 附近的氧化鐵中,而非根表鐵膜上,且根的鉛量會因鐵添加的增加而減少,此處 說明鉛因添加更多的鐵而氧化沉澱於根圈。在不額外添加鉛的土壤中,以 750 ppm 的氯化鐵處理,會降低水稻地上部的鉛,然而添加 300 ppm 鉛的土壤中,地上部 鉛量並未隨著鐵添加而減少。我們推測,過多的鐵可能會危害水稻秧苗,且當土 壤中有較高的有效性鉛時,會因葉片的蒸散作用造成更多的鉛轉運至地上部。

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Table 1. Profiles of Fe in rhizosphere and root plaque and Pb in rhizosphere, root plaque, and plant for the rice seedlings grown in Pb-spiked soils associated with Fe amendments.

Pb spike	Fe	Fe dist	ribution	Pb distribution						
	amendment	Quartz sand	Plaque	Quartz sand	plaque	Root	Shoot			
m	ıg kg⁻¹			mg	g pot <sup>-1</sup>					
0	0	3.063 (0.001) <sup>†</sup>	28.16 (4.80)	0.013 (0.002)	0.014 (0.002)	0.076 (0.019)	0.024 (0.015)			
	750	4.114 (0.001)	23.03 (2.56)	0.020 (0.002)	0.003 (0.001)	0.027 (0.008)	0.005 (0.001)			
300	0	5.160 (0.002)	19.87 (4.39)	0.024 (0.003)	0.015 (0.005)	0.062 (0.015)	0.016 (0.005)			
	750	23.18 (0.02)	2.415 (0.554)	0.122 (0.024)	0.001 (0.001)	0.047 (0.016)	0.097 (0.020)			
	1500	280.5 (0.13)	2.594 (1.945)	0.463 (0.158)	0.004 (0.001)	0.011 (0.002)	0.114 (0.049)			

† Standard error (n=3)

# 鎘逆境對大豆幼苗吸收鈣、鉀之影響

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#### 摘要

隨著工業發展也造成了環境的汙染,提高了作物食用安全風險,為了解其毒性 反應,本研究目的是了解鎘逆境對於大豆幼苗鈣鉀吸收分布的影響,將大豆幼苗 以4種鎘濃度處理,分別在3種鈣濃度及3種鉀濃度背景下。我們發現鎘逆境顯 著降低大豆幼苗對鈣的吸收,然而鉀吸收在鎘處理下影響不明顯,這可能是因為 大豆作物對於鈣有較高的需求相較於鉀,在大豆幼苗中鈣和鎘可能有吸收競爭的 效應。

#### 前言

當耕地土壤受的鎘(Cd) 汙染常導致鎘積累在作物的可食用部位。為改善作物栽培環境降低糧食食用安全的風險,了解植物對於Cd的毒性反應與吸收機制是有必要的。在一般情況下,鎘是能夠與基本營養元素進行交互作用,從而對植物營養一個顯著影響。鎘離子將與大多數營養元素陽離子(例如:K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>等)競爭同一跨膜轉運的結合位(Yang and Juang, 2015)。由於鈣(Ca)和鉀(K)是兩種對植物細胞具有生長調節的直接作用的營養元素,因此對於鎘處理下植物Ca和K的吸收的釐清是有必要的(Zhang et al., 2002; Dong et al., 2006)。本研究目的是調查不同鎘處理下對於大豆幼苗(Glycine max L.)鈣鉀吸收分布差異之影響。

#### 材料與方法

大豆發芽 84 小時候選擇生長均勻的大豆幼苗,然後移植入 50ml 聚丙烯瓶,裝 滿鎘處理溶液,其中包括不同的鈣(0.25,1, and 20 mM) 和鉀(0.3,1.2, and 9.6 mM) 背景,鎘的處理水平分別為 0 (作為對照), 4, 12 and 16  $\mu$ M。7 天的暴露期後,分別 取大豆幼苗的根和枝條,並用去離子水充分洗滌。然後置入烘箱乾燥 65°C 72 小 時,之後測定幹重。將乾燥的樣品以 HNO<sub>3</sub>/HClO<sub>4</sub> (v:v = 4:1)進行消化,消化液的 鈣、鉀和鎘利用火焰式原子吸收光譜儀 (Thermo scientific iCE 3000)測定。

#### 結果與討論

在鎘處理下,大豆植物的鈣、鉀的分佈狀況有顯著不同。增加鎘處理水平,地 上部及地下部 Ca 濃度明顯降低,在地上部更加明顯(圖一),然而在鎘處理下,大 豆鉀的濃度並沒有顯著的影響(圖二)。鎘逆境下大豆的鈣鉀分佈不同反應可能與大 豆植物鈣鉀的吸收機制和利用效率的差異有關。一般雙子葉植物的 Ca 含量比在單 子葉植物更高;例如:豆類比穀類作物有較高的鈣需求量。因此大豆植物在鎘逆境下 Ca 吸收被抑制比 K 明顯。此外,植物鉀和鈣分布受到鎘濃度的影響,大豆幼苗中 鈣和鎘之間可能有顯著的吸收競爭的效應但鎘和鉀之間則不明顯。

