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Influence des propriétés physiques et chimiques du sol et de leur variation saisonnière sur l'occurrence et la distribution de *Burkholderia pseudomallei* dans une rizière au centre du Laos.

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Abstract

Burkholderia pseudomallei (BP) is a pathogenic bacterium commonly found in soils of Southeast Asia and Northern Australia, causing melioidosis, a severe tropical disease. Infection routes of *BP* include direct inoculation from surface waters and soil, through lesions, to which agricultural workers in developing countries are particularly exposed, but also ingestion and inhalation of contaminated water and aerosols. Soil physico-chemistry, microbiology, as well as climate, especially rainfall, are thought to control the occurrence and dissemination of *BP* in the environment, but the ecology of this pathogen remains poorly understood. Our study site is located in Vientiane Province, central Lao PDR, the main region of *BP* endemicity. At odds with the guidelines of the Detection of Environmental *BP* Working Party, which recommends searching at a soil depth of 30 cm, previous investigation at this site showed *BP* to be abundant year-round at depths of 60 and 90 cm. Therefore, one of the objectives of this thesis was to confirm and clarify the causes of the persistence of *BP* in deep soil horizons. Further, we aimed to explore putative links between soil properties and *BP* concentrations, at different scales, and their variability over time.

We applied a multi-scale approach consisting of: (1) a snapshot study along a 300 cm deep soil profile to accurately characterize the distribution of *BP* in relation with soil physical and chemical variability; (2) a monthly monitoring of the 300 cm deep soil profile to highlight seasonal variations in the distribution of *BP*; and (3) a characterisation of soil redoximorphic features in relation to the occurrence of *BP*, to explore effects of potential drivers such as contrasted biogeochemical conditions and substrate availability.

While *BP* occurred at all soil depths from surface to 300 cm, *BP* concentrations spanned over four orders of magnitude along a single profile, unexpectedly reaching peak concentrations between depths of 100 and 200 cm. *BP* persistence correlated with the duration during which soil layers were saturated with water. As a direct consequence of this result, seasonal variations in *BP* concentrations were more pronounced in shallow than in deeper soil horizons. Peaks of *BP* concentrations did not co-occur with rainfall events and periods during which the water table was the shallowest, suggesting that processes other than a simple "piston effect" uplifting *BP* populations along the profile are at work. The distribution of *BP* in soil can vary drastically over distances of a few millimeters only and concentrations of *BP* appear to vary depending on the oxidation state of iron, which may be linked to *BP*'s ability to switch metabolisms at very local scales.

Our results show the importance of considering many aspects of the actual complexity of soil to better understand the environmental conditions favourable to *BP* persistence and proliferation, including soil chemical and textural characteristics, and hydrodynamic properties, its structural features nested over a wide range of scales and its general arrangement in layers. As it sheds new light on *BP* vertical distribution along a soil profile, this work also identifies new potential hazards related to human activities that involve interactions with deep soil layers. Our key recommendations regarding the environmental sampling of *BP* include: collecting samples at successive depth increments along a single soil profile as it maximizes the odds of detecting *BP* at a given location compared to the same number of samples taken at a single soil depth; collecting samples to the depth at which the water table is found at the time of sampling, as groundwater persistence appears to be an important factor for the persistence of *BP*; and recording basic pedological

information such as soil color and macrostructural features, as these simple observations provide valuable information about the redox conditions at the point of sampling.

Résumé

Burkholderia pseudomallei (*BP*), bactérie pathogène répandue dans les sols d'Asie du Sud-Est et d'Australie septentrionale, provoque la mélioïdose, infection tropicale grave. L'infection par *BP* suit l'inoculation par contact direct de lésions avec les eaux de surface et le sol, exposant les travailleurs agricoles des pays en développement, mais aussi l'ingestion et l'inhalation d'eau et d'aérosols contaminés. Divers facteurs édaphiques et climatiques contrôlent probablement la présence et la dissémination de *BP* dans l'environnement, mais son écologie reste mal connue. Le site d'étude choisi, est situé dans la Province de Vientiane, région centrale de la RDP Lao, principale région d'endémicité de *BP* ; des recherches précédentes ont révélé que *BP* y était très abondante toute l'année à des profondeurs de 60 et 90 cm (contrastant avec la recommandation du groupe de travail sur la détection de *BP* dans l'environnement de chercher *BP* à une profondeur de 30 cm). L'un des objectifs de cette thèse a donc été de clarifier les causes de la persistance de *BP* dans les horizons profonds du sol. Par ailleurs, nous avons exploré d'éventuels liens entre propriétés du sol et occurrence de *BP* à différentes échelles, et leur variabilité dans le temps.

Nous avons adopté une approche multi-échelle consistant en: (1) une étude instantanée le long d'un profil de sol de 300 cm de profondeur pour caractériser finement la distribution de *BP* en lien avec la variabilité physique et chimique du sol ; (2) un suivi mensuel de ce profil pour mettre en évidence les variations saisonnières de la distribution de *BP*; et (3) une caractérisation des traits redoximorphiques du sol en relation avec l'occurrence *BP*,

afin d’explorer les effets de conditions biogéochimiques contrastées et de la disponibilité du substrat.

Nous avons détecté *BP* à toutes les profondeurs du sol jusqu’à 300 cm, bien qu’à des concentrations couvrant plus de quatre ordres de grandeur, avec des pics entre les profondeurs de 100 et 200 cm. La persistance de *BP* apparaît corrélée à la durée pendant laquelle ces horizons sont saturés en eau. Conséquence directe de ce résultat, les variations saisonnières des concentrations de *BP* sont plus prononcées dans les horizons superficiels que dans les horizons de sol plus profonds. Les pics de concentration de *BP* ne coïncident pas avec les événements pluvieux et les périodes pendant lesquelles la nappe phréatique était moins profonde, suggérant qu'un simple « effet piston » faisant remonter *BP* le long du profil n’est pas le seul processus déterminant la distribution du pathogène dans le sol. La distribution de *BP* apparaît très variable à l’échelle millimétrique, en fonction de l'état d'oxydation du fer, ce qui reflète peut-être une plasticité métabolique *BP* à des échelles très locales.

Cette thèse démontre l'intérêt de considérer la complexité du sol pour comprendre les conditions environnementales favorables à *BP*, en particulier ses caractéristiques chimiques et texturales, ses propriétés hydrodynamiques, ses caractéristiques multi-échelles et son arrangement en horizons dont la séquence forme le profil. En apportant un nouvel éclairage sur la distribution verticale de *BP*, ce travail identifie de nouveaux risques liés aux activités humaines qui impliquent des interactions avec les horizons pédologiques profonds. Concernant l'échantillonnage environnemental de *BP*, ce travail souligne l’importance de : prélever des échantillons à des profondeurs successives le long d'un même profil pédologique car cela maximise, à nombre d'échantillons égal, les chances de

détecter *BP* par rapport au prélèvement de réplicats à un seul niveau ; prélever jusqu'à la profondeur de la nappe phréatique car la persistance des eaux souterraines semble contrôler la persistance de *BP* ; et enregistrer des informations pédologiques simples (couleur, caractéristiques macrostructurales), ces observations fournissant des éléments sur les conditions redox au point d'échantillonnage.

ບົດຄັດຫຍໍ້

Burkholderia pseudomallei (*BP*) ເປັນເຊື້ອແບັກທີເຣັຍທີ່ມີເຊື້ອພະຍາດສູງ ທີ່ເຮັດໃຫ້ເກີດພະຍາດເມ ລີອອຍ (melioidosis) ໃນພື້ນທີ່ເຂດຮ້ອນ, ພົບທົ່ວໄປໃນດິນ, ໂດຍສະເພາະໃນອາຊີຕາເວັນອອກສຽງໃຕ້ ແລະ ພາກເໜືອຂອງອົດສະຕາລີ. ການຕິດເຊື້ອຂອງ *BP* ສາມາດຮັບເຊື້ອໂດຍກົງຈາກທາງນ້ຳຫນ້າດິນ ແລະ ດິນ, ຜ່ານຮອຍບາດແຜມເທິງຜິວໜັງ, ການດື່ມນ້ຳ ແລະ ການສູດດົມລະອອງນ້ຳ ແລະ ອາກາດທີ່ປົນ ເປື້ອນເຊື້ອພະຍາດຊະນິດນີ້ ໂດຍສະເພາະແມ່ນກຸ່ມຊາວກະສິກອນ ຢູ່ໃນປະເທດທີ່ກຳລັງພັດທະນາ. ປັດໃຈ ຕ່າງໆໃນດິນບໍ່ວ່າຈະເປັນ ທາງກາຍະພາບ - ເຄມີ ແລະ ຈຸລິນຊີວິທະຍາ, ລວມທັງສະພາບອາກາດ ເຊັ່ນ: ນ້ຳ ຝົນ ທີ່ໄດ້ຖືກລາຍງານວ່າເປັນປັດໄຈທີ່ຄວບຄຸມການເກີດຂຶ້ນ ແລະ ການແຜ່ກະຈາຍຂອງ *BP* ໃນ ສິ່ງແວດລ້ອມ, ແຕ່ຄວາມຮູ້ກ່ຽວກັບລະບົບນິເວດຂອງເຊື້ອພະຍາດນີ້ຍັງມີຂອບເຂດຈຳກັດ. ສຳລັບການສຶກ ສາຄັ້ງນີ້, ພວກເຮົາໄດ້ສຸມໃສ່ສະຖານທີ່ສຶກສາທີ່ຕັ້ງຢູ່ໃນແຂວງວຽງຈັນ, ພາກກາງຂອງ ສປປ ລາວ, ເປັນພື້ນ ທີ່ ທີ່ມີການລາຍງານກໍລະນີເກີດພະຍາດ *BP* ຈຳນວນຫລາຍ. ກົງກັນຂ້າມກັບຂໍ້ແນະນຳຂອງຄະນະກວດກາ ສິ່ງແວດລ້ອມ Detection of Environmental *BP* Working Party ທີ່ແນະນຳໃຫ້ຊອກຫາ *BP* ໃນ ຕົວຢ່າງດິນ ໃນຄວາມເລິກຂອງດິນ 30 cm, ແຕ່ການສຳຫຼວດກ່ອນໜ້ານີ້ຢູ່ສະຖານທີ່ສຶກສາຄົ້ນຄວ້ານີ້ ພົບ ວ່າມີເຊື້ອພະຍາດຊະນິດນີ້ເປັນຈຳນວນຫຼາຍຕະຫຼອດປີ ໃນຄວາມເລິກຂອງຊັ້ນ 60 ແລະ 90 cm. ດັ່ງນັ້ນ, ໜຶ່ງໃນຈຸດປະສົງຂອງວິທະຍານິພົນນີ້ແມ່ນເພື່ອຢືນຢັນ ແລະ ພະຍາຍາມຊີ້ແຈງສາເຫດຂອງການຄົງຕົວຂອງ *BP* ໃນຂອບເຂດດິນເລິກ. ນອກຈາກນັ້ນ, ຈຸດປະສົງຫຼັກຂອງວຽກງານນີ້ແມ່ນເພື່ອຄົ້ນຫາການເຊື່ອມໂຍງ ລະຫວ່າງຄຸນສົມບັດຂອງດິນໃນຂອບເຂດທີ່ແຕກຕ່າງກັນ ແລະ ການປະກົດຕົວຂອງເຊື້ອພະຍາດຕາມໄລຍະ ເວລາທີ່ປ່ຽນແປງ.

ຕໍ່ກັບບັນຫານີ້, ຈຶ່ງໄດ້ມີການສຶກສາຫຼາຍຮູບແບບປະສົມປະສານກັນ ປະກອບມີ: (1) ການສຶກສາແບບຖ່າຍ ພາບຕາມລວງຕັ້ງຂອງດິນທີ່ມີຄວາມເລິກ 300 cm ເພື່ອສຶກສາລາຍລະອຽດການແຜ່ກະຈາຍຂອງ *BP* ທີ່ໄດ້ ພິຈາລະນາປັດໄຈກ່ຽວກັບຄວາມແຕກຕ່າງກັນທາງກາຍະພາບ ແລະ ສານເຄມີພາຍໃນດິນ; (2) ຕິດຕາມແຕ່ ລະຊັ້ນດິນເລິກ 300 cm ປະຈຳທຸກເດືອນ ເພື່ອຊີ້ໃຫ້ເຫັນການປະກົດຕົວຂອງ *BP* ຕາມການປ່ຽນແປງຂອງ ລະດູການ; ແລະ (3) ການກຳນົດລັກສະນະຣິດ໋ອກຊີມ໋ອກຟິກ (redoximorphic) ຂອງດິນທີ່ກ່ຽວຂ້ອງກັບ

ການປະກົດຕົວຂອງເຊື້ອພະຍາດ *BP*, ເພື່ອຊອກຫາຜົນກະທົບທີ່ເປັນຕົວຂັບເຄື່ອນ, ເປັນທ່າແຮງໃຫ້ເກີດການແຜ່ກະຈາຍພະຍາດດັ່ງກ່າວເຊັ່ນ: ສະພາບທາງຊີວະເຄມີ ທີ່ມີລັກສະນະກົງກັນຂ້າມກັນ ແລະ ທາດຕ່າງໆທີ່ປະກົດຢູ່ໃນດິນ.

ຜົນການສຶກສານີ້, ພວກເຮົາພົບເຫັນວ່າເຊື້ອ *BP* ປະກົດຕົວໃນທຸກຊັ້ນດິນ ຈາກຫນ້າດິນເຖິງ 300 cm, ປະລິມານສະສົມຂອງເຊື້ອ *BP* ແມ່ນແຕກຕ່າງກັນຢ່າງຫຼວງຫຼາຍ. ປະລິມານສະສົມສູງສຸດແມ່ນພົບຢູ່ຄວາມເລິກ ລະຫວ່າງ 100 ແລະ 200 cm. ການປະຕົວຂອງເຊື້ອ *BP* ໃນຊັ້ນດິນມີຄວາມສໍາພັນກັບຄວາມຕໍ່ເນື່ອງຂອງໄລຍະເວລາທີ່ຊັ້ນດິນອີ່ມຕົວດ້ວຍນ້ໍາ (ອັດຕາການຄົງຕົວຂອງນ້ໍາໃຕ້ດິນ). ດັ່ງນັ້ນ, ການປ່ຽນແປງຕາມລະດູການມີຜົນຕໍ່ກັບປະລິມານສະສົມຂອງ *BP* ເຊິ່ງເຫັນໄດ້ຢ່າງຊັດເຈນຢູ່ໃນຂອບເຂດທີ່ໄກ້ຄຽງກັບຊັ້ນດິນຕື້ນ ຫລາຍກວ່າຊັ້ນດິນເລິກ (ເຊິ່ງຍັງສອດຄ່ອງກັບຄວາມແຕກຕ່າງຂອງໂຄງສ້າງດິນລະຫວ່າງຂອບເຂດດິນຕື້ນ ແລະ ເລິກ). ລະດັບປະລິມານສະສົມສູງສຸດຂອງ *BP* ບໍ່ມີຄວາມສໍາພັນກັບເຫດການຝົນຕົກ ແລະ ລະດັບນ້ໍານ້ໍາດິນທີ່ໄກ້ກັບໜ້າດິນທີ່ສຸດ, ຜົນການສຶກສານີ້ ສະແດງໃຫ້ເຫັນວ່າຍັງມີຂະບວນການອື່ນທີ່ຊັບຊ້ອນ ນອກເຫນືອຈາກກົນໄກທີ່ງ່າຍດາຍເຊັ່ນການເຄື່ອນຍ້າຍປະຊາກອນຂອງເຊື້ອພະຍາດອອກຈາກຊັ້ນດິນທີ່ເລິກກວ່າໃຫ້ຂຶ້ນມາຢູ່ໃກ້ກັບຊັ້ນຫນ້າດິນໂດຍການເຄື່ອນຕົວຂອງລະດັບນ້ໍາໃຕ້ດິນ. ການແຜ່ກະຈາຍຂອງ *BP* ໃນດິນມີຄວາມແຕກຕ່າງກັນຢ່າງຫຼວງຫຼາຍໃນໄລຍະຫ່າງພຽງລະດັບມິນລິແມັດເທົ່ານັ້ນ. ນອກຈາກນັ້ນ, ການສຶກສາຢູ່ໃນຂອບເຂດດິນຂະຫນາດນ້ອຍລະດັບມິນລິແມັດ ແລະ ຄວາມເຂັ້ມຂຸ້ນຂອງ *BP* ອາດແຕກຕ່າງກັນຂຶ້ນສະພາບອ່ອກຊິເດຊັນຂອງທາດເຫຼັກ, ເຊິ່ງອາດຈະກ່ຽວຂ້ອງກັບຄວາມສາມາດຂອງ *BP* ໃນການປ່ຽນປ່ຽງລະບົບການເຜົາຜານອາຫານ (metabolism) ໃນພື້ນທີ່ສະເພາະ.

ຜົນໄດ້ຮັບທີ່ນໍາສະເໜີຄັ້ງນີ້ ສະແດງໃຫ້ເຫັນເຖິງຄວາມສໍາຄັນໃນການພິຈາລະນາຫຼາຍດ້ານຂອງອົງປະກອບທີ່ຊັບຊ້ອນຕົວຈິງຂອງດິນ ເພື່ອຄວາມເຂົ້າໃຈທີ່ຈະແຈ້ງກ່ຽວກັບ ສະພາບສິ່ງແວດລ້ອມທີ່ເອື້ອອໍານວຍໃຫ້ແກ່ການປະກົດຕົວ ແລະ ຂະຫຍາຍຕົວຂອງ *BP* ເຊິ່ງປະກອບມີຄຸນລັກສະນະທາງເຄມີ, ໂຄງສ້າງຂອງດິນ ແລະ ຄຸນສົມບັດທາງກົນລະສາດຂອງໄຫຼ (hydrodynamic), ລັກສະນະໂຄງສ້າງຕາມແຕ່ລະມາດຕາສ່ວນທີ່ແຕກຕ່າງກັນ ລວມເຖິງການຈັດລຽງທົ່ວໄປຢູ່ໃນຊັ້ນດິນ, ລໍາດັບຂອງໂຄງສ້າງຂອງດິນ. ການສຶກສານີ້ແມ່ນສະແດງໃຫ້ເຫັນການຄົ້ນພົບອັນໃໝ່ກ່ຽວກັບວິທີການແຜ່ກະຈາຍຂອງເຊື້ອ *BP* ທີ່ແຈກຢາຍຕາມແນວຕັ້ງ

ພາຍໃນຊັ້ນດິນເລິກ. ນອກນັ້ນ, ຍັງຊີ້ໃຫ້ເຫັນ ແນວໂນ້ມຄວາມເປັນອັນຕະລາຍ ທີ່ກ່ຽວຂ້ອງກັບກິດຈະກຳ ຂອງມະນຸດ ທີ່ມີການພົວພັນກັບຊັ້ນດິນເລິກ. ຄຳແນະນຳທີ່ສຳຄັນຂອງຈາກການສຶກສາຄັ້ງນີ້ ແມ່ນກ່ຽວກັບ ການເກັບຕົວຢ່າງດ້ານສິ່ງແວດລ້ອມຂອງ *BP* ປະກອບມີ: ການເກັບຕົວຢ່າງນຶ່ງຄັ້ງຕາມຄວາມເລິກຊັ້ນດິນທີ່ ເພີ່ມຂຶ້ນຢ່າງຕໍ່ເນື່ອງ ຍ້ອນວ່າການປະຕິບັດແບບນີ້ເຮັດເພີ່ມໂອກາດຄວາມເປັນໄປໄດ້ສູງສຸດຂອງການກວດ ຫາ *BP* ໃນສະຖານທີ່ໃດໜຶ່ງ ເພື່ອທຽບໃສ່ກັບຈຳນວນຕົວຢ່າງທີ່ເອົາຢູ່ໃນຄວາມເລິກຂອງດິນຄືກັນ; ການ ເກັບຕົວຢ່າງຈົນຮອດລະດັບຄວາມເລິກຂອງນ້ຳໃຕ້ດິນ, ເນື່ອງຈາກວ່າຄວາມຄົງຕົວຂອງນ້ຳໃຕ້ດິນປັດໃຈສຳ ຄັນໃນການຄົງຕົວຂອງ *BP*; ແລະ ບັນທຶກຂໍ້ມູນປັດຕະພິວິທະຍາ (pedological information) ເຊັ່ນ: ສີ ຂອງດິນແລະ ລັກສະນະໂຄງສ້າງມະຫາພາກ, ຍ້ອນວ່າການສັງເກດງ່າຍໆເຫຼົ່ານີ້ສາມາດສະໜອງຂໍ້ມູນທີ່ມີ ຄຸນຄ່າກ່ຽວກັບເງື່ອນໄຂ redox ໃນຈຸດຂອງການເກັບຕົວຢ່າງ.

