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SELENATE (SeO_4) AND SELENITE (SeO_3) TOXICITY DISCERNED FROM TRANSCRIPTOMIC ANALYSIS IN THE FRESHWATER INSECT, *Chironomus kiiensis*

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in Aquatic Toxicology in the Institute of Oceanography and Environment Universiti Malaysia Terengganu

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DEDICATION

Dedicated this thesis to:

My everlasting love: Aboh & Ma.

My anchor throughout the storm & turbulence: The BIG ARMY!!

Abang Mie & K.Aisyah, Kak Tie, Abang Yus & Kak Irda, Kak Fiza & Abang Jamal, Kak Yuri & Abang Fizam, Kak Izu & Abang Rudi, Kak Ita & Abang Man, Is & Teha, Zack, Iman, Emy, Ely, Ucu and all nieces & nephews.

My mentor: Prof. Emeritus Dr. Noor Azhar Bin Mohamed Shazili

I owe you guys, big time…

Unconditional love

Endless support

Priceless doa

THANK YOU FOR EVERYTHING!

May Allah bless..

ABSTRACT

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

SELENATE (SeO_4) AND SELENITE (SeO_3) TOXICITY DISCERNED FROM TRANSCRIPTOMIC ANALYSIS IN THE FRESHWATER INSECT, *Chironomus kiiensis*

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School/Institute : Institute of Oceanography and Environment (INOS)

Selenium is an essential micronutrient for living tissues, including aquatic insects but can be very toxic at slightly above the threshold level. Recent evidence indicates that selenite (SeO_3) is more toxic than selenate (SeO_4) to aquatic organisms. However, the question of 'how selenite toxicity differs from selenate' remain unsolved. Therefore, the present study was designed to investigate the toxicity of selenate and selenite to freshwater midges, *Chironomus kiiensis* as well as to address the toxicity and accumulation of Se at different life stages of this insect. The 96-hour acute toxicity test was carried out on 2nd instar larvae which were exposed to 0, 1.0, 10, 32, 56 and 100 mgSeL^{-1}. In sub-lethal tests of 14-d duration, 3-day old post-hatch larvae were exposed to 0, 10%, 25%, 50% and 100% of the 96-h LC_{50} value and the accumulation of Se measured in every life stage of *C. kiiensis*. The 96-h LC_{50} values of SeO_4^{2-} and SeO_3^{2-} were 16.2 mgSeL^{-1} and 10.6 mgSeL^{-1}, respectively, indicating that SeO_3 is more toxic than SeO_4. The selenium accumulation pattern at the different life stages of chironomid also differed and ranked from most to least accumulated, for selenate exposure: Exuviae > Larval > Pupa > Egg > Adult while for selenite exposure:

Larvae > Exuviae > Pupa > Adult. For molecular analysis, the samples of chironomid larvae from the highest concentration of LC_{50} value of both metal exposure were used. All samples were sequenced on the Illumina Hiseq 2000 platform, *de novo* assembled and analyzed for differential expression profiling. A total of 43,493,744 and 45,599,362 raw reads each for selenate and selenite were obtained, generating 6.52 Gb and 6.84 Gb of data, respectively. A total of 186 transcripts were differentially expressed with 144 up-regulated and 42 down-regulated. In response to both selenate and selenite exposure, HSPs, GST, CYP P450, metallothionein and silk protein genes were found differentially expressed, as well as several pathways including proteasome, autophagy regulation, mTOR and MAPK signaling pathway that involve in cell death program. Overall, the findings of this study paves the way for a more in-depth understanding of the underlying molecular mechanism involved *Chironomus kiiensis* in response to different types of inorganic selenium toxicity.

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ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

KETOKSIKAN SELENATE (SeO_4) DAN SELENITE (SeO_3) BERASASKAN ANALISIS TRANSKRIPTOM DALAM SERANGGA AIR TAWAR, *Chironomus kiiensis.*

NOOR AZNAINI BINTI MOHD DAUD

OKTOBER 2019

Penyelia Utama : Professor Emeritus Noor Azhar Bin Mohamed Shazili, PhD

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Selenium (Se) adalah mikronutrien penting untuk tisu hidup, termasuk serangga akuatik tetapi boleh menjadi sangat toksik pada paras melebihi melebihi sedikit aras keperluan. Bukti terkini menunjukkan bahawa selenite (SeO_3) adalah lebih toksik daripada selenate (SeO_4) kepada organisma akuatik. Walau bagaimanapun, persoalan 'bagaimana ketoksikan selenite berbeza daripada selenate' masih tiada jawapan. Oleh itu, kajian ini bertujuan untuk mengkaji ketoksikan selenate dan selenite terhadap agas air tawar, *Chironomus kiiensis* serta untuk menangani keracunan dan pengumpulan Se pada peringkat kitar hidup yang berbeza serangga ini. Ujian 96-jam ketoksikan akut telah dijalankan ke atas larva instar ke-2 yang didedahkan kepada 0, 1.0, 10, 32, 56 dan 100 $mgSeL^{-1}$. Dalam ujian 14-hari sub-kronik, larva berusia 3 hari selepas menetas didedahkan kepada 0, 10%, 25%, 50% dan 100% daripada nilai 96-jam LC_{50} dan pengumpulan Se diukur dalam setiap peringkat kitar hidup *C. kiiensis*. Nilai 96-jam LC_{50} bagi SeO_4^{2-} dan SeO_3^{2-} adalah 16.2 $mgSeL^{-1}$ dan 10.6 $mgSeL^{-1}$, setiap satu, yang menunjukkan bahawa SeO_3 adalah lebih toksik daripada SeO_4. Corak pengumpulan selenium adalah berbeza bagi setiap peringkat kitar hidup chironomid

yang berbeza dan kedudukan pengumpulan Se disusun daripada paling banyak ke paling sedikit, untuk pendedahan selenate: Exuviae> Larva> Pupa> Telur> Nyamuk (Dewasa) manakala bagi pendedahan selenite: Larva> Exuviae> Pupa> Nyamuk (Dewasa). Untuk analisis molekular, sampel larva chironomid daripada kepekatan tertinggi nilai LC_{50} bagi kedua-dua logam telah digunakan. Semua sampel telah disusun pada platform Illumina Hiseq 2000, dihimpun secara *de novo* dan dianalisis untuk ungkapan profil berbeza. Sebanyak 43,493,744 dan 45,599,362 *raw reads* setiap satu untuk selenate dan selenite diperolehi, masing-masing menjana 6.52 Gb dan 6.84 Gb data. Sebanyak 186 transkrip telah terekspres dengan 144 *up-regulated* dan 42 *down-regulated*. Dalam tindak balas terhadap kedua-dua pendedahan selenate dan selenite, HSPs, GST, CYP P450, *metallothionein* (MT) dan gen protein sutera ditemui terekspres serta beberapa laluan termasuklah *proteasome*, *autophagy regulation*, *mTOR* dan *MAPK signaling pathway* yang terlibat dalam program *cell death*. Secara keseluruhan, dapatan kajian ini telah membuka jalan untuk pemahaman yang lebih mendalam mengenai asas mekanisme molekul yang terlibat dalam *Chironomus kiiensis* sebagai tindak balas kepada pelbagai jenis keracunan selenium bukan organik.