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圖一、大豆幼苗地下部地上部鈣濃度暴露於 Cd 溶液(0, 4, 8 and 12  $\mu$ M)在不同 Ca 背景下(0.25, 1, and 20 mM)。鎘處理間的差異由不同小寫字母表示由 Duncan's test (p<0.05); 鈣處理間的差異由不同大寫字母表示由 Duncan's test (p<0.05)。誤差槓= 標準差(n = 3)。



圖二、大豆幼苗地下部地上部鉀濃度暴露於 Cd 溶液 $(0, 4, 8 \text{ and } 12 \mu M)$ 在不同 K 背景下(0.3, 1.2, and 9.6 mM)。鎘處理間的差異由不同小寫字母表示由 Duncan's test (p<0.05); 鉀處理間的差異由不同大寫字母表示由 Duncan's test (p<0.05)。誤差槓=標準差(n=3)。

# Co-precipitation of dissolved organic matter with Fe(III) and Al

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### Abstract

Soils consist of minerals and organic matters as well as porous phase that contain gases and water. A portion of organic matters are soluble, known as dissolved organic matter (DOM), which can be transferred along with rainfall into subsoil/groundwater or through a surface runoff to water bodies. The DOM, bearing many oxygen-containing groups, is capable of modifying the surfaces of soil minerals (e.g., Fe, Al hydroxides) or even forming stable co-precipitates with Fe/Al ions in a slight acidic solution. Besides, the DOM/Fe/Al co-precipitate, a nanoparticle, may serve as a carrier for nutrients or environmental pollutants while being transferred through the soil pores with water. Therefore, it is vital important to understand the formations and stabilities of DOM/Fe or DOM/Al co-precipitates in the soils; however, the structures and the reactivities of C/Fe+Al co-precipitates receive less scientific attentions. In this study, we used DOM (8.33 mM C) obtained from Changhua peat soil to coprecipitate with Fe(III) or/and Al(III) (0.25~1 mM), and the structural changes of C/Fe(III) co-precipitates with different Fe/(Fe+Al) molar ratios of 0.25, 0.50, 0.75, and 1 at pH 3 and 6 were systematically examined using spectroscopy techniques (XRD, FTIR, XPS and XAS). The preliminary results show XRD studies indicated that an amorphous C/metal co-precipitate was produced at pH 3 indifferent to the presence of Al(III). However, a discernible two-line ferrihydrite was formed with C and initial Fe/(Fe+Al) molar ratios of 0.25, 0.50, 0.75, and 1 when the solution pH was adjusted to 6. FTIR results indicated that carbohydrates, C=O bond of carboxyl groups disappeared on all C/Fe+Al coprecipitates; nonetheless, the peak intensity decreased with a decrease in Fe/Al ratios. XPS results suggested that two different structures of C/metal co-precipitates may be formed in the DOM/Fe(III)/Al systems: (1) Al ions associated with DOM, which was attached on the surfaces of central cores, mainly dominated by Fe hydroxides, and the size of central Fe domain in the C/metal co-precipitates increased with an increase in Fe/(Fe+Al) molar ratio and pH 6 ; (2) DOM complexed with Fe(III)/Al(III), which was distributed evenly on the surfaces of co-precipitates, formed at pH 6 with a Fe/(Fe+Al) molar ratio of 0.25, and at pH 3 for all samples. Based on the EXAFS results of DOM/Fe(III)/Al systems, the coordination environments of Fe in the DOM/Fe(III)/Al co-precipitates changed along with different Fe/(Fe+Al) molar ratios. We found that Fe

coordination number of Fe-C bond (2.98 Å) and Fe-Fe (corner-sharing) bond (3.05 Å) gradually decreased with an decrease in Fe/(Fe+Al) ratios when the co-precipitates were produced at pH 6. We also found no Al substitution for all C/Fe+Al samples. Further study is required to clarify the effects of Al or the compositions of DOM on the structures and coordination environments of Fe in the C/Fe+Al co-precipitates. *Keywords – DOM, Fe(III), Al, coprecipitate* 

# **Experiments**



# Results

Figure 1. XRD patterns for CHA co-precipitated with different Fe(III)/Al(III) concentrations (C/Fe+Al=8.3) at pH 6.

Figure 2. FTIR spectras for CHA co-precipitated with different Fe(III)/Al concentrations (C/Fe+Al=8.3) at pH 6.

Figure 3. Magnitude of Fourier transformed Fe K-edge  $k^3 \chi(k)$  data for CHA co-precipitated with different Fe(III)/Al(III) concentrations (C/Fe+Al=8.3) at pH 6.