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List of abbreviations

A horizon	Surface (topsoil) horizon
Al	Aluminum
Aw	Tropical savanna climate
B. pseudomallei /*BP*	*Burkholderia pseudomallei*
BD	Bulk density
B (Brt and Bcr) horizon	Subsoil horizon
C (Cr) horizon	Parent material horizon
Ca_e	Exchangeable calcium
CEC	Cation exchange capacity
CFU/g	Colony Forming Unit gram
CTD	Conductivity, Temperature, and Depth
DALaM	Department of Agricultural Land Management
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
EA	Exchange acidity
EC	Electrical conductivity
Er or E horizon	Eluvium soil horizon
Fe	Iron
GET	Geosciences Environment Toulouse
GPR	Groundwater persistence rate
K	Potassium
K_e	Exchangeable potassium
Ks	Saturated hydraulic conductivity
PAFO	Provincial Administration of Agriculture and Forestry
Poro	Porosity

Lao PDR	Lao People's Democratic Republic
r(RNA)	Ribosomal (Ribonucleic acid)
LOMWRU	Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit
MAF	Ministry of Agriculture and Forestry
Mg_e	Exchangeable magnesium
Mn	Manganese
Na_e	Exchangeable sodium
OM	Organic matter content
ORP	Oxidation-reduction potential
PLS-R	Partial least square regression
PVC	Polyvinyl chloride
Qz	Quartz
S	Sulfur
Si	Silicium
spp	Species
IRD	Institut de recherche pour le développement
TDS	Total dissolved solids
Ti	Titanium
VIP	Variable importance in projection
WC	Water content
XRD	X-ray diffraction

Chapter 1: Introduction

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Le fardeau mondial de la mélioïdose représente un problème majeur de santé publique. Les cas de mélioïdose humaine ont tendance à être liés à la présence de *Burkholderia pseudomallei* (*B. pseudomallei*) dans le sol (Vuddhakul et al., 1999). *B. pseudomallei* est une bactérie intracellulaire facultative Gram-négative, découverte au Myanmar par A. Whitmore et son assistant C. S. Krishnaswami en 1911(Limmathurotsakul et al., 2016; Wiersinga et al., 2018). Cette bactérie est connue comme l'agent causal de la mélioïdose, une infection sévère dans les régions tropicales. *B. pseudomallei* reste un défi sanitaire pour les humains et les animaux (Wiersinga et al., 2018). Les cas rapportés ont augmenté ces dernières années et on estime qu'ils s'élèvent au total à 165 000 cas dans le monde et 89 000 décès par an (Limmathurotsakul et al., 2016). Les voies d'infection de *B. pseudomallei* incluent l'ingestion et l'inhalation d'eau et d'aérosols contaminés ainsi que l'inoculation via contact des lésions avec les eaux de surface et le sol (Wiersinga et al., 2018).

La distribution dans l'environnement de *B. pseudomallei* reste mal connue, notamment dans le sol. Des études récentes rapportent que la présence de cette bactérie est contrôlée par de nombreux facteurs environnementaux tels que les propriétés physico-chimiques et biologiques des sols (Currie and Jacups, 2003; Duangurai et al., 2018; Liu et al., 2015), ainsi que des facteurs climatiques, notamment les précipitations. En effet, lors d'événements pluvieux extrêmes, une détection plus élevée de *B. pseudomallei* a été observée à la fois dans le sol et dans les eaux de surface (Bulterys et al., 2018; Mukhopadhyay et al., 2018; Ong et al., 2017). Certains micronutriments, comme le fer,

qui est suspecté de jouer un rôle important dans l'expression des enzymes de respiration (Tuanyok et al., 2005), sont probablement favorables à la persistance de *B. pseudomallei* dans le sol (Musa et al., 2016). *B. pseudomallei* est connue pour ses facultés d'adaptation à divers stress environnementaux tels que la salinité, le stress oxydatif ou la faible teneur en fer, lui permettant d'occuper diverses niches écologiques, notamment dans le sol (Duangurai et al., 2018; Hantrakun et al., 2016). Il existe potentiellement plusieurs réservoirs environnementaux susceptibles d'offrir des niches pour *B. pseudomallei*. Par exemple, il a été proposé que les plans d'eau stagnante, les sols gorgés d'eau, les plans d'eau salée, la rhizosphère des plantes, la plupart des animaux vertébrés ainsi que l'être humain, constituent autant de niches écologiques pouvant favoriser la persistance de différents génotypes de *B. pseudomallei* (Seng et al., 2019; Yip et al., 2020). La bactérie peut être disséminée à partir de ces niches écologiques sur de grandes distances par le biais de processus hydrologiques (Ribolzi et al., 2016) tels que le ruissellement de surface (Rachlin et al., 2021), augmentant ainsi le risque d'infection humaine, en particulier dans les pays en développement où la population utilise souvent des eaux de surface non traitées pour les besoins domestiques (Boithias et al., 2016). De plus, il existe des éléments controversés indiquant que les amibes peuvent représenter un réservoir dans lequel *B. pseudomallei* serait susceptible de persister (Inglis et al., 2000; Noinarin et al., 2016).

A ce jour, de nombreuses études environnementales ont porté sur la distribution de *B. pseudomallei* dans les eaux de surface et les eaux souterraines, ainsi que dans les sols peu profonds (<90 cm), mais aucune n'a pris en compte l'organisation spatiale des sols ni la variabilité de certaines propriétés du sol dans le temps. Par conséquent, des études complémentaires, en particulier dans les horizons profonds du sol et à l'échelle des unités

pédologiques, sont nécessaires pour suivre la distribution de ce pathogène et son adaptation aux conditions environnementales.

Dans ce contexte, cette étude vise à comprendre la présence et la distribution de *B. pseudomallei* dans le sol des rizières du sud de la province de Vientiane, en République Démocratique Populaire Lao (RDP Lao). Une approche multi-échelle a été adoptée, consistant en : (1) une étude instantanée le long d'un profil de sol de 300 cm de profondeur visant à étudier en détail la distribution de *B. pseudomallei* en relation avec la variabilité physique et chimique du sol, à des profondeurs rarement prises en compte; (2) un suivi mensuel du profil du sol pour mettre en évidence les variations saisonnières de la distribution de *B. pseudomallei* le long du profil du sol à 300 cm de profondeur ; et (3) une caractérisation des traits redoximorphiques du sol en relation avec l'occurrence de l'agent pathogène, afin d'explorer les effets de facteurs tels que des conditions biogéochimiques contrastées et la disponibilité du substrat.

Outre l'introduction, qui fait l'objet de ce premier chapitre, le manuscrit de la thèse comprend quatre autres chapitres, qui couvrent les sujets suivants :

Le chapitre 2 présente un état de l'art basé sur une revue de la littérature sur la mélioïdose et les connaissances actuelles sur *B. pseudomallei*.

Le chapitre 3 donne un aperçu du site de l'étude et de la méthodologie utilisée pour collecter et analyser les données de cette étude.

Le chapitre 4 présente et discute les résultats de l'analyse multi-échelle conduite dans le cadre de ce travail. Ce chapitre comprend :

Les résultats de l’analyse ponctuelle détaillée de la distribution de *B. pseudomallei* en fonction des propriétés physico-chimiques du sol le long du profil de 300 cm de profondeur ; l’évolution de cette distribution de *B. pseudomallei* au cours d'une période continue de 10 mois recouvrant les saisons sèche et humide ; et enfin les observations détaillées à une échelle millimétrique, de la distribution du pathogène en fonction des caractéristiques redoximorphes du sol le long d’un profil de sol de 90 cm de profondeur.

Le chapitre 5 conclut cette thèse en résumant les résultats les plus significatifs et en proposant quelques perspectives pour de futures investigations sur l’écologie de *B. pseudomallei*. Ce dernier chapitre souligne en outre l'importance de considérer de nombreux aspects de la complexité du sol pour mieux comprendre les conditions environnementales favorables à la persistance et à la prolifération de *B. pseudomallei*, un des messages clé de cette recherche.

Chapter 1: Introduction

The global burden of melioidosis worldwide represents a major public health problem, and Human melioidosis cases tend to be related to the presence of *Burkholderia pseudomallei (B. pseudomallei)* in soil (Vuddhakul et al., 1999). *B. pseudomallei* is a Gram-negative facultative intracellular bacterium, found in Myanmar by A. Whitmore and his assistant C. S. Krishnaswami in 1911 (Limmathurotsakul et al., 2016; Wiersinga et al., 2018). This bacterium is known as the causative agent of melioidosis, a severe infection in tropical regions. *B. pseudomallei* remains a health challenge for humans and animals (Wiersinga et al., 2018). Case reports have been increasing in recent years with an estimated 165,000 cases worldwide and to 89,000 death annually (Limmathurotsakul et al., 2016). The infection routes of *B. pseudomallei* include ingestion and inhalation of contaminated water and aerosols as well as inoculation via contact of lesions with surface waters and soil (Wiersinga et al., 2018).

The distribution in the environment of *B. pseudomallei* remains poorly understood, particularly in soil. Recent studies reported the presence of this bacterium under the control of many environmental factors such as physico-chemical properties (Currie and Jacups, 2003; Liu et al., 2015), and biological factors of soil (Duangurai et al., 2018; Tuanyok et al., 2005), as well as climate factors, especially rainfall. Indeed, during extreme rainfall events, a higher detection of *B. pseudomallei* was observed in both soil and surface water (Bulterys et al., 2018; Mukhopadhyay et al., 2018; Ong et al., 2017). Some micro-nutrients, such as iron, which is suspected to play an important role in the expression of respiration enzymes (Tuanyok et al., 2005), are likely favorable to the

persistence of *B. pseudomallei* in soil (Musa et al., 2016). *B. pseudomallei* is known to adapt to various environmental stresses such as salinity, oxidative stress, or low iron content, allowing it to occupy a variety of ecological niches, particularly in soil (Duangurai et al., 2018; Hantrakun et al., 2016). There is potential for several environmental reservoirs to offer niches for *B. pseudomallei*. For example, stagnant water bodies, waterlogged soil, salt water bodies, human and animals, as well as plant rhizosphere, have been proposed as ecological niches that may support the persistence of different genotypes of *B. pseudomallei* (Seng et al., 2019; Yip et al., 2020). The bacterium can disseminate from these ecological niches over large distances through hydrological processes (Ribolzi et al., 2016) such as soil surface runoff (Rachlin et al., 2021), thus increasing the risk of human infection, especially in developing countries where population often uses untreated surface water for domestic needs (Boithias et al., 2016). Additionally there is controversial evidence that amoeba may represent a reservoir in which *B. pseudomallei* can persist (Inglis et al., 2000; Noinarin et al., 2016).

To date, there have been extensive environmental studies dealing with the distribution of *B. pseudomallei* in surface water and groundwater, as well as in shallow soil (<90 cm), but none considered the spatial arrangement of soils and its variation of certain soil properties over time. Hence, further studies in soil, particularly in deep soil horizons and at the scale of pedo-features, are needed to track the distribution of this pathogen and its adaptation to environmental conditions.

In this context, this study aims to understand the presence and distribution of *B. pseudomallei* in the soil of rice paddy fields of southern Vientiane Province, Lao People's Democratic Republic (Lao PDR). A multi-spatial approach is considered, consisting of:

(1) a snapshot study along a 300 cm deep soil profile to investigate in detail the distribution of *B. pseudomallei* in relation with soil physical and chemical variability within soils, at depths rarely taken into account; (2) a monthly monitoring of the soil profile to highlight seasonal variations in the distribution of *B. pseudomallei* along the 300 cm deep soil profile; and (3) a characterisation of soil redoximorphic features in relation to the occurrence of the pathogen, to explore effects of potential drivers such as contrasted biogeochemical conditions and substrate availability.

Aside from the Introduction, which is the subject of this first chapter, the thesis manuscript includes four other chapters, which cover the following topics:

Chapter 2 presents a state of the art based on a review of literature on melioidosis and current knowledge on *B. pseudomallei*.

Chapter 3 provides an overview of the study site and of the methodology used to collect and analyze the data for this study.

Chapter 4 presents and discusses the results of the multi-scale analysis conducted in this work. The chapter includes:

The results of the detailed ad hoc analysis of the distribution of *B. pseudomallei* as a function of soil physico-chemical properties along the 300 cm deep profile;

The evolution of the distribution of *B. pseudomallei* throughout a continuous 10-month period covering both dry and wet seasons; and finally

The detailed observations of soil redoximorphic features at millimeter scale of pathogens according to the redoximorphic characteristics of the soil along 90-cm deep soil profile.

Chapter 5 concludes this thesis with a summary of the most significant results and proposes some perspectives for future investigations on the ecology of *B. pseudomallei*. This last chapter also highlights the importance of considering many aspects of soil complexity to better understand the environmental conditions favorable to the persistence and proliferation of *B. pseudomallei*, one of the key messages of this research.

Chapter 2: State of the art and objectives

Chapter 2 - State of the art and objectives

2.1 Burkholderia genus

Burkholderia was named after Walter Burkholder who found *Pseudomonas cepacia* which caused plant diseases (sour skin of onion) in the 1940s and 1950s (Yabuuchi et al., 1992). The genus of *Burkholderia* is under the genus *Pseudomonas* and divided into five groups based on the DNA-DNA and rRNA-DNA hybridization (Palleroni et al., 1973). When the *Burkholderia* genus was first defined in 1992 it then comprised seven species: *B. caryophylli, B. cepacia, B. gladioli, B. mallei, B. pseudomallei, B. solanacearum*, and *B. pickettii* (Yabuuchi et al., 1992).

Members of the *Burkholderia* genus are known for their ability to use a wide range of organic compounds as carbon sources (Coenye and Vandamme, 2003). At present, there are over 60 species of the *Burkholderia* genus named *Burkholderia* spp. These bacteria are known as zoonotic and plant pathogens (including fungi and insects), found in diverse ecological niches (Vandamme and Dawyndt, 2011) in soil, water, plants, insects, animal, and humans (Coenye and Vandamme, 2003).

Previous studies on *Burkholderia* spp. led to the finding of many novel species; some *Burkholderia* spp. are known to be plant growth promoting agents; other are plant commensals, or can be of use as biocontrol agents or for bioremediation to degrade environmental pollutants (Mahenthiralingam et al., 2005). *Burkholderia* species responsible for pathologies in humans are divided into two groups (Coenye and Vandamme, 2003).

Group 1: *B. pseudomallei and B. mallei* (causative agents of melioidosis and glanders respectively (Wiersinga et al., 2006)).

Group 2: *B. cepacia* complex (Bcc) (Mahenthiralingam et al., 2005), particularly dangerous for patient suffering cystic fibrosis.

The distribution and the survival mechanism of *B. pseudomallei*, the causative agent of melioidosis in the environment, remains poorly understood.

2.2 *Burkholderia pseudomallei*

Melioidosis, or Whitmore's disease, is caused by *Burkholderia pseudomallei*. In 1912, this bacterium was first described by Whitmore and Krishnaswami in Rangoon, Myanmar (Whitmore and Krishnaswami, 1912). This bacterium has been known by different names over time. It was named *Bacillus pseudomallei* because it looks similar to *Bacillus mallei* which causes glanders disease. It is also known as *Whitmore's Bacillus*, *Malleomyces pseudomallei*, *Pseudomonas pseudomallei* and finally as *B. pseudomallei* since 1992 (Yabuuchi et al., 1992). *Burkholderia pseudomallei* has a typical size of 0.5 – 2 µm and is a motile, non-spore forming, gram-negative bacillus with bipolar staining with a safety pin appearance (Cheng and Currie, 2005).

2.3 Global burden of melioidosis

In 2016, the prediction of the global distribution of melioidosis revealed the global spread of melioidosis, showing that millions of people worldwide are at risk of melioidosis infection. The misdiagnosis of this pathology concerns 79 countries, including 34

countries that have never reported any melioidosis case. It is currently estimated that there are of the order of 165,000 cases of melioidosis per year worldwide, of which 54% prove fatal (**Figure 2.1**) (Limmathurotsakul et al., 2016). Endemic areas are mainly in Southeast Asia, Northern Australia, South Asia, and China. This infectious disease can spread from endemic areas to non-endemic areas worldwide. Both developed and developing countries of the tropics are potentially exposed to emerging melioidosis due to international travel resulting in imported infectious cases (Currie et al., 2008; Le Tohic et al., 2019; Limmathurotsakul et al., 2016).

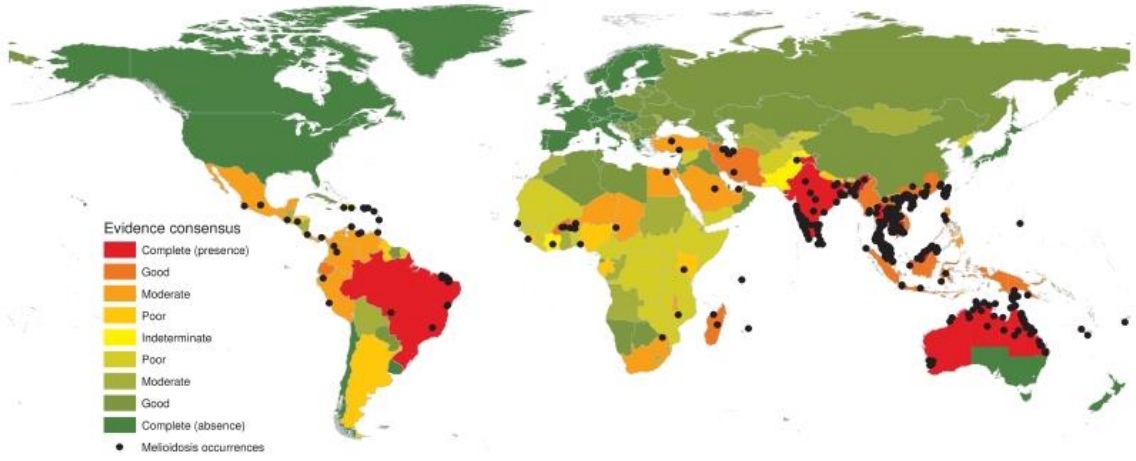

Figure 2.1: A global evidence consensus and geographic locations of melioidosis occurrence data from 1910 to 2014 (Limmathurotsakul et al., 2016).

Southeast Asia is recognized as a major endemic region for melioidosis. In northeast Thailand and northern Australia, the annual incidence rate can reach up to 50 cases per 100,000. In Thailand, 2,000 infectious cases have been confirmed, of which 40% were fatal, and the median age of the patients is 50 years, while only 5-20% are younger (Currie et al., 2010; Limmathurotsakul et al., 2010a). Besides Thailand and Australia, other

countries such as Malaysia, Singapore, Vietnam, Cambodia, and Lao PDR, are considered endemic regions. (Cheng and Currie, 2005; Southern et al., 2015).

Under-diagnosed and under-reported cases of melioidosis are of major concern. Especially, reports of melioidosis cases in South Asia and Africa remain entirely unreliable (Limmathurotsakul et al., 2016), although melioidosis in human and animal and the presence of *B. pseudomallei* in the environment have been reported for many decades in these regions (Birnie et al., 2015). Remarkably, there are only a few documented cases of melioidosis infections in Africa (Limmathurotsakul et al., 2016; Tipre et al., 2018). The first known African case was found in 1959 in a soldier who previously served in Indochina. Later in 1985, the first melioidosis patient was reported in western Africa. In 2005, further cases were reported in Mauritius, Madagascar, Nigeria, and Malawi (Birnie et al., 2015).

One of the factors that potentially influences the global distribution of melioidosis to non-endemic areas is the increasing number of long-distance travelers. A review from the years 2000 – 2018 reported that the melioidosis cases imported to Europe amounted to 77 cases mainly from Asia (66 cases or 86%), Africa (7 cases or 9%), America (4 cases or 5%), and Oceania (2 cases or 3%) and concerned leisure travelers who contracted the disease during holidays of less than one month. It is reported that countries where travelers have contracted the infection are mainly Thailand, with 41 cases (53%), Vietnam and Cambodia 7 cases (9%) (Le Tohic et al., 2019).

2.4 Occurrence of melioidosis in Lao PDR

2.4.1 Known cases of human melioidosis

In Lao PDR, a landlocked country of Southeast Asia with a population of approximately 7.2 million people (Lao Statistics Bureau, Ministry of Planning and Investment, Lao PDR, 2021) where the majority of the population are farmers, melioidosis was first recognized in 1999 (Phetsouvanh et al., 2001). The major region of known endemicity in Lao PDR spans along the Mekong river valley which defines the border between northeast Thailand and Lao PDR (Wuthiekanun et al., 1995). Earlier, during military action in 1954, a case corresponding almost certainly to a contamination that occurred in Lao PDR was diagnosed and reported in a hospital in Vietnam (Besseige et al., 1959). These reports are consistent with the presence of *B. pseudomallei* in the environment in Lao PDR for several decades at least. The lack of laboratory equipment and awareness amongst healthcare staff have resulted in the unrecognition of this bacterium earlier (Dance et al., 2018b). In 1986, a research collaboration between the Microbiology Laboratory of Mahosot Hospital and the Mahidol-Oxford-Research Unit in Thailand was established to work on melioidosis in Ubon Ratchathani, northeast Thailand (Phetsouvanh et al., 2001). Later, the melioidosis disease was confirmed as an important public health problem in Lao PDR. Microbiology diagnostic services for human infectious disease was developed in Lao PDR. The Microbiology Laboratory of Mahosot Hospital under the name Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) was established in 1999 and mainly supported the provision of diagnostic services for melioidosis using selective culture media and reagents (Dance et al., 2018b).

From 1999 to 2017, the total number of documented melioidosis cases has increased from 1 case to 1,359 cases. The highest numbers of melioidosis cases are found in the central part of Lao PDR including Vientiane Province, Vientiane Capital, Bolikhamxay Province and in the south of Lao PDR, in particular Khammouan and Saravane Provinces (**Figure 2.2**). The number of infected patients increased dramatically from 35 cases per year in 2004 to 167 cases per year in 2017 (**Table 2.1**). Documented infection cases have spread over the country. This regularly increasing number of diagnosed cases each year reflects the greater awareness of the disease amongst healthcare workers and the public likewise (Dance et al., 2018b).

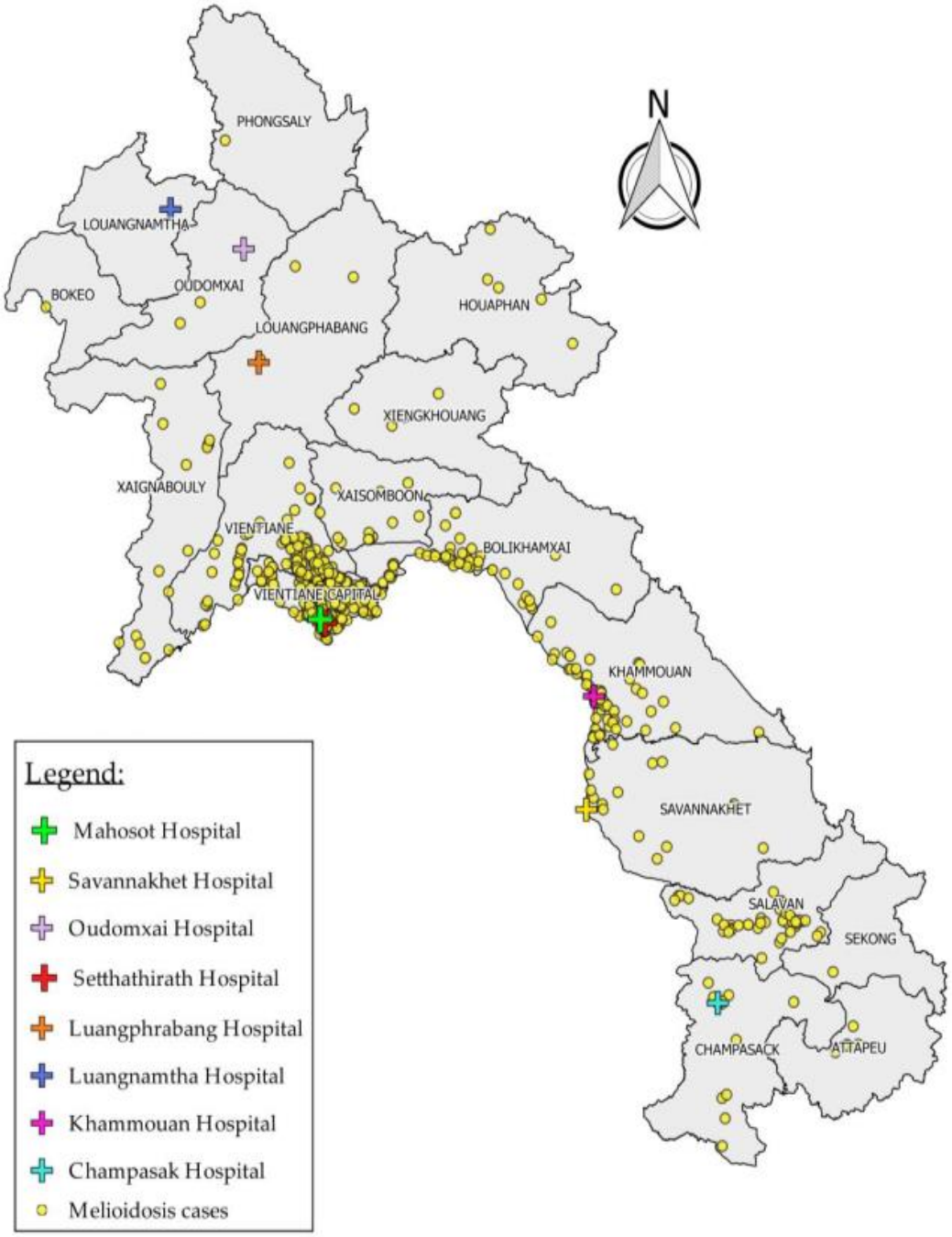

Figure 2.2: Location of 1,310 cases of melioidosis and hospital laboratories capable of making a diagnosis of melioidosis in Lao PDR *(Dance et al., 2018b)*.

Table 2.1: Case culture-positive melioidosis in Lao PDR[1;2] from 1999 to 2017 *(Dance et al., 2018b)*

Province	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	TOTAL
Attapeu									1	1		1			2	3			1	9
Bokeo												1								1
Bolikhamxai [1]					1	2	3	3	3	3	2	3	2	4	8	8	7	7	12	68
Champasak [1]			1	1				1		2					1	4		3	1	14
Houaphan												1	1	1		1			1	5
Khammouan [1]							1	3		2		10	12	19	12	10	6	8	17	100
Luangnamtha																				0
Luangphrabang																1	2			3
Oudomxai												1							1	2
Phongsali																1				1
Salavan										2	6	4	4	3	3	11	9	4	15	61
Savannakhet [1]												1	1		1	3	6	1	2	15
Vientiane Province [1]		1	1	1	1	7	7	9	15	11	19	33	10	13	28	32	33	30	36	287
Vientiane Capital [1]		2	6	4	5	25	20	27	51	31	22	58	41	57	66	65	81	108	73	742
Xaignaburi					1			1	1	1		2		1		1		4	4	16
Xekong										1										1
Xaisomboun [3]	1				1				1		1	2			2	2	3	2	2	17
Xiangkhouang [1]												1			1	1		1	1	5
Unknown [1]						1	2						1				2	5	1	12
TOTAL	1	3	8	6	9	35	33	44	72	54	50	118	72	98	124	143	149	173	167	1359

[1] Pooled data from LOMWRU, Thakek hospital and NCLE; [2] Data reflect patient's home village (where known) but not necessarily place of contraction of melioidosis;
[3] Province established in 2014.

2.4.2 Known cases of animal melioidosis

The finding of melioidosis in humans in Lao PDR suggests that animal may also be infected. At the Lao Conservation Trust for Wildlife, five cases of melioidosis were confirmed in macaques, three of which were fatal (M. Bomon, LCTW, personal comm.). Up to date, LOMWRU laboratory team has confirmed only one goat infectious case in Lao PDR in 2003 (Dance et al., 2018b). In Lao PDR, the study of melioidosis in animals is at a very early stage. Further studies of melioidosis in the animal are required.

2.4.3 Occurrence of *B. pseudomallei* in the environment

B. pseudomallei was found in both soil and water in Lao PDR (Manivanh et al., 2017; Wuthiekanun et al., 2005) and surrounding border of neighboring Thailand (Hantrakun et al., 2016), Myanmar (Swe et al., 2021; Win et al., 2018), Vietnam (Parry et al., 1999), and Cambodia (Wuthiekanun et al., 2008).

Several recent studies have focused on characterizing the nationwide occurrence of *B. pseudomallei* in Lao PDR. A study in the area surrounding Vientiane Capital based on 110 soil samples taken at the depth of 20 – 30 cm from 55 geographical locations showed that 12 out of 55 sites were positive with the mean concentration of 39 CFU/g soil (Wuthiekanun et al., 2005). Later, Rattanavong et al., (2011) found a positivity rate of 44% in 6 provinces of Lao PDR, using soil samples collected at the depth of 30 cm. The highest detection frequency was in the East of the southern province of Saravane where 94% of soil samples were positive with an average concentration of 464 CFU/g soil (Rattanavong et al., 2011). The investigation of Ribolzi et al. (2016) along the Xe Don

attempted to explain the link between the occurrence of *B. pseudomallei* in the hydrographic network and upstream land use and soil type. Another study by of Manivanh et al. (2017) conducted in a paddy field of southern Vientiane Province reported a positivity rate of nearly 30% (195 out of 653 samples) based on soil samples from 5 to 90 cm deep collected throughout a period of 13 months. The authors suggested that *B. pseudomallei* occurred more consistently at soil depths greater than 30 cm throughout the year (Manivanh et al., 2017).

Waterways are considered as potential environmental pathways along which bacteria can be carried over long distances. The study of Vongphayloth et al. (2012) showed that surface water samples taken in the East and West of Saravane province, southern Lao PDR, were found to be 36% and 6% positive, respectively, and highly positive water samples were found in the Xe Don River, downstream of the East Saravane Province. The authors hypothesized that this high positivity rate for *B. pseudomallei* was related to low pH and high turbidity. Other authors proposed that high positivity rates for *B. pseudomallei* in water samples was associated with the turbidity, Total Dissolved Solids, and cooler water temperature (Rachlin et al., 2021).

2.5 Current knowledge gaps about the presence of *Burkholderia Pseudomallei* in soil

B. pseudomallei is known as an environmental Gram-negative, motile, non-sporulating oxidase-positive bacillus, which causes melioidosis. Humans can be infected from skin inoculation, inhalation, or ingestion of the bacterium in the environment (**Figure 2.3**).

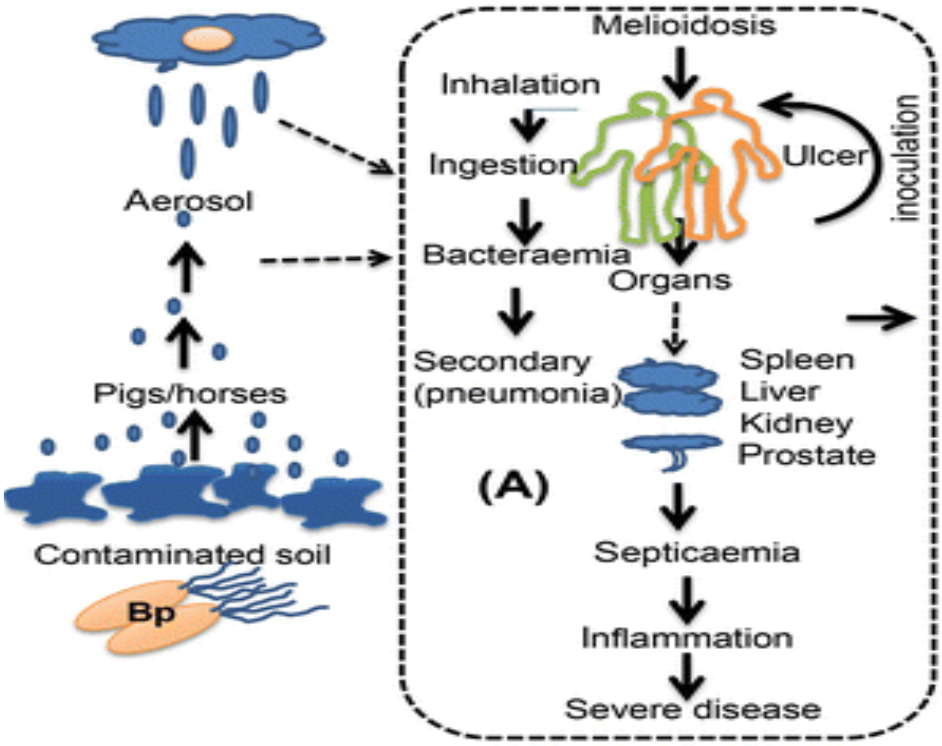

Figure 2.3: *B. pseudomallei dissemination in the environment and* melioidosis infection of humans (Burkholderia pseudomallei Toxins and Clinical Implications) (Samy et al., 2018).

The environmental distribution of *B. pseudomallei* and the true burden of melioidosis remain poorly understood (Astier-Théfenne et al., 2015; Vuddhakul et al., 1999). A geostatistical analysis of the distribution of *B. pseudomallei* in disused land and rice field in Thailand concluded that the bacterium was not distributed uniformly in soil (Limmathurotsakul et al., 2010b). Furthermore, soil is considered as the major reservoir to offer niches for *B. pseudomallei* (Seng et al., 2019).

The presence of *B. pseudomallei* is reportedly correlated with environmental factors such as climate, as well as soil physico-chemical and biological factors (Currie and Jacups, 2003; Duangurai et al., 2018; Liu et al., 2015). Extreme weather such as heavy rainfall events and flooding are commonly associated with both increased environmental detection

of *B. pseudomallei* (Bulterys et al., 2018; Mukhopadhyay et al., 2018; Ong et al., 2017) and increased incidence of melioidosis, although it is unclear what precise mechanism drives the behaviour of *B. pseudomallei*. The uneven spatial distribution of bacterial communities in soil may be influenced by an uneven distribution of organic matter from diffusing of soluble compounds resulting in higher bacterial density close to points enriched in organic matter (Ranjard and Richaume, 2001; Schoeneberger et al., 2012).

The structure of soil displays a mosaic of microenvironments that have specific physical, chemical and biological properties and structural characteristics. At the surface of the earth, soils are at the interface between the lithosphere and the atmosphere. At the microscale level, there are different indigenous bacteria that are heterogeneously distributed. Some studies have highlighted the fact that landscape features may influence the occurrence and spread of *B. pseudomallei* (Kaestli et al., 2015, 2009; Ribolzi et al., 2016) and that anthropic factors, particularly land use, can induce shifts in the microbial community structure that may affect its presence in soils (Kaestli et al., 2015; Ribolzi et al., 2016). In a rice paddy field in central Lao PDR, *B. pseudomallei* was found to occur preferentially in soil with high moisture content but low organic matter and nitrogen contents (Manivanh et al., 2017). Likewise, based on a survey of 61 rice fields in Thailand, Hantrakun et al. (2016) found that *B. pseudomallei* was associated with nutrient-depleted soils and suggested that agricultural practices that induce soil degradation may increase the presence and amount of *B. pseudomallei* in endemic areas.

Soil moisture is a variable of prime importance to the survival of *B. pseudomallei* (Pumpuang et al., 2011). *B. pseudomallei* can survive over a year in soil at a 20% moisture content, but only 30 days in dry soil (Tong et al., 1996). In the field, *B. pseudomallei* has

nevertheless been identified in humid habitats of arid environments (Alvarez-Hernandez et al., 2021) and in places where dry conditions prevail after heavy rains (Rolim et al., 2005; Yip et al., 2015).

Some micro-nutrients, such as iron, which is suspected to play an important role in the expression of respiration enzymes (Tuanyok et al., 2005), are likely favorable to the persistence of *B. pseudomallei* (Musa et al., 2016). *B. pseudomallei* is nevertheless known to adapt to various environmental stresses such as salinity, oxidative stress, or low iron content, and to occupy a variety of ecological niches, particularly in soil (Duangurai et al., 2018; Hantrakun et al., 2016).

Attempts to characterize such niches, particularly in soil, remain few and lack details, or were undertaken at a relatively coarse scale, despite well documented evidence that soil encompasses intricately nested biotic and abiotic components and functions, thus forming a complex ecosystem (Ponge, 2015). Up to now, environmental surveys of *B. pseudomallei* have relied on the recommendations of the Detection of Environmental *B. pseudomallei* Working Party (DEBWorP) (Limmathurotsakul et al., 2013) which suggest collecting a minimum of 100 individual soil samples, taken from point locations 2.5 to 5 m apart from an area of about 50 x 50 m, at a depth of 30 cm below the soil surface. While these recommendations were established based on an exhaustive analysis of environmental studies on *B. pseudomallei*, they did not include any consideration of the intrinsically universal arrangement of soils as so-called profiles (FOA, 2006), nor the associated processes of soil formation (pedogenesis). Soil profiles consist, from ground surface litter to bedrock, of superimposed and laterally homogeneous layers, known as soil horizons. Along the vertical axis, soil horizons of a given profile differ in their

biogeochemical makeup at scales of a few to tens of centimeters, while within each horizon, biogeochemical conditions can also be very different in ways of utmost relevance to microbial activity (soil structure and microstructure (Baveye et al., 2018)). Current knowledge of soil microbiology relies on sampling of the topsoil layers, based on the assumption that these layers concentrate the majority of microbial biomass, activity and diversity. However, recent studies indicate that some microbial taxa preferentially adapted to low-nutrient availability are more abundant in deep soils, suggesting that the depth-wise variability of soil physico-chemical properties may be the most important factor shaping the structure of soil microbial communities (Brewer et al., 2019; Frey et al., 2021).

Since the beginning of the 2000s, there have been several studies on the presence and distribution of *B. pseudomallei* in the environment (Currie and Jacups, 2003; Limmathurotsakul et al., 2010b; Manivanh et al., 2017; Ong et al., 2017). While information about the vertical distribution of *B. pseudomallei* in soils is rare, a few studies have suggested that *B. pseudomallei* may occur at soil depths > 30 cm. For example, a survey of 360 sampling sites in Hainan, China, consistently found *B. pseudomallei* in soil samples taken at depths of 30 to 60 cm and suggested that documenting soil heterogeneity would help to better understand the distribution and the prevalence of this pathogen in soil (Dong et al., 2018). Studies conducted in Myanmar, in Northeastern Thailand, and in the plain of Vientiane, Lao PDR, also indicated that *B. pseudomallei* can be more abundant at soil depths of 90 cm than nearer the soil surface (Manivanh et al., 2017; Win et al., 2019; Wuthiekanun et al., 1995). In an attempt to provide new detail about the heterogeneity of the distribution of *B. pseudomallei* within soils, particularly along the vertical axis, and to document whether this putative heterogeneity covaries with soil biogeochemical

properties, it is important to assess if concentration of *B. pseudomallei* are higher at soil depths greater than 90 cm.

2.6 Detection methodology

Due to the potential severity of melioidosis, laboratory investigations are important to shed light on the nature of this organism. Various laboratory methods have been developed that can be useful in both routine clinical laboratory diagnoses and in scientific research of *B. pseudomallei* while other methods are more applicable solely in scientific research settings. These laboratory method used in this work is briefly described below.

For all soil samples processed as part of this thesis, *B. pseudomallei* was isolated following the methods described by Manivanh et al. (2017) and Wuthiekanun et al. (2005). The choice of this methodological approach was motivated by two factors : 1) the fact that it was readily accessible at the Mahosot Hospital Microbiology Laboratory in Vientiane, Lao PDR and 2) the excessive logistical difficulties which would have resulted from relying upon methods that are not routinely implemented in Lao PDR and that would have necessitated the export of this pathogenic bacterium (*B. pseudomallei* is considered a Class B biothreat agent by the CDC (Centers for diseases control and prevention) and an MOT (Highly Pathogenic Microorganisms and Toxins) agent in France).

Briefly, 100 g of each soil sample was added to 100 ml of sterile de-ionised water, and mixed well by agitation, for semi-quantitative culture of *B. pseudomallei* on Ashdown agar plates (Dance et al., 2018a). Ashdown agar is a selective culture medium for *B. pseudomallei*, *B. mallei* and *B. thailandensis* which combined with a growth temperature

of 42 °C ensures minimal to no growth of competitive flora. After overnight sedimentation at room temperature, the supernatant was transferred to a separate sterile container. Supernatant aliquots in duplicate of 10, 100, and 500 µL were plated and spread onto Ashdown's agar, incubated at 42 °C in air, and inspected for up to 4 days. Suspect colonies (selected based on their morphology) were identified as presumptive *B. pseudomallei* if they were colistin and gentamicin resistant, co-amoxiclav susceptible, and specific latex agglutination-positive. The number of presumptive *B. pseudomallei* colonies on each plate was counted. A subset of presumptive *B. pseudomallei* isolates were confirmed by API20NE (bioMérieux) phenotypic testing. The only other *Burkholderia* sp. that might have caused confusion is *B. thailandensis* that expresses *B. pseudomallei*-like capsular polysaccharide (CPS; known as BTCV). Such isolates have been frequently found in Thailand, although only once in Lao PDR (Hantrakun et al., 2018). This is why a subset of isolates were tested by API20NE, as these two organisms can be distinguished by arabinose assimilation (*B. pseudomallei* being negative while *B. thailandensis* is positive). No BTCV were found.

Molecular methods are increasingly being used worldwide to detect *B. pseudomallei*. Such new methods offer promising prospects to increase the sensitivity of environmental screening methods for B. pseudomallei (Dance et al., 2018a; Göhler et al., 2017; Knappik et al., 2015). Combined with new medium containing erythritol as a single carbon source, such methods also show prospects for quantification of *B. pseudomallei* loads from environmental samples (Trinh et al., 2019).

2.7 Research objectives

This study considers the interactions between *B. pseudomallei* and soil as a key to understand the distribution in space and time of the bacterium. Thus, variations in physical and chemical properties, at various space scales (down to the millimeter scale) were taken into account along the vertical axis of the soil profile, as well as time variation related to the seasons of the year, in an attempt to clarify the determinants of the presence of *B. pseudomallei* in soil (including the putative occurrence of hotspots).

This overarching goal was pursued according to three main objectives, namely:

Objective 1: this objective aims at studying the physical and chemical characteristics, and the environmental conditions to understand the distribution of *B. pseudomallei* in a soil profile from 0 to 300 cm depth. The hypotheses are that *B. pseudomallei* occurs at a soil depth greater than 30 cm and that soil properties such as pH, iron, and soil texture play an important role in determining the presence and abundance of *B. pseudomallei.*

Objective 2: as an extension of Objective 1, this objective aims to bring new knowledge about changes in the distribution of *B. pseudomallei* along the soil profile depending on seasonal conditions. The observation of the distribution of *B. pseudomallei* in soil profile from 0 to 300 cm depth were conducted at monthly intervals, including both dry and wet seasons throughout the year. The hypothesis is that concentration of *B. pseudomallei* along 300 cm depth are different and their distribution relates to the rainfall regime which itself determines groundwater level and moisture (saturation rate) of soil horizons, thus affecting the properties of niches favorable to the bacterium.

Objective 3: this objective aims to characterize the distribution of *B. pseudomallei* at the millimeter scale along a 90 cm deep soil profile. This investigation aims to provide original information about putative hotspots of *B. pseudomallei* in soil.

Based on these three axes of investigations, I finally draw general conclusions about the environmental reservoirs of *B. pseudomallei*, including soil physical and chemical characteristics and their variations in space and time.