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# 施用生質炭對土壤特性及青江菜生產之影響

# The effect of applying biochar on soil characteristics and production of bok choy

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#### 摘要

生質炭施用於農地後常有提高作物品質、產量、改良土壤物化性質、降低溫 室氣體排放及增進土壤碳匯等效益。本試驗即探討炭化油棕果串與炭化棧板木屑, 進行施用 5%、20%及 40%用量混合於砂質和黏質土壤時,對土壤 pH、EC 及青江 菜生長之影響。試驗結果顯示,炭化資材的施用能提高土壤 pH,且隨著用量增加 而提升,其中以黏土較為明顯;土壤 EC 方面則以炭化油棕果串的施用提升最多。 所有處理組合以炭化棧板木屑 40%施用於黏土之青江菜產量最高;炭化油棕果串 則以 5%施用於黏土之產量最佳,僅次於黏土/炭化棧板木屑 40%處理者,其餘炭 化油棕果串處理組合大多全數凋亡,凋亡植株之土壤 EC 皆超過 2 mS/cm。

關鍵詞:生質炭、炭化油棕果串、炭化棧板木屑

#### 前言

生質炭(biochar)為藉由限制氧氣環境下,將有機物質以相對低溫裂解形成之 炭化資材(Lehmann and Joseph, 2009),其不易被分解,可作為捕獲或儲存二氧化碳 之方式之一。為了解不同生質炭量的添加對於作物生長之反應,本研究進行不同 生質炭不同施用量對土壤 pH、EC 及青江菜生長之影響調查。

#### 材料與方法

試驗使用黏質土與砂質土,添加的資材包含炭化、未炭化油棕果串與棧板木 屑共4種資材(表1),並以重量比0%、5%、20%、40%用量與土壤混合,另外加 砂質土/2%、4%炭化油棕果串與黏質土/10%、15%炭化油棕果串4種組合,每種處 理進行4重複,後種植青江菜,每盆種植3株。

## 結果與討論

施用生質炭可提升土壤 pH 與 EC,且隨著用量增加而提高。生質炭對於提升 黏土之 pH 效果較為顯著(圖 1a)。炭化油棕果串的 EC 高達 12 mS/cm,施用 5%於 砂質土時,土壤 EC 即已達 2 mS/cm,青江菜已受鹽害;施用 40%於砂質土,土壤 EC 更達 12 mS/cm (圖 1b),青江菜已呈現凋亡。此結果顯示栽培青江菜之土壤 EC 應控制在 2 mS/cm 以下。產量方面,所有處理組合以炭化棧板木屑 40%用量施於 黏土之產量最高,與黏土對照組相較為 318%;炭化油棕果串 5%用量施於黏土之 產量僅次於黏土/炭化棧板木屑 40%,其產量為對照組的 270% (表 2)。

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表 1. 生質炭與未炭化資材之特性與營養元素含量

	pH <sub>1:10</sub>	EC <sub>1:10</sub>	CEC	OM	С	Ν	Р	K	Ca	Mg	
		μS/cm	cmole/ kg	%			mg/kg				
炭化油棕果串	10.6	12013	41.5	65.2	56.4	1.80	4074	62933	14990	5015	
炭化棧板木屑	8.4	411	10.7	94.5	66.8	0.26	263	3506	4189	1124	
油棕果串	8.6	7350	30.6	76.6	42.0	1.35	1230	24396	4340	2057	
棧板木屑	7.6	608	9.90	97.3	46.3	ND	133	3143	2342	439	

表 2. 施用不同資材與不同施用量對青江菜增產效益(盆栽試驗)

		砂土		_		黏土			
	炭化	炭化	棧板	油棕	-	炭化	炭化	棧板	油棕
	栈板木屑	油棕果串	木屑	果串		栈板木屑	油棕果串	木屑	果串
0%	100	100	100	100	0%	100	100	100	100
2%		140			5%	106	270	166	144
4%		83			10%		96		
5%	85 <sup>a</sup>	5	57	43	15%		17		
20%	120	0	35	16	20%	229	0	71	53
40%	101	0	16	8	40%	318	0	37	39

<sup>a</sup> 數值之表示為相較同種不施用任何資材之土壤對照組作基準之產量百分比(%)。





圖 1. 青江菜種植前盆栽土壤 (a) pH (b) EC。(WB:炭化棧板木屑;FB:炭化油棕果 串;W:棧板木屑;F:油棕果串)

# Comparison of K speciation by traditional method and modified step of traditional method

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#### Abstract

Potassium (K) is an essential element for plant growth. The estimation available K is one of the most common practices in the agriculture field. The traditional method is the popular use for estimation amount of various K speciation for potash fertilizer recommendation. However, this method is many steps process for estimation various K speciation. This study investigated to 1) compare the amount of K speciation in soil by traditional method and modified step of the traditional method. 2) determine the content of various K speciation in different management agriculture soil and forest soil. The tropical soil in Taiwan from farming without K fertilizer (10 years without applied K fertilizer), long term (more than 10 years) intensive applied K fertilizer (168-240 kg K2O ha-1 year-1), red soil (450-500 kg K2O ha-1 year-1) and forest soil were selected. The results showed that the concentration of various K speciations extracted by traditional method and modified step of the traditional method were not difference. In applied K fertilizer soils (long term intensive applied K fertilizer and red soil) were higher K concentration than without K fertilizer soils (long term without applied K fertilizer soil and forest soil). This indicated that the K fertilizer can be built up the concentration of various K in soil.

Keywords : Potassium, Soil fertility, Agriculture, Fertilizer