Chapter 3: Site description and methodology

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3.1 Site description

3.1.1 Study site location and main features

Lao PDR is a landlocked country of Southeast Asia with approximately 7.2 million inhabitants (Lao Statistics Bureau, Ministry of Planning and Investment, Lao PDR, 2021). Melioidosis cases have been found nationwide while the highest number of cases occurred in Vientiane Capital and Vientiane Province (**Table 2.1**) (Dance et al., 2018b). This site was selected because it is close to the site where *B. pseudomallei* was previously detected in 1998 (Wuthiekanun et al., 2005) and in 2011 (Manivanh et al., 2017).

The area studied in this work is located in the Vientiane plain, west of the lower reaches of Nam Ngum River (one of the main tributaries of the Mekong River in Lao PDR). The area is one of the largest food production hubs of the nation with a total rice production land area of 64,668 hectares and production yields of around 279,065 tons of rice per year (Lao Statistics Bureau, Ministry of Planning and Investment, Lao PDR, 2021). The sampling site for this study is located in a lowland paddy rice field of Nabone village (18°22'59.02" N, 102°25'22.02" E; altitude 180 m above mean sea level), Phonhong district, Vientiane Province, Lao PDR (**Figure 3.1**). *B. pseudomallei* had been detected at this location on the occasion of previous studies (Manivanh et al., 2017; Wuthiekanun et al., 2005). Sample collection was conducted following the approval of Provincial Administration of Agriculture and Forestry (PAFO) under the request of the Department

of Agricultural Land Management (DALaM) of the Ministry of Agriculture and Forestry (MAF) and with the agreement of both the land owner and the chief of the Nabone village.

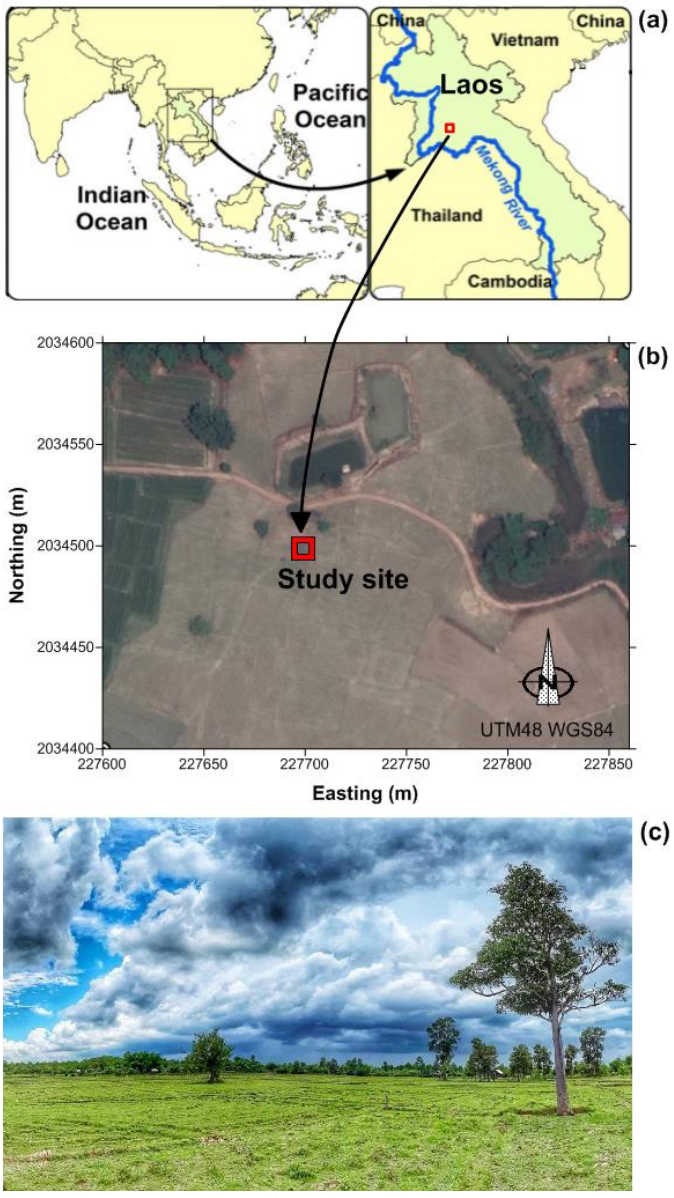


Figure 3.1: Study site: (a) location of the site in northern Lao PDR; (b) situation within the cultivated plain of the Nabone village (Satellite image 2019-02-22, Image © 2021 Maxar Technologies, Google Earth); (c) photograph of the paddy rice field where the soil profile was observed, monitored, and sampled (photo credit: A.Pierret, IRD).

3.1.2 Geological characteristics

The studied area is located in the northern margin of the Khorat plateau basin and belongs to the Vientiane sub-basin, a topographic plain that is an extension of the Upper Cretaceous Sakhon Nakhon basin. Rocks in the area consist of Cretaceous continental deposits (Saysomboun formation), mainly red-brown fluvial claystone to fine sandstone sedimentary formations partially covered by quaternary fluvial deposits containing gravel, sand, and clays (Lovatt Smith et al., 1996). Soils that develop over these sedimentary formations are mainly Acrisol (ferric Acrisols, gleyic Acrisol, and haplic Acrisols) and are for the most cultivated (mainly rice based cropping system).

3.1.3 Climate characteristics

The area experiences a Tropical wet-dry climate (*Aw* – Köppen climate classification) with three main seasons: a rainy season from May to October, a cool dry season from November to February and a hot dry season from March to April. Over the 2010-2016 period, the average annual rainfall at nearby Phonhong meteorological station was 1,519 mm, and the mean annual temperature was 27.4 °C (Department of Meteorology and Hydrology, Lao PDR).

3.2 Soil sampling and field observations

To answer the three main objectives of the thesis, the soil samples have been divided and taken during three main field campaigns:

Campaign 1: observe the distribution of *B. pseudomallei* along soil profile (vertical direction) by collecting soil samples along a 300 cm deep soil profile

Campaign 2: understand the change of *B. pseudomalle*i's distribution along the 300 cm deep soil profile, depending on the seasonal variation.

Campaign 3: characterize the distribution of *B. pseudomallei* at millimeter scale along a 90 cm deep soil profile.

These three campaigns are described in detail below.

3.2.1 Soil sampling and description

In order to meet the first objective of the thesis, which aims to expand more the knowledge about the distribution of *B. pseudomallei* along the vertical direction of a 300 cm deep soil profile, a motorized percussion corer (Cobra TT; 14.19SD HM1400 - https://www.atlascopco.com) was used at the onset of the 2018 rainy season (early in May) to collect three soil cores (100 cm long, 10 cm inner diameter), so as to sample an overall depth of 300 cm below the soil surface (**Figure 3.2**).

Every 20 cm, from 10 to 290 cm below the soil surface, undisturbed triplicate 40 cm^3 soil sub-samples were taken from the soil cores with small PVC cylinders. In total, 45 subsamples were collected along the vertical soil profile. Chemical and mineralogical analyses were performed on air-dried samples. At each soil depth increment, 100 g of soil were also taken for subsequent *B. pseudomallei* determination. These samples were temporarily stored in sterile plastic bags, sealed and placed in a cool box in the shade prior to being transported to the laboratory within 6 hours. Sampling instruments were cleaned after each sample collection, using water to remove soil and subsequently disinfected with 70% ethyl alcohol.

Figure 3.2: Collecting soil samples from a 300-cm deep soil profile in May 2018 at the rice paddy field of Nabone Village, Vientiane Province, Lao PDR (photo credits: A. Pierret, IRD).

Morphological characteristics of soil horizons were observed and described in the field, immediately after core extraction, in order to avoid re-oxidation of samples taken from horizons where reducing conditions may prevail. These morphological characteristics include: soil matrix color, soil structure and macroporous features, reductimorphic and redoximorphic features (Schoeneberger, P.J. et al., 2012), mottled colors, identification of clay leaching and iron oxide nodules pedofeatures (as indicators of clay and iron dynamics). Soil color was estimated using the Munsell colors chart (Munsell Color Compagny, 1994).

An additional 300-cm vertical profile was collected in December 2018, following the end of the rainy season, for the purpose of assessing depth-wise *B. pseudomallei* concentrations only. This second profile was sampled at a distance shorter than 5 m from the location of the May 2018 profile. No further pedological nor physico-chemical characterization was attempted on this second sampling. Rather, this sampling was intended to assess whether the order of magnitude of the semi-quantitative estimates of *B. pseudomallei* varied substantially at the seasonal scale.

In order to meet the second objective of the thesis, which aims to understand more the distribution of *B. pseudomallei* during the seasonal change in both wet and dry season, soil samples of the 300-cm vertical profile were collected each month during 10 months, from mid of May 2019 to mid of February 2020. Each soil profile was collected at a distance shorter than 5 m surrounding the first profile location of May 2018.

From the beginning of May 2018 to the end of April 2019, selected physico-chemical properties of the soil solution (i.e. water with dissolved gases, minerals, and organic matter that make up the liquid phase of soil) were monitored monthly, directly in a 210 cm deep piezometer using a multi-parameter probe (YSI 556 MPS; www.ysi.com). Measured variables were temperature, electrical conductance at 25 °C (EC), dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP).

In order to meet the third objective of the thesis, which aims to understand more the distribution of *B. pseudomallei* at millimeter scale, four soil profile samples with an overall depth of 90 cm below the soil surface were collected at the end of November 2017 in the same paddy field as that where 300 cm cores were taken (**Figure 3.3**).

Figure 3.3: Collecting soil samples from a 300-cm deep soil profile in November 2017 at the rice paddy field of Nabone Village, Vientiane Province, Lao PDR (photo credits: A. Pierret, IRD).

The purpose of this sampling set was to estimate the *B. pseudomallei* concentration in relation with soil redoximorphic features. The soil profile sample was divided into five soil depths from 0 to 90 cm. The first soil depth was 0-10 cm and the second to fifth soil depths were every 20 cm (**Figure 3.4**).

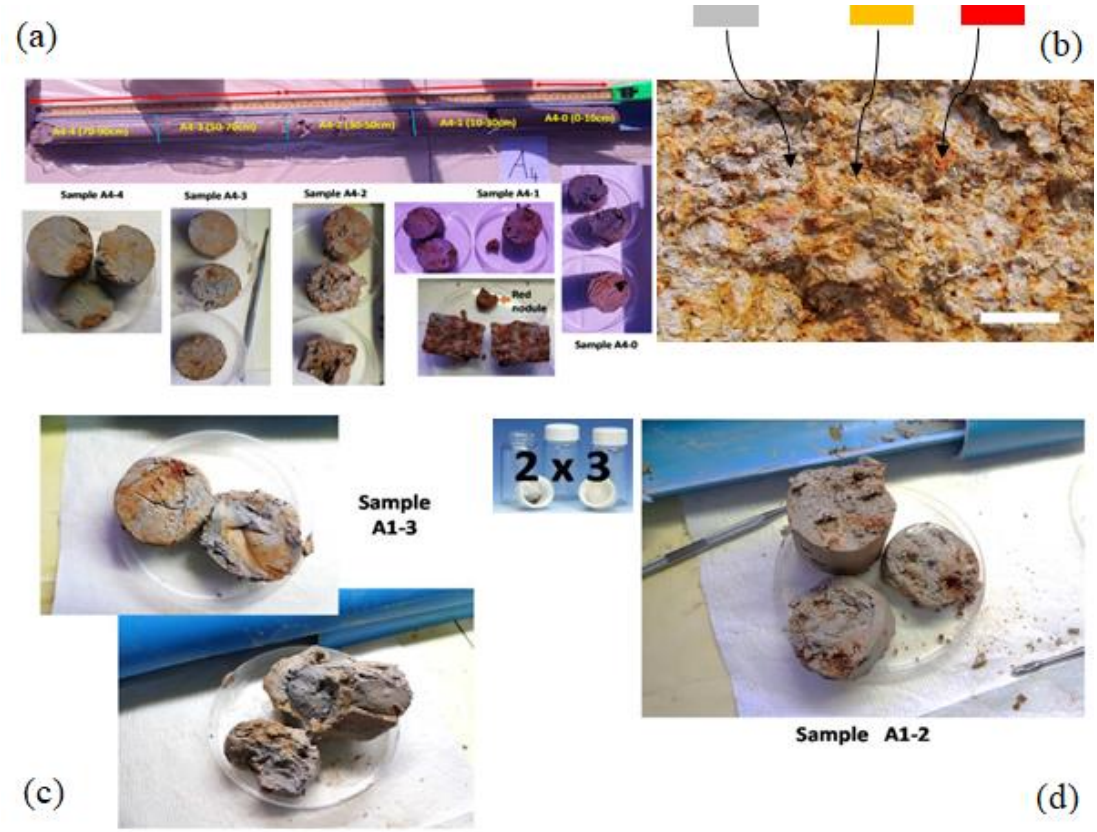


Figure 3.4: Soil redoximorphic features along a soil profile of 90 cm depth. (a) soil profile along 90 cm depth is divided in five sections to observe soil redoximorphic features; (b) soil colors reflect iron distribution in the form of ferrous (grey) and ferric (orange – red) iron; (c) observed sample A1-3 at the depth of 50-70 cm; (d) observed sample A1-2 at the depth of 10-30 cm (photo credits: A. Pando & A. Pierret, IRD).

3.2.2 Physico-chemical monitoring in field

In parallel with semi-quantitative cultures of *B. pseudomallei*, the soil 300-cm soil core collected in May 2018 was used for measurements of soil physico-chemical properties. Physical variables such as soil water content (WC), bulk density (BD), soil pH, organic matter content (OM), and particle size analysis (soil texture) were conducted at the DALaM soil analyses laboratory.

Chemical variables including soil elemental concentrations of major elements (Na, Mg, Al, Si, P, S, K, Ca, Ti, Mn, and Fe) were analysed at Geosciences Environment Toulouse (GET) laboratory, Toulouse, France. The mineralogical composition of soil samples was determined by using X-ray diffraction for 6 selected soil depth increments (at 10, 30, 90, 170, 210 and 250 cm depth), analyses also conducted at GET. Exchangeable Ca^{2+} (Ca_e), Mg^{2+} (Mg_e), Na^{+} (Na_e) and K^{+} (K_e) concentrations of soil from 0 – 300 cm deep soil profile were calculated.

3.2.3 Groundwater level monitoring and hydrodynamic properties of soil

Groundwater level was monitored using a 210 cm deep piezometer installed in the field within less than 5 meters of the location where all the soil samples analysed as part of this work have been collected. A water level pressure sensor (CTD-Diver datalogger), submerged at the base of the piezometer, was used to automatically record the pressure at 15-minute intervals during one year, starting from early May 2018. Water levels were derived from these measurements after correcting the atmospheric pressure variations using data from a barometric probe (Baro-Diver datalogger) installed next to the piezometer. Manual groundwater level measurements were also taken using this piezometer, monthly and bi-monthly during the dry and rainy seasons, respectively.

Saturated hydraulic conductivity (Ks) of soil was measured in the field at 5 depth increments (namely 10, 50, 90, 130, and 170 cm), in 6 replicate test boreholes, using a Wiltschut permeameter (Klute, 1986). These measurements were conducted at a distance shorter than 2 m of the soil profile sampled for *B. pseudomallei* and associated physico-chemical variables.

3.3 Laboratory measurements

3.3.1 Soil physico-chemical variables

WC, BD, pH, OM and texture were measured at each sampling depth. WC and BD were measured using intact 40 cm^3 soil cores collected with small PVC rings; moist soil cores were first weighed, then oven dried at 105 °C for 48 h, then reweighed; WC is calculated as the ratio of the mass of water lost by drying divided by the mass of dry soil whereas BD is the ratio of the mass of dry soil divided by the volume of the core (McKenzie et al., 2004). For soil pH, 20 g of soil was diluted into 50 ml of deionised water and the pH of this solution was subsequently measured with a laboratory pH-meter (Hanna, Woonsocket, RI, USA). The standard Walkley-Black operating procedure was used for OM determination (FAO, 2019). Soil texture (i.e. Clay < 2 μm; Silt 2–50 μm; Sand 50–2000 μm) was determined at each sampling depth following the standard pipette method (Olmstead et al., 1930).

3.3.2 Soil elemental concentrations and mineralogy

Elemental concentration measurements and mineralogical properties of soil samples were performed at GET. Soil elemental concentrations of major elements (Na, Mg, Al, Si, P, S, K, Ca, Ti, Mn, and Fe) were determined on 1 g of finely ground powder samples fused with 10 g of $Li_2B_4O_7$:$LiBO_2$ (mass ratio of 66:34) into Pt crucibles to produce glass discs for X-ray fluorescence analysis by the way of a Bruker S2 Ranger energy-dispersive X-ray fluorescence analyser, using Pd X-ray tube and Peltier-cooled silicon drift detector. Measurements were run with the X-ray tube successively tuned to 20, 40 and 50 keV.

The mineralogical composition of soil samples (bulk samples and clay fraction) was determined using X-ray diffraction (XRD). XRD measurements for random powder analysis were performed on the Bruker D2 diffractometer (Cu-Kα radiation, Brag Brentano theta/theta setup, 2-80°) after crushing the total fraction in an agate mortar. XRD measurements were made on the clay granulometric fraction (0–2 µm) from oriented samples for 6 selected samples (at 10, 30, 90, 170, 210 and 250 cm depth) and were performed using D8 Advance (www.bruker.com) diffractometers (Cu-Kα radiation, Brag Brentano theta/theta setup, 2-30°). Diffraction patterns of powder samples were interpreted with reference to the ICDD database (PDF-2^{TCM}) and COD databases using EVA software (Bruker).

Exchangeable Ca^{2+} (Ca_e), Mg^{2+} (Mg_e), Na^{+} (Na_e), and K^{+} (K_e) concentrations of soil were estimated using the 1N ammonium acetate method (Chapman, 1965). Cation exchange capacity (CEC) of the soil and exchange acidity (EA) were estimated based on calculations considering the "sum of base cations" and the "sum of acid cations" approach using both pHKCl and pHH_2O values (Schwertfeger and Hendershot, 2009).

3.3.3 Microbiological analysis

For all soil samples, namely subsamples taken from 90 cm deep soil cores taken in November 2017, from 300 cm deep soil cores taken in May and December 2018, and from 300 cm deep soil cores collected monthly between May 2019 – February 2020, *B. pseudomallei* were isolated from soil as described by Manivanh et al. (2017) and Wuthiekanun et al. (2005) (see section 2.6).

3.4 Data analysis

As some datasets were not normally distributed, a Spearman's rank-order correlation, also known as a non-parametric measure of rank correlation, was applied (XLSTAT Premium version 20.1.1.) for all data analysis and test putative interactions between semi-quantitative estimates of *B. pseudomallei* in samples with measured soil properties.

We conducted a Partial Least Square Regression (PLS-R) analysis using the natural logarithm of the semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei*, and physico-chemical variables determined at all sampled soil depths along the 300-cm deep profile on May 2018 (XLSTAT Premium version 20.1.1.). PLS-R is well-suited to the analysis of a dataset with few observations and many variables such as that corresponding to the 300-cm soil profile studied in this work. The variable importance in projection (VIP) number was used to assess the relative importance of projected variables, with variables having a VIP below 1 considered to be unimportant in the analysis (Ribolzi et al., 2016).

Chapter 4: Results and discussion

Chapter 4: Results and discussion

4.1 Distribution of *Burkholderia pseudomallei* along a 300-soil profile in relation to soil physico-chemical properties, in a lowland paddy rice field in Lao PDR

4.1.1 Semi-quantitative vertical distribution of *Burkholderia pseudomallei*

Samples have been collected in lowland rice paddy (**Figure 3.1**). Field semi-quantitative cultures of soil samples taken along a profile in May 2018 allowed the detection of *B. pseudomallei* at nearly all depths except for 50, 80 and 230 cm. Although counts averaged 1,179 CFU.g^{-1} over the whole profile, values varied drastically spanning four orders of magnitude, from 1 to 12,600 CFU.g^{-1}, with the highest *B. pseudomallei* concentrations between the soil depths of 110 cm and 250 cm. The maximum *B. pseudomallei* concentration was observed at 170 cm soil depth (**Figure 4.1**)

The additional 300-cm vertical profile collected in December 2018 revealed that, below 100 cm, depth-wise *B. pseudomallei* concentrations were of the same order of magnitude as that observed in May 2018. In the first meter of the profile, CFU counts were more dissimilar between the two sampling dates, which might be related to the re-wetting of the profile in December, following the end of the rainy season, in line with the previous results of Manivanh et al. (2017) (**Figure 4.1**).

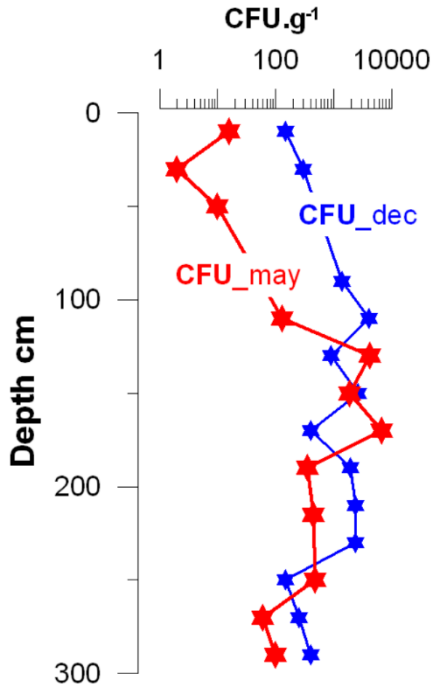
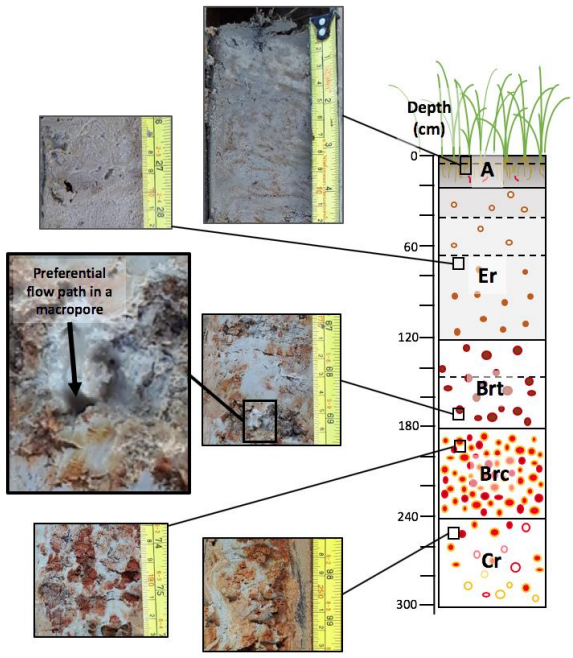


Figure 4.1: Semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) along the 300-cm deep profile, in May (CFU_may) and December (CFU_dec) 2018.

4.1.2 Morpho-pedological features

The pedological description (**Figure 4.2**) revealed that the studied soil belongs to the gleyic plinthic Acrisol type according to the international soil classification (FOA, 2006).



Horizon	Structure	Pedofeatures	Color	
			Matrix	Pedofeatures
Ploughed, stagnic	Polyhedral	Redoxymorphic features along root system	10YR5/2	5YR3/4 to 4/6
Eluvial, gleyic	Polyhedral	Diffuse mottles	10YR6/2	5YR5/3
	Massive to single grained	Diffuse mottles dismantled Fe nodules	10YR6/2	5YR5/3
			10YR7/2	5YR5/6 to 4/4 5YR3/2 to 4/6
Mottled	Polyedral	Mottles and Fe nodules	10YR7/1	5YR4/6
	Massive	Mottles, Fe nodules Mn nodules		5YR3/4 to 4/6 5YR5/8
Nodular	Massive	Two types of Fe nodules	10YR7/1	5YR5/6 2,5YR4/8
Mottled, gleyic	Polyhedral	Mottles and dismantled nodules	10YR7/1	5YR5/6 7,5YR5/6

Figure 4.2: Morpho-pedological features of the studied soil profile: center, schematic of the soil log showing the vertical succession and thickness of the main horizons (i.e., A, Er, Brt, Brc, and Cr); left, pictures showing colored features mainly related to iron redistribution, and a preferential water flow path in a macropore; right, synthesis table of the soil horizons structure, pedofeatures and colors (Munsell color chart; Munsell Color Compagny, 1994).

Such a soil is characterized by intense clay leaching processes inducing clay depletion in surface horizons and clay accumulation in deeper horizons (i.e., argic horizon). Morphological features indicate that the soil is highly weathered and has experienced intense oxidation-reduction processes, as evidenced by the dismantling of iron-containing nodules. In the argic horizons, soil shows non-homogeneous porosity features with visible preferential pathways (**Figure 4.2**, picture taken at 169 cm depth). From the surface to the depth of 300 cm, the soil profile can be divided into 5 main soil horizons, with some sub-horizons being distinguished based on variations in pedofeatures (**Figure 4.2**):

The first soil horizon mainly resulted from anthropo-pedogenetic (Richter, 2007) processes (hydragric A-horizon, 0-20 cm), had a sandy-loam texture and a dark greyish

brown colour (10YR5/2) related to high organic matter content and displayed ploughing and stagnic traits. Redoximorphic features displaying specific reddish colours from 5YR3/4 to 4/6 were observed along biopores and root channels;

The underlying albic horizon (Er-horizon), extending between depths of 20 to 120 cm, was characterized by a pronounced sandy texture with sand contents exceeding 70 % between 30 and 110 cm, and a lighter grey colour (10YR6/2 to 10YR7/2) corresponding to a lower organic matter content than in the A horizon. Iron nodules occurred increasingly towards the lower part of the horizon with colours from 5YR5/3 to 5YR3/2;

The third soil horizon (Brt horizon), an argic B horizon extending between the depths of 120 and 180 cm, had a high clay content, reaching a maximum of 46% at 170 cm, a light grey matrix colour of 10YR7/1, and included increasing amounts of iron and manganese nodules with colours mainly yellowish red (5YR4/6) giving it an apparent light yellow-red colour. Nodules in this horizon showed signs of dismantling;

The fourth horizon (Bcr horizon) is a plinthic nodular horizon, extending from 180 cm to 240 cm and characterized by the presence of two types of iron nodules with notable colour differences (2.5YR4/8 and 5YR5/6), in amounts even higher than in the Brt horizon, resulting in an even more pronounced orange overall coloration despite of similar matrix colour (10YR7/1). Clay content decreased regularly from the top to the bottom of this horizon, mirrored by increasing amounts of sand;

Finally, between 240 and 300 cm lays the last horizon (Cr-horizon) of sandy-clay-loam texture with overall a much lighter grey-white colour due to lower content of apparently fragmented and dismantled iron nodules.

4.1.3 Physico-chemical variables

Particle size analysis revealed that sand fraction (60-84%) prevailed in the surface and subsurface horizons (A and Er) to a depth of 110 cm (**Figure 4.3**). Variations in particle size distribution with depth indicated a depletion of both clay and silt in the surface horizons (10 to 16% for clays and 10 to 26% for silts) which resulted in an enrichment of the deeper B horizons (33 to 44% clays) from 120 to 230 cm. Such a particle size evolution with depth, a typical pattern for soil experiencing clay leaching such as Acrisol, confirms the horizon succession that our pedological observations revealed, in particular, the presence of argic horizons between 120 and 240 cm. Below 230 cm, the decrease in clay content (28 to 21%) indicated the transition between the argic horizon and the saprolite (Cr horizon).

Variations in porosity with depth indicated that the highest porosity value (~52%) is in the argic horizon (**Figure 4.3**). If textural micropores probably constitute the major fraction of total porosity in this horizon, structural macropores, revealed by field observations, are also part of the soil porosity. This well-developed network of macropores allows preferential flow pathways, hence likely explains the higher hydraulic conductivity values measured in the third soil horizon compared to the first and the second ones (**Figure 4.3**).

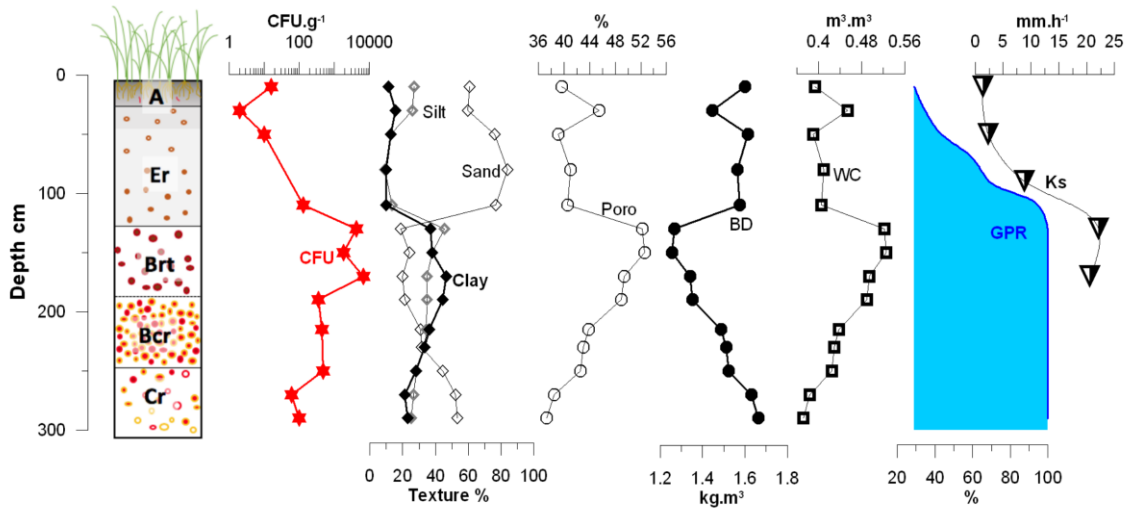


Figure 4.3: Soil log showing the main pedological horizons i.e., A, Er, Brt, Bcr and Cr – A: surface (topsoil) horizon; E: eluvium soil horizon (horizon leached of its mineral and/or organic content, B: subsoil horizon (layers which are significantly altered by pedogenesis, mostly with the formation of iron oxides and clay mineral); C: parent material horizon (layer marginally affected by pedogenesis). When indicated, abbreviations for master soil horizon subdivisions are r: weathered or soft; t: accumulation of silicate clays; c: concretions or hard nodules, semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}), and soil physical variables along the 300-cm deep profile: texture (i.e. percentages of silt, sand, and clay), porosity (Poro; %), bulk density (BD; $kg.m^3$), volumetric water content (WC; $m^3.m^3$), groundwater persistence rate (GPR; %) and saturated hydraulic conductivity (Ks; $mm.h^{-1}$).

Variations in porosity with depth indicated that the highest porosity value (~52%) is in the argic horizon (**Figure 4.3**). If textural micropores probably constitute the major fraction of total porosity in this horizon, structural macropores, revealed by field observations, are also part of the soil porosity. This well-developed network of macropores allows preferential flow pathways, hence likely explains the higher hydraulic conductivity values measured in the third soil horizon compared to the first and the second ones (**Figure 4.3**).

OM was low (0.1 to 0.7%) in the studied soil profile of May 2018, with the highest values observed in the surface horizon (**Figure 4.4**). However, the profile of OM suggested a relative enrichment of organic compounds in the argic horizons (about 0.37 to 0.47 % of

OM between 120 and 240 cm). The origin of such organic matter enrichment at depth of 120 – 230 cm cannot be fully determined based on the analysis made in this study (Gleixner, 2013). It can either result from the migration and incorporation of mobile organic compounds from the Er horizon toward the B horizons, similarly to the clay and silt fractions, or from the persistence of organic matter compounds in relation with past ecosystem functioning prior to rice cultivation (e.g., forest ecosystem). Soil pH was acidic with pH values ranging from 4.58 to 5.02 (**Figure 4.4**) with the exception of the surface horizon being less acidic (pH = 5.79) probably due to higher OM in the A soil horizon (**Figure 4.4**) and the temporary puddling of the rice crop (Sahrawat, 2005).

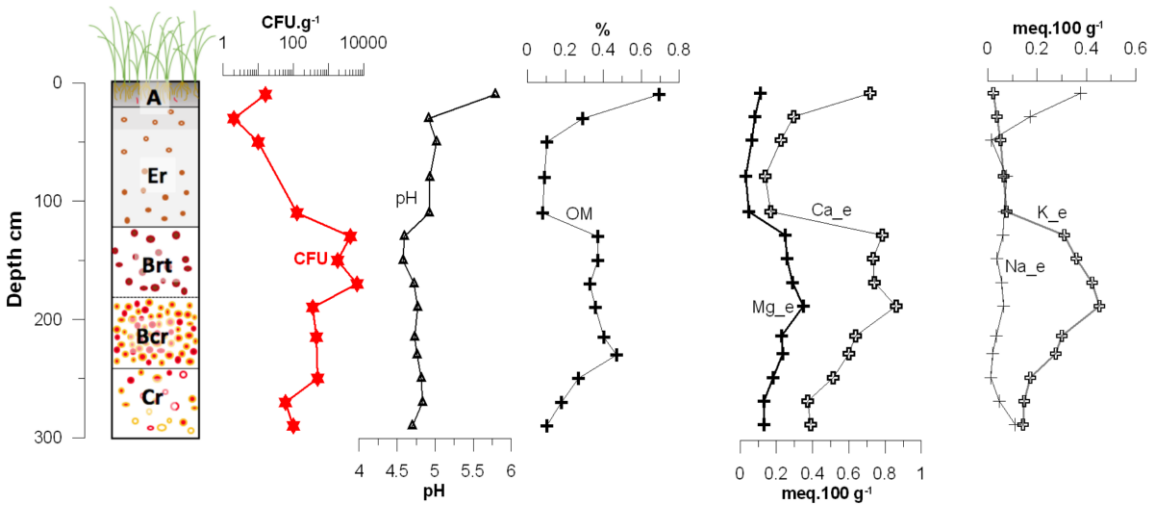


Figure 4.4: Soil log showing the main pedological horizons (i.e. A, Er, Brt, Bcr and Cr see caption of Figure 4.3 for meaning of abbreviations), semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}), and soil physico-chemical variables along the 300-cm deep profile: pH, organic matter content (OM; %), exchangeable magnesium (Mg_e; meq.$100g^{-1}$), exchangeable calcium (Ca_e; meq.$100g^{-1}$), exchangeable sodium (Na_e; meq.$100g^{-1}$) and exchangeable potassium (K_e; meq.$100g^{-1}$).

The very low CEC of this soil (i.e. < 2.5 meq.$100g^{-1}$) coupled with a relatively high exchange acidity (between 20 and 35% of the total CEC) except for the surface horizon,

creates favorable conditions for clay leaching. Ca_e, Mg_e, Na_e and K_e are low and reach a maximum value of 1.7 meq.100 g^{-1} at 290 cm depth (**Figure 4.4**). These results are in line with the previous study of Matsuo et al. (2015) for soils from the Phonghong district, Lao PDR.

Major element concentrations showed inverted profiles for the major elements Al and Si (**Figure 4.5**). Si concentration was depleted in the argic horizons in contrast with Al and K concentrations that displayed significant increases. These results are consistent with clay leaching processes occurring in this soil and the clay rich nature of horizons between 120 and 240 cm. Fe concentration gradually increases from surface down the soil profile, reaching a maximum at a depth of 215 cm, corresponding to a deep nodular horizon.

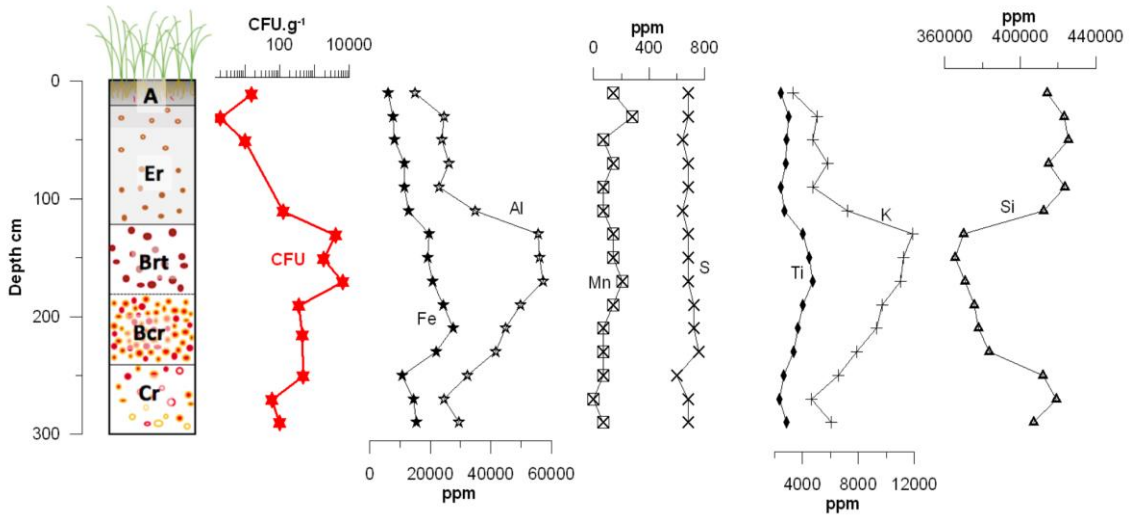


Figure 4.5: Soil log showing the main pedological horizons (i.e. A, Er, Brt, Bcr and Cr – see caption of Figure 4.3 for meaning of abbreviations), semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}), and elemental concentration along the 300-cm deep profile: iron (Fe; ppm), aluminum (Al; ppm), manganese (Mn; ppm), sulfur (S; ppm), titanium (Ti; ppm), potassium (K; ppm), silicium (Si; ppm).

Parallel to major element concentrations, mineralogical investigations (powder XRD patterns) showed that quartz was the dominant mineral at the whole soil profile scale,

regardless of the horizons (**Figure 4.6**). XRD patterns show the high intensity peaks of quartz (Qz), chlorite, illite, kaolinite, felspar and goethite, quartz being the major mineralogical phase for all bulk soil samples. However, not unexpectedly, the intensity of peaks corresponding to clay minerals increased in argic horizons samples. XRD analysis of the mineralogical composition of the <2µm clay fraction revealed a similar clay minerals assemblage for all soil horizons, consisting of Mg vermiculite, chlorite, illite, kaolinite, and interstratified phases of illite and vermiculite type. Mg vermiculite and chlorite phases are common in paddy field soils prone to redox processes. Indeed, mineralogical transformations can occur in such soils, such as chloritization and the loss of K from micaceous clays (Kögel-Knabner et al., 2010). Iron rich phases were only apparent in powder XRD patterns from 120 cm depth and were mainly ferrihydrite and goethite for the fine earth fraction. Nodules were composed of hematite. Specific analysis of the <2µm clay fraction also revealed the existence of lepidocrocite. Both ferrihydrite and lepidocrocite are iron bearing phases that form during *in situ* soil processes and which are commonly observed within redoximorphic features of rice paddy fields. The observed mineralogical assemblages for the studied soil are similar to those observed by Egashira et al. (1997) from soils developed on similar geological substrate in the Vientiane plain.

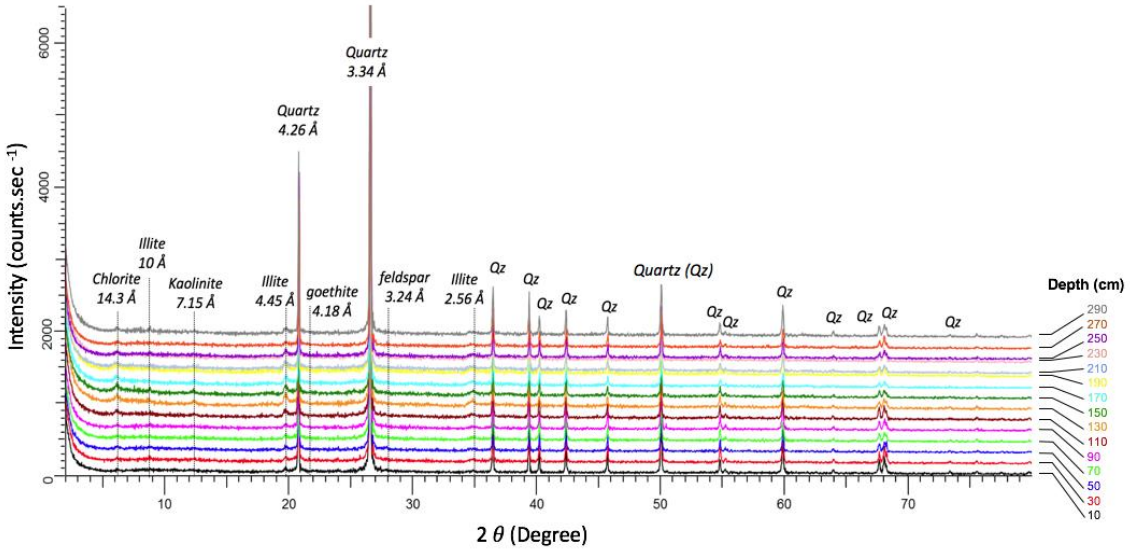


Figure 4.6: X-ray diffractograms (XRD) of the bulk soil samples collected every 20 cm along the 300-cm deep soil profile.

4.1.4 Groundwater persistence

The average groundwater persistence rate (GPR) (Bouzigues et al., 1997), defined as the number of days during which soil was saturated with water, divided by the number of days in a year, was calculated (**Figure 4.3**) based on the water level data of groundwater from both automatic and manual measurements within one year (May 2018 - May 2019). GPR was equal to 100% at all soil depths below 120 cm. Groundwater depths varied between a minimum of less than 15 cm between June and September and a maximum of 175 cm at the end of the dry season (May). GPR was at least 99% in the 130 - 290 cm depth range where the highest concentrations of *B. pseudomallei* were measured.

4.1.5 Statistical analysis

The PLS-R analysis considering all soil depths from 10 to 290 cm and all measured variables (**Figure 4.7**), including Ks, with values interpolated to 290 cm, slightly

outperformed a first PLS-R that did not take Ks into account. This confirms the importance of Ks as an explanatory variable throughout the profile and had a cumulated Q^2 of 0.70 and 0.64 on the first two components, respectively, indicating an overall good quality of the fit. The cumulated R^2Y and R^2X also ranged from 0.60 to 0.79, indicating that the first two components summarize well both the Xs and the Ys. The best explanatory variables of this PLS regression were: GPR, Ks, Ca, Al, Si, Clay, Mg, K, Mg_e, K_e, Sand, Ti and Fe.

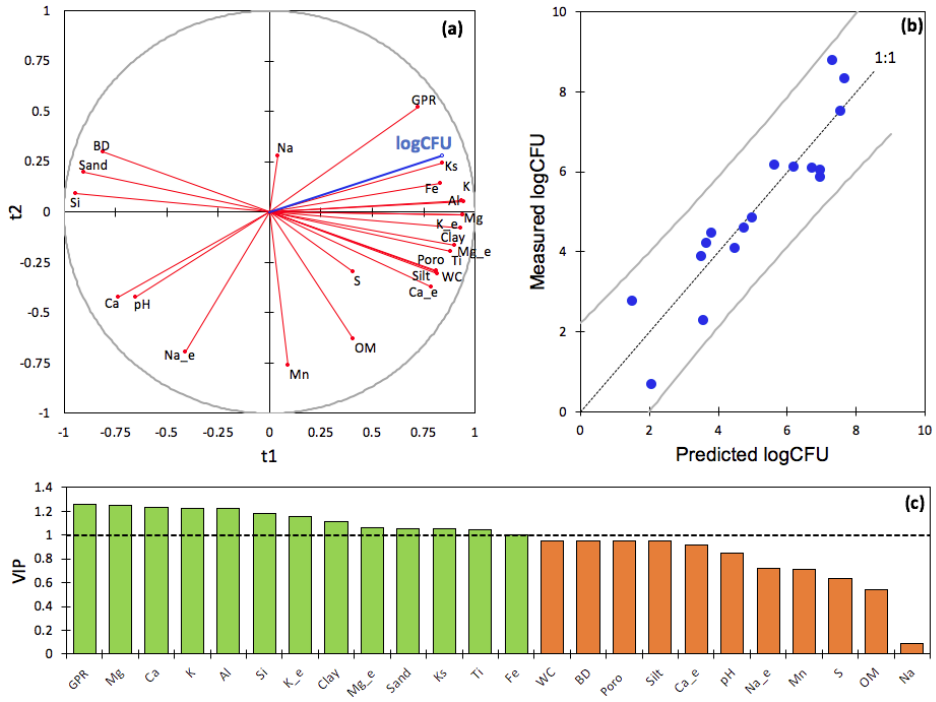


Figure 4.7: Partial Least Squares Regression (PLS-R) analysis where the natural logarithm of the semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (logCFU) are compared with soil physico-chemical variables: (a) logCFU in the correlation circle with the 24 soil variables; (b) Measured and predicted log CFU using the PLS-R model; (c) Variable importance for the projection (VIP) score plot of the 24 soil physico-chemical variables. Fe: total iron concentration; Al: total aluminum concentration; Mn: total manganese concentration; S: total sulfur concentration; Ti: total titanium concentration; K: total potassium concentration; Si total silicium concentration; pH; OM: organic matter content; Mg_e: exchangeable magnesium fraction; Ca_e: exchangeable calcium fraction; Na_e: exchangeable sodium fraction; and K_e: exchangeable potassium; silt, sand, and clay textural fractions; Poro: soil porosity; BD:

bulk density; WC: volumetric water content; GPR: groundwater persistence rate; Ks: saturated hydraulic conductivity.

Overall, there was a strong contrast between the Spearman's rank-order correlations computed considering on the one hand, the first meter of the profile only and on the other hand, the deeper layers from 110 to 290 cm (**Table 4.1**). Only three of the measured soil properties (namely, OM, clay, and Ks) were significantly correlated with *B. pseudomallei* counts in the first meter of the soil profile, while 18 such significant correlations were found when considering the full soil profile (10 to 290 cm). CFU counts from semi-quantitative cultures of *B. pseudomallei* thus appeared significantly correlated with:

- Clay (ρ=0.71; p=0.002); silt (ρ=0.76; p<0.001); sand (ρ= -0.76; p<0.001);
- Fe (ρ=0.68; p=0.003); Mg (ρ=0.90; p<0.0001); Ti (ρ=0.62; p=0.009); Al (ρ=0.87; p<0.0001); K (ρ = 0.87; p<0.0001); Si (ρ=-0.86; p=<0.0001); Ca (ρ=-0.78; p=0.0003);
- Exchangeable Ca (ρ=0.63; p=0.008); Mg (ρ=0.72; p=0.0013); K (ρ=0.86; p<0.0001);
- Soil BD (ρ=-0.66; p<0.005); Poro (ρ=0.66; p<0.005); GPR (ρ=0.60; p<0.05);
- Soil pH (ρ=-0.80; p<0.001).

Additionally, when considering the soil profile from 10 to 170 cm only, i.e., the maximum depth at which Ks was measured, semi-quantitative cultures of *B. pseudomallei* were significantly correlated with Ks (ρ=0.94; p<0.0001).

Table 4.1: Correlation (Spearman r coefficient) between the semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) and soil physico-chemical characteristics at the soil profile depth of 10 to 290 cm and 10 to 170 cm. Listed variables are pH; organic matter content (OM, %); exchangeable calcium (Ca_e, meq.100 g^{-1}); exchangeable magnesium (Mg_e, meq.100 g^{-1}); exchangeable potassium (K_e, meq.100 g^{-1}); exchangeable sodium (Na_e, meq.100 g^{-1}); Sodium (Na; mg/l); Magnesium (Mg; mg/l); Calcium (Ca; mg/l) sand, silt, and clay textural fractions (%); bulk density (BD, $g.cm^3$); soil porosity (poro, %); volumetric water content (WC, $m^3.m^{-3}$); groundwater persistence rate (GPR, %); iron content (Fe, ppm); aluminum content (Al, ppm); manganese content (Mn, ppm); sulfur content (S, ppm); titanium content (Ti, ppm); potassium content (K, ppm); silicium content (Si, ppm); saturated hydraulic conductivity (Ks, $mm.h^{-1}$).

Physico-chemical variables	Soil profile depth		Physico-chemical variables	Soil profile depth	
	10 to 290 cm	10 to 170 cm		10 to 290 cm	10 to 170 cm
pH	**-0.80*** **	**-0.69***	GPR	**0.61***	**0.95*** **
OM	0.37	0.10	Na	-0.03	-0.12
Ca_e	**0.63****	0.43	Mg	**0.90*** **	**0.94*** **
Mg_e	**0.73****	0.43	Al	**0.87*** **	**0.78***
K_e	**0.86*** **	**0.95*** **	Si	**-0.86*** **	**-0.76***
Na_e	-0.42	-0.30	S	0.24	0.19
Sand	**-0.76*** **	-0.41	K	**0.87*** **	**0.78***
Silt	**0.76*** **	0.49	Ca	**-0.78*** **	**-0.87****
Clay	**0.71****	0.36	Ti	**0.62****	0.45
BD	**-0.66****	**-0.65***	Mn	0.01	0.04
Poro	**0.66****	**0.65***	Fe	**0.68****	**0.94*** **
WC	**0.66****	**0.65***	Ks	x	**0.94*** **

Significance level: $***$ $p < 0.001$, $**$ $p < 0.01$, $*$ $p < 0.05$.

The soil physico-chemical variables presented in this study that appear to be of importance with regards to the vertical distribution of *B. pseudomallei* are those with the highest VIP values in the PLS-R, namely, GPR, Mg, Ca, K, Al, Si, K_e, Clay, Mg_e, Sand, Ks, Ti, and Fe. The group of chemical elements thus outlined is consistent with the apparent importance of clay minerals. Otherwise, GPR and soil structure (Ks - hence aerobic conditions) appear to be the variables that are the most significantly correlated with the observed vertical distribution of *B. pseudomallei*.

Apart from considerations related to the ecology of *B. pseudomallei*, an essential contribution of this work is the reminder that soils are not restricted to a few centimeters below the surface and that their physico-chemical and mineralogical properties vary with depth, consistent with changes in morphopedological features. Recent studies of *B. pseudomallei* emphasize that different interacting factors (climate, physico-chemical, and biological conditions) in soil and groundwater depth may influence the occurrence and spread of the organism (Rachlin et al., 2021; Zimmermann et al., 2018). However, most investigations of the ecology of *B. pseudomallei* have so far overlooked altogether the fact that soils do not correspond to large volumes of homogeneous material below the ground surface but are instead one of the most complex ecosystems on Earth. As a direct consequence of the over-simplistic representation of soils that emerges from the literature on the ecology of *B. pseudomallei*, the overwhelming majority of the thousands of samples that have been specifically collected to detect this bacterium were collected from a depth of at most 30 cm, and very occasionally up to 90 cm. As a first attempt to take a different look at the distribution of *B. pseudomallei* in soil, here we have, for the first time, examined the semi-quantitative distribution of *B. pseudomallei* along a 300-cm deep soil profile, in relation to environmental covariates related to soil physico-chemical properties.

Indeed, soils generally consist of successive layers (i.e., horizons), each having its own biogeochemical makeup and dynamics. Within each horizon, the arrangement of solid particles creates several levels of nested structures, representing a myriad of potential niches for communities of living organisms, including (i) bacteria, fungi, protozoa, and nematodes at scales <100 μm; (ii) acari, springtails, diplura, symphylans, and enchytraeids

at scales >100 μm and <2 mm; and (iii) mollusc, spiders, insects, earthworms at scales >2 mm (Briones, 2014).

Soil texture. Along the studied soil profile, soil texture, which reflects the relative proportions of sand, silt and clay, varied substantially as a function of soil depth, as a consequence of clay leaching processes. Soil texture was predominantly sandy in the first meter of the profile, then markedly clayey between 120 and 210 cm and finally gradually sandier again deeper in the profile. Such variations in soil texture were well correlated with *B. pseudomallei* counts, with fine-textured material (and clay <2 μm and silt 2–50 μm) and coarser material (sand 0.05–2.0 mm) positively and negatively correlated with *B. pseudomallei* counts, respectively. Information suggesting the existence of a correlation between soil texture and the presence of *B. pseudomallei* has previously been reported (Baker et al., 2015; Dong et al., 2018; Hantrakun et al., 2016; Ribolzi et al., 2016).

Based on an analysis of soil samples from southern US states, Hall et al. (2015) found *Burkholderia* spp. to be much more abundant in sandy soils than in clay soils, hypothesizing that clay-based soils are more prone to anoxia which may limit the survival and growth of Burkholderiaceae. A recent study in Myanmar did not find any conclusive results of such an association between soil texture and *B. pseudomallei* due to a very low positivity rate (Win et al., 2019), while in peninsular Malaysia, Musa et al. (2016) found that the odds of isolating *B. pseudomallei* were significantly higher for samples with higher clay content. Such apparent contradictions between reports further outlines the need for a description of the structural and pedomorphological features of soils layers that accounts for the depth-wise variability of soil physical and biogeochemical conditions and their complex interactions, beyond the mere consideration of soil types or of simple

descriptors such as particle size distribution. In this study, even though *B. pseudomallei* preferentially occurred in clay-rich horizons, it must be noticed that these horizons also had a well-developed structure, as indicated by their low BD (hence high porosity) and high hydraulic conductivity at saturation.

Groundwater level, groundwater persistence rate, water content, bulk density, hydraulic conductivity. Laboratory investigations showed that *B. pseudomallei* can survive about a year in soil with a moisture content of 20% while its survival is reduced to 30 days in dry soil (Tong et al., 1996). Soil, at least near the ground surface, is a medium that is exposed to extreme variations in moisture content, depending on rainfall, air temperature, and wind. While some studies reported higher rates of soil sample positivity for *B. pseudomallei* in the dry season compared to the rainy season (Rolim et al., 2009), previous environmental studies generally suggest that the persistence of *B. pseudomallei* is associated with moist soils (Currie and Jacups, 2003; Kaestli et al., 2016; Liu et al., 2015) and groundwater. Several studies indicate that melioidosis is a disease that prevails in the rainy season (Suebrasri et al., 2013; Wiersinga et al., 2018), when the moisture of soil surface layers is high, increasing the likelihood of agricultural workers to be exposed to the organism, or when *B. pseudomallei* is discharged from naturally occurring seasonal groundwater seeps (Baker et al., 2011).

At the time of sampling the 300 cm soil core analysed and discussed in this chapter (May 2018 - beginning of the rainy season), the ground water table was at a depth of 70 cm, and although *B. pseudomallei* was detected at almost all soil depths, bacteria counts were consistently higher below the depth of 110 cm. This depth of 110 cm is also the depth at which the groundwater persistence rate reaches 100%, indicating that below 110 cm soil

was saturated with water all year-round. Such a co-occurrence of high *B. pseudomallei* counts with high soil moisture content is in agreement with results already obtained in Lao PDR (Manivanh et al., 2017; Rattanavong et al., 2011).

Putative processes that could explain why *B. pseudomallei* is able to survive in dry soil and then become more abundant when the soil is rewetted could include survival in specific niches, locally differing in texture and water-holding capacity hence offering "micro-islands" of relatively wet soil within otherwise dry soil horizons. Another possibility suggested by others would be the upward migration of bacteria from a deep, round-year moist reservoir to shallower horizons, concomitantly with a rising water table (Baker et al., 2011; Thomas et al., 1979).

BD measured along the soil profile varied substantially from soil surface to the depth of 290 cm. There was a strong negative correlation between *B. pseudomallei* counts and BD, indicating that *B. pseudomallei* occurred preferentially in parts of the soil that had a higher porosity (i.e., void/solid ratio). Soil density dropped abruptly from values of about 1.6 $Mg.m^{-3}$ to values of about 1.3 $Mg.m^{-3}$ between 110 and 130 cm, which, quite strikingly, coincides with the depth where both *B. pseudomallei* dramatically increases and where the groundwater persistence rate reaches 100%. Additionally, we found a significant correlation between *B. pseudomallei* counts and Ks. Together with data of soil texture, BD and GPR, the correlation between *B. pseudomallei* counts and Ks indicates that, at that location, *B. pseudomallei* was more abundant in deep clay silt soil layers with a porosity sufficient to allow for a good diffusion of water and gases. Indeed, it was also observed that the more porous horizons where *B. pseudomallei* prevails, between depths of 120 to 180 cm, are characterized by the presence of preferential flow pathways

consisting mostly of biopores. Such an interpretation is corroborated by *in situ* measurements of dissolved oxygen at 210 cm: DO only dropped transiently below 0.5 mg/l (**Figure 4.8**), the threshold below which water is considered as anoxic (Zogorski et al., 2006). Therefore, it can be assumed that oxic conditions, under which organisms can use oxygen for their metabolism, prevailed at the time of sampling at the soil depths where high concentrations of *B. pseudomallei* were observed.

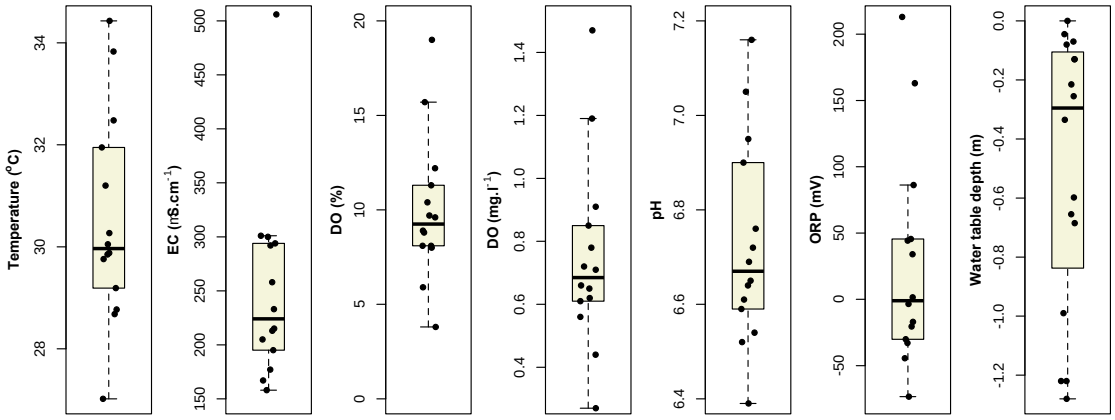


Figure 4.8: Box plots of physico-chemical variables measured in the groundwater using a 210 cm deep piezometer: temperature (^{o}C); electrical conductivity (EC; $\mu S.cm^{-1}$); disssolved oxygen (DO; % and mg/l^{-1}); pH ; oxydo-reduction potential (ORP; mV) ; Water table depth (m). The central horizontal line indicates the median values, and the upper and lower edges of the boxes (hinges) indicate the 25th and 75th percentile values, while the whiskers extend 1.5 × the spread of the hinges. Data points outside this range are indicated with black dots circles.

It has been experimentally observed that the number of soil microorganisms declined linearly with increasing soil density from 1.00 to 1.60 $Mg.m^{-3}$ (Li et al., 2002). Different soil bulk densities correspond to different arrangements of the organic and inorganic constituents of soil, hence different types of porosity, the connectivity and tortuosity of which eventually governs the movement of fluids and associated solutes, particles and

organisms, through soil (Ritz, 2011). Such an arrangement of pores and solids, referred to as soil structure, results in a diversity of niches with contrasted biogeochemical conditions, including substrate availability, hence harbouring diverse microbial communities (Gupta, 2011; Juyal et al., 2014). In turn, metabolic processes associated with these microbial communities are one of the main drivers of soil structure and fertility formation and maintenance (Strong et al., 1998).

Physico-chemical factors. Our experimental results point out several correlations between soil physico-chemical variables and *B. pseudomallei* counts. Soil pH is known to be a strong predictor of soil bacterial community structure (Kim et al., 2016; O'Brien et al., 2019). In this study, soil pH was acidic throughout the profile (average pH=4.86), reaching values above 5 only near the soil surface (resulting in a strong negative correlation with *B. pseudomallei* counts). This result contrasts slightly with results from microcosm experiments that indicate better survival of *B. pseudomallei* within the 5 to 7 soil pH range, with survival reducing below pH = 4 (Wang-Ngarm et al., 2014). Overall, the genus *Burkholderia* appears to be acid-tolerant and field surveys of *B. pseudomallei* indicate that it is indeed generally associated with low pH soils (Kaestli et al., 2009; Stopnisek et al., 2014).

The vertical distribution of *B. pseudomallei* counts in the soil profile was positively correlated with the total concentrations of iron, magnesium, potassium, aluminium, titanium, and negatively correlated with silica and calcium, which might mirror a correlation of *B. pseudomallei* counts with the mineralogical composition of different soil layers. Indeed, *B. pseudomallei* counts were also positively correlated with the finer textured soil material (clay and silt) and anticorrelated with coarse-textured material

(sand), which corresponds to different minerals associations with different surface properties. XRD investigations revealed that the granulometric clay fraction (i.e., <2µm) was mainly composed by a complex clay minerals assemblage (illite, Mg-vermiculite, chlorite, kaolinite, and inter-stratified phases) associated to iron oxyhydroxides (goethite and lepidocrocite). In this granulometric fraction, quartz remained a minor phase although it largely dominated X-ray patterns for bulk soil samples, particularly for the sandiest horizons. Further mineralogical quantification would be needed to unravel this putative correlation between *B. pseudomallei* counts and the different mineralogical phases.

Previous field investigations indicated that, at a depth of 30 cm, the probability of finding *B. pseudomallei* in soil was higher when clay and iron contents were higher (Musa et al., 2016), a result that these authors interpreted as being related to the water and nutrient retention properties of clay minerals, despite their tendency to increase waterlogged, hence anoxic conditions. Other authors found, based on replicate soil sampling at 30 cm depth, that *B. pseudomallei* is more common in soils with low organic matter and nutrients contents, including phosphorus, potassium, calcium, magnesium, and iron (Manivanh et al., 2017), and even that growth-limiting conditions such as nutrient and oxygen limitation can lead to formation of persister cells (O'Rourke et al., 2017).

Iron is known as an essential nutrient for most living organisms, including bacterial pathogens, as it is pivotal to many enzymatic and metabolic processes. *Burkholderia* species, including *B. pseudomallei*, are known to have evolved several iron uptake pathways, including the production of siderophores, and the ability to take up heme in infected hosts (Butt and Thomas, 2017), making them perfectly equipped to mobilize Ferric iron (Fe^{3+}), the less bio-available but most common form of environmental iron in

aerobic environments (Andrews et al., 2013; Duangurai et al., 2018; Schmidt et al., 2018). Reports regarding the association of *B. pseudomallei* with iron in soil are highly contradictory, with positive correlations (Andrews et al., 2013; Musa et al., 2016; Strong et al., 1998) or negative correlations (Hantrakun et al., 2016; Ngamsang et al., 2015) being seemingly equally likely. Yet, most reports did not include any indication of the bioavailability of iron or the oxidation state of the prevailing redox conditions of the environments in which such putative correlations were assessed. While known to be aerobic, *B. pseudomallei* can also survive anaerobiosis and stable subpopulations have been observed to survive under anaerobic conditions for at least one year, although growth was inhibited and metabolism most likely minimal (Hamad et al., 2011). In light of such findings, it is therefore quite possible that, in soils, depending on oxic conditions at the very local scale, *B. pseudomallei* switches metabolism and uses various mixes of ferrous and ferric iron, hence rendering the interpretation of correlations between the organism and total iron very uncertain – and to some extent, meaningless - in the absence of additional information. Indeed, in infected hosts, *B. pseudomallei* is able to cope with iron-restricted conditions by up-regulating its iron-acquisition system and use alternative metabolic pathways (i.e., other available electron donors/receptors) for energy production (Tuanyok et al., 2005).

In the case of the soil profiles studied in this work, we observed that there was a positive correlation between *B. pseudomallei* counts and several metallic element contents, including iron and fine textured soil material in association with a positive correlation with Ks and OM, and a negative correlation with BD, while measured DO values indicated that strictly anoxic conditions rarely occurred at the soil depths where *B. pseudomallei* was the

most abundant (**Figure 4.8**). Together, these observations suggest that, within a given soil profile, *B. pseudomallei* may primarily thrive in horizons where sufficient organic substrates, metallic elements (among which ferric iron), and moisture, are available year-round, and where oxic conditions prevail, as a result of a soil structure (pore network of minimal connectivity and low tortuosity) sufficiently developed to allow minimal circulation of groundwater and supply of oxygen in dissolved form. This does not exclude, as previously reported, the simultaneous occurrence of other subpopulations of *B. pseudomallei* in other soil horizons or subsets (niches) of the same soil horizons where overall conditions substantially differ from that where the bacterium was found to be the most abundant in this study.

4.2 Seasonal variation of *Burkhoderia pseudomallei* distribution along a 300 cm deep soil profile

Recent studies highlighted that the detection of *B. pseudomallei* often correlates with rainfall events (Bulterys et al., 2018; Mukhopadhyay et al., 2018; Ong et al., 2017). The 16 years consensus study on climatic drivers of melioidosis in Lao PDR indicated that melioidosis cases mainly associate with high air humidity and wind speed and not significantly with rainfall event (Bulterys et al., 2018). The rising of water table and sewer discharge can be a source of surface water contamination with *B. pseudomallei*; subsequently, contaminated aerosols may be inhaled and infect people and animals (Chen et al., 2015; Cheng et al., 2006).

Clarifying the effect of seasonal weather conditions the persistence and growth of on bacterial communities in soil, especially for pathogenic agents such as *B. pseudomallei*,

may provide useful indications relative to the putative existence of a soil reservoir for such pathogens and is important to better predict contamination hazard in areas of endemicity. Previous studies have reported high concentrations of *B. pseudomallei* near the soil surface in the rainy season (Palasatien et al., 2008), putatively as a result of the bacterium being uplifted from deeper soil horizons by the rising water table (Wuthiekanun et al., 1995). It must however be noted that most bacterial transport is strongly correlated with solid particle transport (as the majority of bacteria are attached to solids (Bai et al., 2016; Cheng and Currie, 2005) and that most solid transport processes in soil profiles are driven by water infiltration under the effect of gravity.

Microbial communities in soil are influenced by environmental conditions such as pH, moisture, temperature, oxygen concentration, etc. and previous studies have suggested that the occurrence of *B. pseudomallei* in soil is associated with soil moisture and ground water fluctuations (Kaestli et al., 2016; Liu et al., 2015). Previous laboratory investigations showed that *B. pseudomallei* needs a moisture content of 20% to survive year-round in soil (Tong et al., 1996). Plant roots reaching deeper soil layers where ground water is perennially available has been suggested as a feature favourable to the survival *B. pseudomallei* (Kaestli et al., 2009). The relationship between seasonal weather patterns and bacteria persistence and growth are more difficult to clarify in agroecosystems than in forest or grassland because agricultural practices specifically alters physico-chemical factors (Luo et al., 2020). As such, low detection of *B. pseudomallei* near the soil surface could result from agricultural practices at the onset of the rainy season, such as ploughing which is known to influence the distribution of bacteria in the topsoil (Baker et al., 2011) and increases, at least temporarily the likelihood of transport by runoff (Rachlin et al.,

2021). Some authors interpreted high rates of detection *B. pseudomallei* near the ground surface as a positive effect of water and nutrient retention from clay minerals, despite their tendency to increase waterlogged, hence anoxic conditions (Musa et al., 2016).

In **Figure 4.9**, we report the average monthly concentrations of *B. pseudomallei* over the full 300 cm profile according to three soil depth increments, together with average monthly rainfall and average monthly water table depth.

In **Figure 4.10**, we consider the average monthly concentrations of *B. pseudomallei* according to three soil depth increments, namely 0-100 cm, 100-200 cm and 200-300 cm.

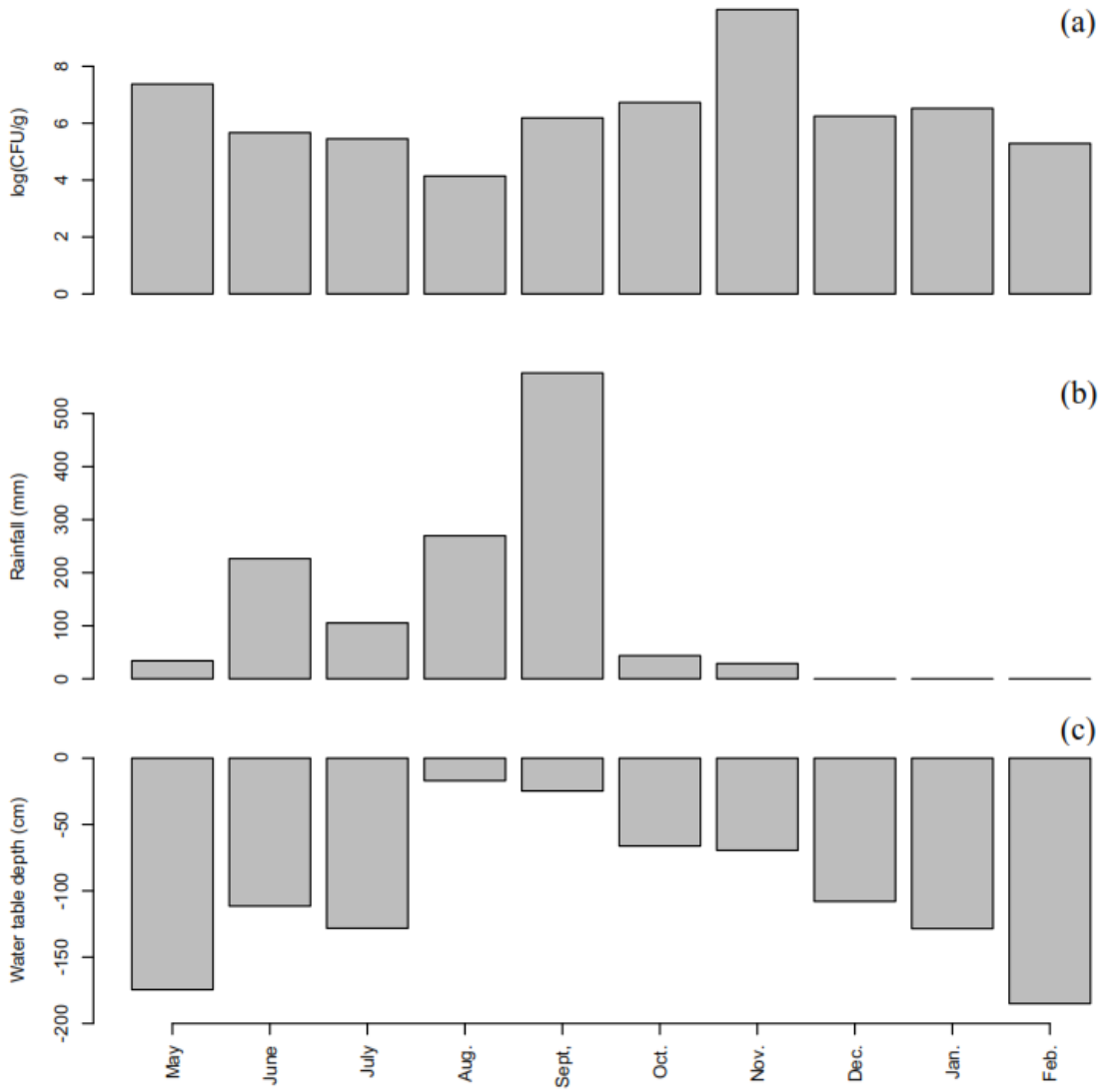


Figure 4.9: Monthly observations – from May 2019 to February 2020 - of (a) average semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) along the 300-cm deep profile; (b) Monthly rainfall (mm); (c) Water table depth (cm).

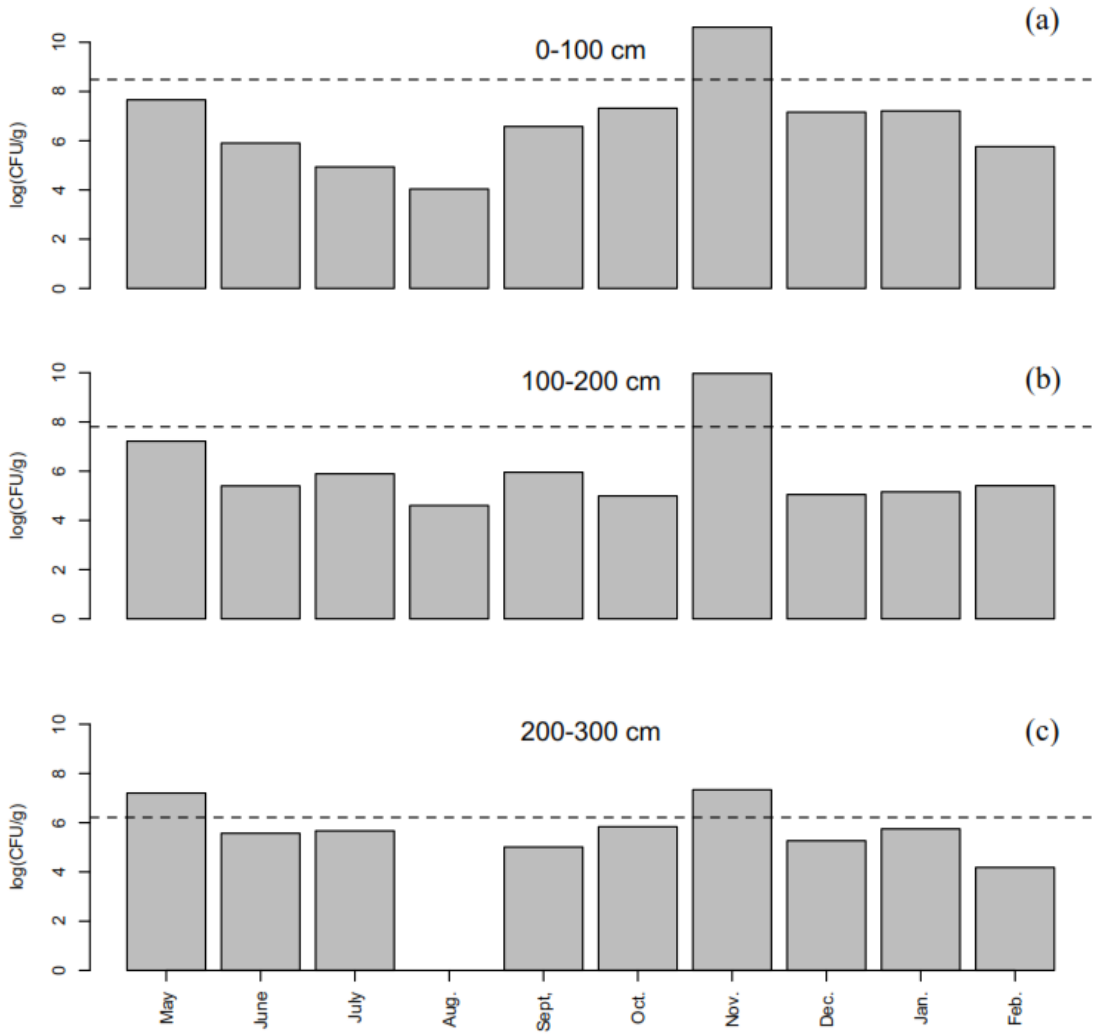


Figure 4.10: (a) Semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) along the 0 - 100-cm deep soil profile; (b) Semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) along the 100 - 200-cm deep soil profile; (c) Semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) along the 200 - 300-cm deep soil profile from May 2019 to February 2020.

The results of the semi-quantitative culture of soil samples taken at monthly intervals along the 300-cm deep profile over a 10-month period from May 2019 – February 2020 are shown in **Figure 4.9**. *B. pseudomallei* was consistently detected in both the dry and the rainy seasons, but was found at all soil depths but one (positivity rate of 96.7%) only in May 2019, November 2019, and February 2020. The positivity rate was less than 80%

(dropping to 23% in August) from June to October while it rose again above 80% from November to February. While the lowest positivity rates could result, to some extent form technical issues (false negatives), a clear trend characterized by overall lower positivity rates during the rainy season emerges from this dataset.

The average concentrations of *B. pseudomallei* varied drastically, spanning four orders of magnitude, from 5 to over 50,000 CFU.g^{-1}. The highest counts were detected in November 2019 towards the final stages of the wet season while the second highest concentration was found in mid of May 2019, i.e., early in the rainy season. Indeed, *B. pseudomallei* detection has often been reported to be higher in the rainy season (Suebrasri et al., 2013; Swe et al., 2021). However, other studies have reported higher rate of *B. pseudomallei* detection in the dry season (Kaestli et al., 2007; Wuthiekanun et al., 1995).

CFU values averaged for successive 30-cm soil layers and over the May 2019 to February 2020 period (**Figure 4.11**) indicate that the highest *B. pseudomallei* concentrations were detected between the soil depths of 120 and 250 cm with a maximum observed between 120 and 150 cm, i.e., in soil layers with the highest clay content (**Figure 4.3**) as previously observed when analysing the 300 cm soil cores collected in May and December 2018 (**Figure 4.1**).

Importantly, extreme *B. pseudomallei* concentration of the order of 10^9 - 10^{10} CFU.g^{-1} were found on several occasions, which raises the question of potential methodological issues as it is generally accepted that the maximum concentration of total bacteria in one gram of soil, encompassing between $4 \cdot 10^3$ to $5 \cdot 10^4$ species, is of the order of 10^{10} bacterial cells (Roesch et al., 2007; Torsvik et al., 1990).

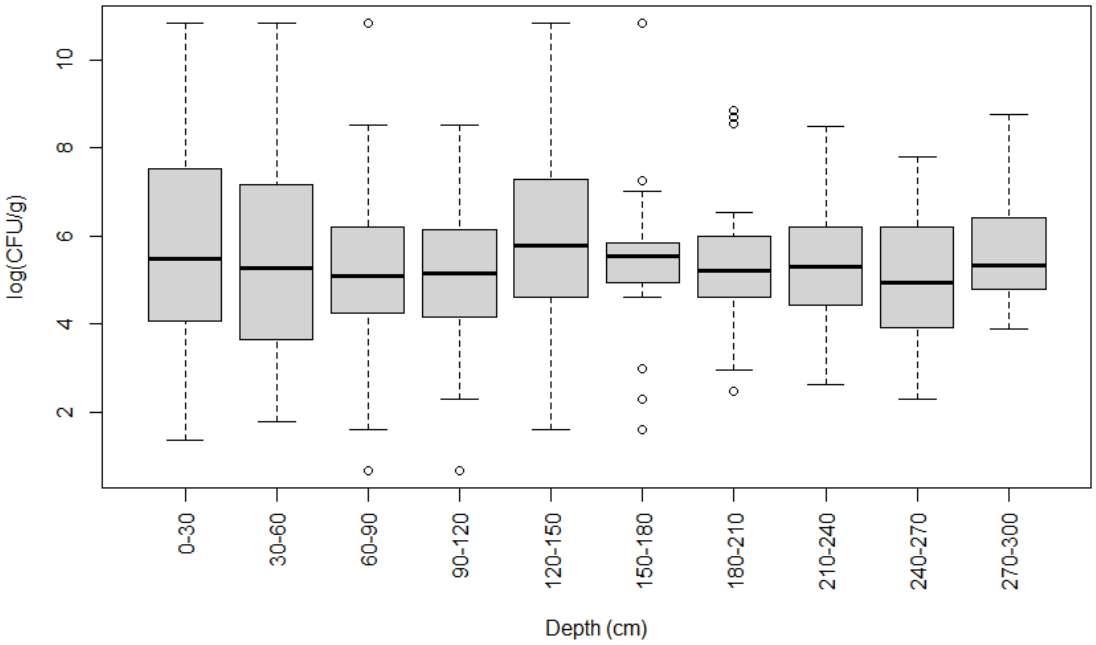


Figure 4.11: Box-whisker plots of log-transformed semi-quantitative colony forming unit (CFU) counts of *B. pseudomallei* (CFU g^{-1}) for successive 30-cm soil layers, observed from May 2019 to February 2020. The central horizontal line indicates the median value, and the upper and lower edges of boxes (hinges) correspond to the 25th and 75th percentile values, while the whiskers extend 1.5× beyond the spread of the hinges. Data points outside this range (outliers) are indicated with circles.

It is also noteworthy that the seasonal variability of *B. pseudomallei* was higher from the surface down to the depth of 150 cm than between 150 and 210 cm, while it increased again between 210 and 300 cm. This finding is in agreement with the hypothesis that a higher soil water content and groundwater persistence during dry season could be favorable for the organism to persist throughout the year and be transported toward the soil surface with the rising water table during the rainy season (Thomas et al., 1979). The observed higher variability of CFU values near the soil surface and within the first meter of the profile could result from a higher variability of physico-chemical conditions in the corresponding soil layers, particularly soil moisture, temperature, and oxygen concentration.

When considering the whole soil profile, concentrations of *B. pseudomallei* varied over the course of the 10-month period of observation. In a first phase, from May to August, average monthly concentrations over the full 300-cm profile decreased (**Figure 4.9**). The lowest concentration was observed in August, with detection of the bacterium between the soil depths of 0 to 200 cm only (**Figure 4.10**). In a second phase, from August to November, average concentrations increased and reached a maximum towards the end of the rainy season, in November. Subsequently, from December to February, average concentrations decreased again to a level comparable to that observed early in the rainy season. While it cannot be dismissed that the absolute maximum of May and November may correspond to localized hot-spot of *B. pseudomallei*, as successive soil cores could not be collected exactly at the same position, it remains that these results suggest a clear seasonal pattern of the concentrations of *B. pseudomallei* over the whole 300-cm soil profile. These trends are to some extent consistent with the results of Manivanh et al. (2017) who reported, for the first 90 cm of the same soil profile, higher average CFU counts towards the end and the beginning of the dry season than during the rainy season.

When considering this seasonal pattern in relation to monthly rainfall and water table depth, it appears that higher average values of *B. pseudomallei* concentration (over the whole depth of the 300 cm profile) occurred both in May and November, in months when the rainfall was modest, with the water table rising in May and subsiding in November (**Figure 4.9**). Such an observation apparently contradicts the model according to which the organism would merely migrate upward following the rise of the water table, subsequently to periods of heavy rainfall. While such a model could possibly correspond to the November peak, it is not consistent with the May maximum which occurred

following the prolonged seasonal drought characteristic of the climate at the study location. Further, *B. pseudomallei* counts at soil depths > 100 cm in the months preceding the November peak were not higher at depth than nearer the soil surface (**Figure 4.10**).

When considering the three successive 100-cm soil layers, as in the case of average values computed over the whole soil profile, higher concentrations were found towards the end of the dry season in May 2019 and towards the end of the wet season, in November 2019.

B. pseudomallei concentrations displayed much more pronounced seasonal variations in the first 100 cm of the profile than in deeper horizons while the 10-month average (horizontal dotted lines in **Figure 4.10**) decreased with depth. While the November 2019 peak was apparent in both the 0-100 and 100-200 cm layers, it did not differ in magnitude from the May 2019 maximum in the 200-300 cm layer. Apart from these two extreme values, average concentrations observed between 100 and 200 cm appeared rather constant (which also holds, to a lesser extent for the 200-300 cm layer).

4.3 Distribution of *Burkhoderia pseudomallei* according to soil redoximorphic features

4.3.1 Soil features

Four 90-cm soil cores, ~50 mm in diameter were taken at the Nabone village site in November 2017, i.e., at the transition between rainy and dry season. These soil cores were divided into five subsamples, each approximately 20 cm long, from which three soil fractions were selectively collected, according to soil color (**Figure 3.4**). Observation of these cores at the millimeter scale revealed two main types of redoximorphic features

distinguished on the basis of their colour: orange to red (oxidized features), corresponding to the predominance of ferric iron (Fe^{3+}), and grey colour (reduced features) corresponding to the predominance of ferrous iron (Fe^{2+}). The first 20-30 cm of the cores encompassed the hydragric A-horizon with a sandy-loam texture and high organic matter content resulting in a dark greyish brown color of the soil matrix. Dominant redoximorphic features in this horizon have a reddish color. From 30 to 90 cm, the second soil horizon, or albic horizon (Er-horizon) of predominantly sandy texture with a light grey color corresponding to lower organic matters than the A-horizon, displayed iron red nodules that started to be observed at the depth of 70-90 cm.

Selective sampling of these oxidized and reduced soil fractions was carried out manually using a scalpel to carefully dissect very small volumes of soil based on their apparent colour (as checked under the binocular microscope). As long as the colour did not appear to change substantially from the colour of the soil first sampled, careful scraping of more soil was carried out until a mass of about 1 g was gathered. Often, sampling from several separate redoximorphic features had to be pooled together to obtain the desired amount of soil. Even though carried out with care, such a sampling remains far from perfect as, due to the complex spatial arrangement of redoximporphic features, it is in practice impossible to totally avoid a partial mixing of the different fractions.

4.3.2 Presence of *Burkholderia pseudomallei* at the millimeter scale

The soil system is a complex environment that displays a mosaic of micro-environments each with specific physical, chemical, biological, and structural characteristics, that may favour/challenge the survival of microbial communities, depending on prevailing

biological process and biogeochemical function (Ranjard and Richaume, 2001). Unfavorable environmental conditions may force *B. pseudomallei* to adapt to survive in environmental niches. However, there are no clear reports about their adaptation response to multiple sources of stress in the environment. In the environment, *B. pseudomallei* can be isolated as a free-living organism in different ecological niches such as soil, water, and plant rhizosphere (Inglis and Sagripanti, 2006), as well as under parasitic form living in host organisms (amoeba, plants, fungi, and animal) where its adaptation to survive in ecological niches is a complex process that depends on various environmental factors (Duangurai et al., 2018). The presence of iron, a microelement that is plentiful in soils, plays an important role as a cofactor of enzymes in cell, and metabolic processes essential to the survival of many bacteria. Therefore, it has been proposed that high iron concentrations could support the growth of *B. pseudomallei* in soil (Gerhardy and Simpson, 2013; Schmidt et al., 2018). Nevertheless, to our knowledge, only total iron has been considered in previous environmental studies of *B. pseudomallei* and no consideration has yet been given to the potentially contrasted role of the oxidation states of iron with regards to the occurrence and growth of this organism in soil. Indeed, it is known that some bacteria generate energy from ferrous iron which they use as an electron donor while some other rely on ferric iron as an electron acceptor (Bird et al., 2011). Gaining knowledge about the reliance/flexibility of *B. pseudomallei* relative to these two forms of iron may shed new light upon environmental niches in which the organism is most likely to persist and be found.

The semi-quantitative culture method was applied to estimate *B. pseudomallei* concentration in two different types of soil redoximorphic feature: oxidized and reduced

zones (**Figure 4.12**). *B. pseudomallei* concentrations observed in our November 2017 samples ranged from 0 to 5,400 CFU.g^{-1}. The average detection of *B. pseudomallei* in this set of samples was 1,169 CFU.g^{-1}.

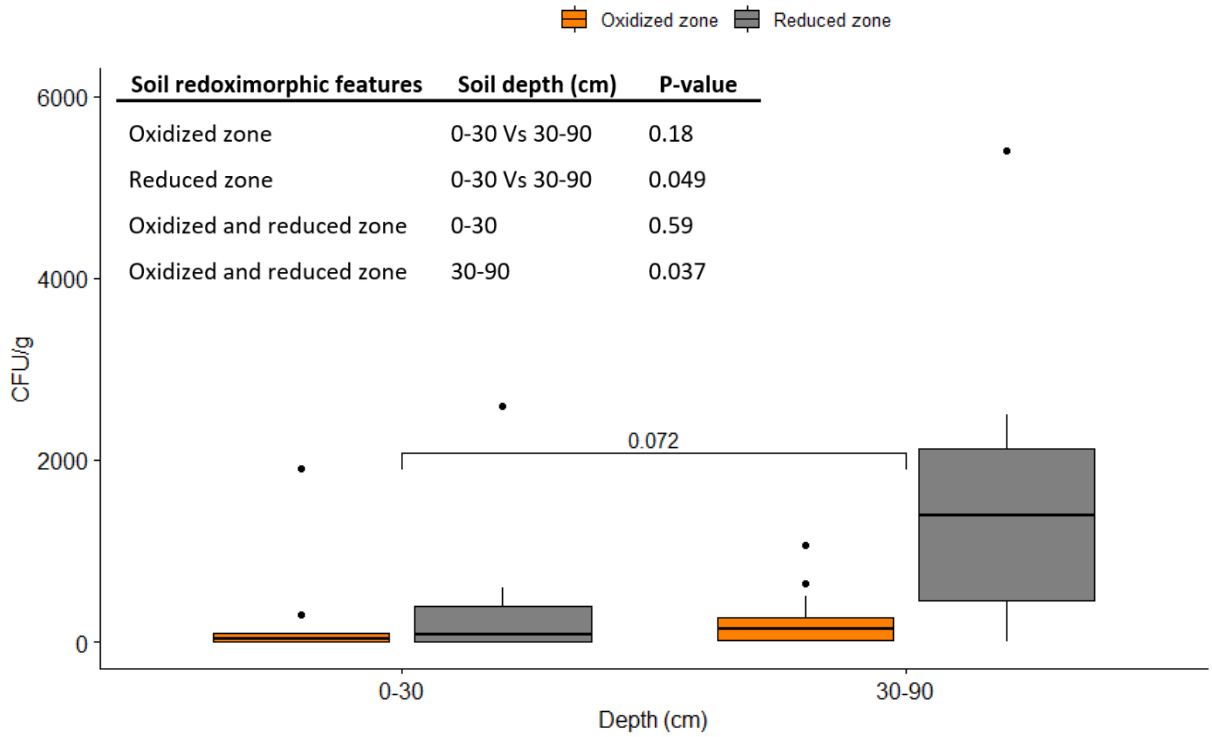


Figure 4.12: Distribution of *B. pseudomallei* (CFU/g) in oxidized redoximorphic feature (orange and red soil color) and reduced redoximorphic feature (grey soil color) along 0 – 90 cm deep soil profile.

In the A soil horizon (0 - 30 cm), *B. pseudomallei* was found at a concentration of 4 - 1,899 CFU.g^{-1} and 10 – 2,600 CFU.g^{-1} in the oxidized (predominance of ferric iron – Fe^{3+}) and reduced (predominance of ferrous iron Fe^{2+}) fractions, respectively. Although higher on average in reduced redoximorphic features, the *B. pseudomallei* concentrations were not significantly higher within this soil horizon.

At the depth of 30 - 90 cm, *B. pseudomallei* was detected in the two types of soil fractions with significantly lower ($p<0.05$) concentrations in the oxidized (4 – 1,650 $CFU.g^{-1}$), than in the reduced redoximorphic features (10 – 5,400 $CFU.g^{-1}$).

Iron is an essential nutrient for most living organisms, including bacterial pathogens, as it is pivotal to many enzymatic and metabolic processes. *Burkholderia* species, including *B. pseudomallei*, are known to have evolved several iron uptake pathways, including the production of siderophores, and the ability to take up heme in infected hosts (Butt and Thomas, 2017), which allows then to mobilize ferric iron (Fe^{3+}), the less bio-available but most common form of environmental iron in aerobic environments (Andrews et al., 2013; Duangurai et al., 2018; Schmidt et al., 2018).

Reports regarding the association of *B. pseudomallei* with iron in soil are highly contradictory, with positive correlations (Andrews et al., 2013; Musa et al., 2016; Strong et al., 1998) or negative correlations (Suebrasri et al., 2013; Hantrakun et al., 2016; Ngamsang et al., 2015) reported in the literature. Remarkably though, none of these reports includes any indication of the bioavailability of iron or the redox conditions of the environments in which the observations were made. While known to be aerobic, *B. pseudomallei* is also capable of stable long-term anaerobic survival (Hamad et al., 2011).

Moreover, in infected hosts, *B. pseudomallei* is able to cope with iron-restricted conditions by up-regulating its iron-acquisition system and use alternative metabolic pathways (i.e., other available electron donors/receptors) for energy production (Tuanyok et al., 2005).

Consistently with such survival in anaerobiosis observed in lab conditions, our study of *B. pseudomallei* distribution at the millimeter scale shows that the pathogen was

significantly more abundant in soil redoximorphic features where ferrous iron prevail, at least within the eluvial horizon. This might indicate that, while it can also grow and is indeed present in oxic parts of the soil system, *B. pseudomallei* might proliferate more easily in hypo- and anoxic compartments where competitive pressure from other organisms might be less intense.

In light of such findings, we propose that, in soils, depending on oxic conditions at the very local scale, *B. pseudomallei* has the ability to switch metabolism to obtain energy from various mixes of ferrous and ferric iron. Such a result also outlines that interpretation of correlations between the presence/concentration of *B. pseudomallei* and total iron is most likely misleading. We conclude that future studies about the role of iron with regards to the survival and growth of *B. pseudomallei* in soil should include information about the redox status of the iron compounds considered and their spatial distribution relative to that of the bacterium.

4.4 Limitations of the methodological approach

An important methodological limitation of the results presented in this thesis stems from the lack of internationally-validated methods for accurately detecting and quantifying *B. pseudomallei* in environmental samples such as soil or water. Molecular methods have generally given higher yields than culture-based methods and have the ability to detect bacteria that are in a viable but non-culturable state (as well as non-viable organisms), whilst cultural methods have varied considerably in their sensitivity in different studies (Dance et al., 2018a).

All detection methods lack precision and none will reliably determine which soil samples contain *B. pseudomallei*. We therefore chose to use a well-established semi-quantitative culture method for this study, rather than introducing a molecular assay, which would have required additional validation in this setting.

Chapter 5: Conclusion and perspectives

Chapter 5: Conclusions and perspectives

5.1 Conclusions

In this study, we have presented and discussed the space and time distributions of *B. pseudomallei* according to three different research axes**:** (1) "snapshot" distribution of *B. pseudomallei* along a 300 cm deep soil profile in relation to soil physico-chemical variables; (2) seasonal variations in the distribution of *B. pseudomallei* along a 300 cm deep soil profile; (3) distribution of *B. pseudomallei* at the millimeter scale in the first 90 cm of the soil profile. Previous reports of *B. pseudomallei* occurrence in deep bore water do not directly compare with our study as they did not attempt to elucidate the associated soil geochemical environment. The novelty of the results presented in this thesis is that unravelling the environmental conditions favourable to *B. pseudomallei* persistence and proliferation entails considering many aspects of the actual complexity of soil. This complexity includes not only soil chemical and textural characteristics, but also its hydrodynamic properties, its structural features nested over a wide range of scales, as well as its general arrangement in layers, the sequence of which forms the soil profile. As a direct consequence of the work presented in this thesis, it can be reasonably assumed that the odds of isolating *B. pseudomallei* in dry environments would often been higher from deep than shallow soil layers. However, our observations along the 300 cm deep soil profile showed that the concentration of *B. pseudomallei* varied drastically throughout the year. Such observations of seasonal variations of *B. pseudomallei* in soil provide useful indications to clarify the existence of a soil reservoir for this organisms and to better predict the risk of contamination in endemic areas. This 10-month campaign enabled us

to observe that the *B. pseudomallei* seasonality of *B. pseudomallei* concentrations in the first 100 cm of the profile was more pronounced than in deeper horizons. Several environmental reservoirs potentially offer niches for *B. pseudomallei* (Seng et al., 2019); while uneven distribution of *B. pseudomallei* in soil has been previously reported (Limmathurotsakul et al., 2010b), our results shed new light, at unprecedented detail, in terms of scale and physico-chemistry, on conditions that may favour or limit the survival of *B. pseudomallei* in soil. In particular, our results indicate that the spatial distribution of *B. pseudomallei* does not relate on total iron concentrations but rather on the redox status of iron compounds. Our results also suggest that the pathogen may switch metabolism to obtain energy from various form of iron such as ferrous and ferric iron. Finally, even though this point was only very marginally considered in this study, the inherently dynamic nature of the complex biogeochemical interactions that constantly transform the soil environment must also be taken into account, as transient changes in variables of central importance for the metabolism of microorganisms, such as DO, can vary drastically within a matter of hours under the influence of weather conditions.

Key recommendations from this work regarding the environmental sampling of *B. pseudomallei* include:

- Collecting samples at successive depth increments from the soil surface, as many soil properties vary much more drastically vertically than horizontally. Hence a single soil profile maximizes the odds of detecting *B. pseudomallei* at a given location compared to the same number of samples taken at a single soil depth;

- Collecting samples down to the depth at which the water table is found at the time of sampling, as groundwater persistence appears to be an important factor for the persistence of *B. pseudomallei*;

- Recording basic pedological information such as soil colors and macrostructural features (redoximorphic features), as these simple observations provide valuable information about the redox conditions at the point of sampling.

Despite the significant advances that this study brings for a better understanding of the ecology of *B. pseudomallei*, it still leaves important questions unaddressed; for example, while we were able to find that lateral variations in soil properties in association with different pedological features along a soil profile influence the distribution of *B. pseudomallei*, we did not elucidate whether populations detected in different parts of the soil profile correspond to a single or several strains.

As it sheds new light on how *B. pseudomallei* is vertically distributed within a soil profile, including at depths generally not taken into account in environmental *B. pseudomallei* studies, this work also identifies new potential hazards related to human activities that involve interactions with deep soil layers, seasonal change and redoximorphic soil features. This is potentially clinically significant because human activities have become the main geomorphological process on the Earth's surface, disturbing and displacing globally ten times more soil, sediment, and rock material than natural geological processes (Wilkinson, 2005). Hence, beyond agriculture, which generally involves no or limited interactions between humans and deep soil layers, earthworks across the globe are now dramatically increasing chances of people being in contact with material extracted from

several meters under the soil surface, potentially hosting pathogenic bacteria, as shown in this study. As part of future studies, recent advances in hydrological modelling might prove an avenue to predict the occurrence, concentrations and transport as a function of rainfall and hydrological conditions.

5.2 Perspectives

The dataset presented in this thesis, particularly the data corresponding to the 300-cm profiles collected in May 2018, December 2018, and from May 2019 to February 2020, converge in indicating that:

1. There is some degree of persistence (lower time variability) of the *B. pseudomallei* concentrations in soil layers where GPR was 100% with the highest clay content;

2. Peaks of *B. pseudomallei* concentrations are not synchronous with rainfall and periods during which the water table is the shallowest, suggesting that processes other than a simple “piston” effect that displaces populations of the pathogen from deeper soil layers nearer the soil surface are at work; and

3. Seasonality mostly affects the distribution of *B. pseudomallei* in shallow soil horizons, i.e., in the case of the study site, within the first meter of the soil profile, which might also be reinforced by the contrast in soil texture that characterizes the studied soil profile.

Further, the observations made at the millimeter scale conducted in November 2017 with an overall depth of 90 cm indicate that:

- The distribution of *B. pseudomallei* in soil can vary drastically within a few millimeters only;

- Considering total iron as an environmental determinant of the occurrence of *B. pseudomallei* is probably misleading as concentrations of the organism appear to vary depending on the oxidation state of iron;

- In relation to the previous point, *B. pseudomallei* likely switches metabolism at very local scales depending on where it sits in the soil system.

Finally, the consistency between results obtained on the occasion of three separate experiments over a period of over two years points out that, despite the methodological limitations of the semi-quantitative approach that was used, the results reported in this thesis can be regarded with a good degree of confidence.

Chapter 5: Conclusions and perspectives

5.3 Conclusions

Dans cette thèse, nous avons présenté et discuté les distributions spatiales et temporelles de *B. pseudomallei* selon trois axes de recherche : (1) distribution " instantanée " de *B. pseudomallei* le long d'un profil de sol de 300 cm de profondeur en relation avec les variables physico-chimiques du sol ; (2) variations saisonnières de la distribution de *B. pseudomallei* le long d'un profil de sol de 300 cm de profondeur ; (3) distribution de *B. pseudomallei* à l'échelle millimétrique dans les 90 premiers cm du profil de sol. Les études précédentes sur la présence de *B. pseudomallei* dans les eaux de forage profondes ne sont pas directement comparables à notre étude car ils ne visaient pas élucider l'environnement géochimique du sol associé à la présence de la bactérie. Un aspect novateur des résultats présentés dans cette thèse réside dans le fait que nous montrons que les conditions environnementales favorables à la persistance et à la prolifération de *B. pseudomallei* recouvrent de nombreux aspects de la complexité réelle du sol. Cette complexité comprend non seulement les caractéristiques chimiques et texturales du sol, mais aussi ses propriétés hydrodynamiques, ses caractéristiques structurelles imbriquées sur une large gamme d'échelles, ainsi que sa disposition générale en horizons, dont la séquence forme le profil du sol. En lien direct avec le travail présenté dans cette thèse, on peut raisonnablement supposer que la probabilité d'isoler *B. pseudomallei* dans des environnements arides est plus élevée dans les horizons profonds que près de la surface du sol. Cependant, nos observations le long du profil de sol de 300 cm de profondeur ont aussi montré que la concentration de *B. pseudomallei* variait drastiquement tout au long de l'année. De telles observations des variations saisonnières de *B. pseudomallei* dans le

sol clarifient l'existence d'un réservoir dans le sol pour cet organisme et fournit des éléments pour mieux prédire le risque de contamination dans les zones endémiques. Le suivi de 10 mois que nous avons conduit nous a permis d'observer que la saisonnalité des concentrations de *B. pseudomallei* dans les 100 premiers cm du profil était plus prononcée que dans les horizons plus profonds. Plusieurs réservoirs environnementaux offrent potentiellement des niches pour *B. pseudomallei* (Seng et al., 2019); alors qu'une distribution inégale de *B. pseudomallei* dans le sol a déjà été rapportée (Limmathurotsakul et al., 2010b), nos résultats apportent un éclairage nouveau, à un niveau de détail sans précédent, en termes d'échelle et de physico-chimie, sur les conditions qui peuvent favoriser ou limiter la survie de *B. pseudomallei* dans le sol. En particulier, nos résultats indiquent que la distribution spatiale de *B. pseudomallei* ne dépend pas des concentrations totales en fer mais plutôt de l'état d'oxydo-réduction des composés du fer. Nos résultats suggèrent également que le pathogène peut changer de métabolisme pour obtenir de l'énergie à partir de différentes formes de fer telles que le fer ferreux et le fer ferrique. Enfin, même si ce point n'a été que très marginalement pris en compte dans cette étude, la nature intrinsèquement dynamique des interactions biogéochimiques complexes qui transforment constamment l'environnement du sol doit également être prise en compte ; en effets, des variables d'une importance centrale pour le métabolisme des microorganismes, comme la concentration en oxygène dissout dans la solution du sol, peuvent varier drastiquement en quelques heures sous l'influence des conditions météorologiques.

Plusieurs recommandations importantes concernant l'échantillonnage environnemental de *B. pseudomallei* peuvent être formulées sur la base de ce travail de thèse :

- Effectuer des prélèvement à des profondeurs successives à partir de la surface du sol, car de nombreuses propriétés du sol varient beaucoup plus suivant la direction verticale que latéralement. Par conséquent, un seul profil de sol maximise les chances de détecter *B. pseudomallei* à un endroit donné par rapport au même nombre d'échantillons prélevés à une seule profondeur de sol;
- Prélever des échantillons jusqu'à la profondeur à laquelle se trouve la nappe phréatique au moment de l'échantillonnage, car la persistance des eaux souterraines semble être un facteur important pour la persistance de *B. pseudomallei*;
- Enregistrer les informations pédologiques de base telles que les couleurs du sol et les caractéristiques macrostructurales (traits redoximorphiques), car ces observations simples fournissent des informations précieuses sur les conditions redox au point d'échantillonnage.

Malgré les avancées significatives que cette étude apporte pour une meilleure compréhension de l'écologie de *B. pseudomallei*, elle laisse encore d'importantes questions en suspens ; par exemple, si nous avons pu constater que les variations latérales des propriétés du sol en association avec différentes traits pédologiques le long d'un profil de sol influencent la distribution de *B. pseudomallei*, nous n'avons pas elucide si les populations détectées dans différentes parties du profil de sol correspondent à une seule ou plusieurs souches.

En apportant un nouvel éclairage sur la façon dont *B. pseudomallei* est distribuée verticalement dans un profil de sol, y compris à des profondeurs généralement non prises en compte dans les études environnementales sur *B. pseudomallei*, ce travail identifie également de nouveaux risques potentiels liés aux activités humaines qui impliquent des

interactions avec les couches profondes du sol, les changements saisonniers et les caractéristiques redoxymorphes du sol. Cela peut avoir une importance clinique car les activités humaines sont devenues le principal processus géomorphologique à la surface de la Terre, perturbant et déplaçant globalement dix fois plus de sol, de sédiments et de matériaux rocheux que les processus géologiques naturels (Wilkinson, 2005). Par conséquent, au-delà de l'agriculture, qui n'implique généralement pas ou peu d'interactions entre les humains et les couches profondes du sol, les travaux de terrassement à travers le monde augmentent considérablement les chances que les populations humaines entrent en contact avec des matériaux extraits de plusieurs mètres sous la surface du sol, potentiellement chargés de bactéries pathogènes, comme le montre cette étude. Dans le cadre d'études futures, les progrès récents en matière de modélisation hydrologique pourraient permettre de prédire l'occurrence, les concentrations et le transport du pathogène en fonction des précipitations et des conditions hydrologiques.

5.4 Perspectives

L'ensemble des données présentées dans cette thèse, en particulier les données correspondant aux profils de 300 cm collectés en mai 2018, décembre 2018 et de mai 2019 à février 2020, convergent pour indiquer que:

1. Il y a un certain degré de persistance (variabilité temporelle plus faible) des concentrations de *B. pseudomallei* dans les couches de sol les plus argileuses où le taux de persistance des eaux souterraines atteint 100%;

2. Les pics de concentration de *B. pseudomallei* ne sont pas synchronisés avec les précipitations et les périodes pendant lesquelles la nappe phréatique est la moins profonde, ce qui suggère que des processus autres qu'un simple effet de "piston" qui déplacerait les populations de l'agent pathogène des couches de sol plus profondes vers les horizons plus proches de la surface du sol sont à l'œuvre ; et

3. La saisonnalité affecte principalement la distribution de *B. pseudomallei* dans les horizons de sol peu profonds, c'est-à-dire, dans le cas du site étudié, dans le premier mètre du profil de sol, ce qui pourrait également être renforcé par le contraste de texture du sol qui caractérise le profil de sol étudié.

De plus, les observations réalisées à l'échelle millimétrique menées en novembre 2017 dans les premiers 90 cm du profil indiquent que:

- La distribution de *B. pseudomallei* dans le sol peut varier drastiquement en l'espace de quelques millimètres seulement;
- Considérer le fer total comme un déterminant environnemental de la présence de *B. pseudomallei* est probablement insuffisant car les concentrations de l'organisme semblent varier en fonction de l'état d'oxydation du fer;
- En relation avec le point précédent, *B. pseudomallei* change probablement de métabolisme à des échelles très locales en fonction de sa position dans le système sol.
- Enfin, la cohérence entre les résultats obtenus à l'occasion des trois expériences de terrain distinctes menées sur une période de plus de deux ans dans le cadre de cette thèse indique que, malgré les limites méthodologiques de l'approche semi-quantitative qui a été utilisée, nos résultats peuvent être considérés avec un bon degré de confiance.

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