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BIOACCUMULATION TRENDS OF Cs-134 AND Cd-109 IN THE ASIAN TIGER SHRIMP *Penaeus monodon* VIA WATERBORNE EXPOSURE PATHWAY

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ABSTRACT

Accumulation kinetic trends of cesium and cadmium in the Penaeus monodon were studied using Cs-134 and Cd-109 as a tracer. The objective of this study was to quantify the uptake and loss/depuration kinetic of these two radionuclides in the Penaeus monodon. Uptake and loss/depuration kinetic of these two radionuclides in the Penaeus monodon were varied widely, displayed a simple double kinetic model of linear and exponential trend with time unless modified by moulting at the stage in the mount cycle. Therefore, the variation of Cs-134 and Cd-109 bio-concentration factor could be concluded considerably influence by moulting cycle, environmental and biological condition as well as physico-chemical that direct effects on their uptake and loss/depuration kinetic.

Keywords: Bioaccumulation, Cd-109, Cs-134, exposure, loss, uptake

INTRODUCTION

Various research works have been conducted regarding the Cs-134 and Cd-109 accumulation in marine organisms. Crustaceans, bivalves and fishes are of particular interests in this regard since they serve as important seafood for human consumption and bio-indicators of contaminants exposure (Ke et al., 2016). The bio-accumulation of many radionuclides including Cs-134 and Cd-109 in aquatic organisms has been examined in many previous studies (Nolan and Dahlgaard, 1991) and indicated their bioaccumulation varies among different species of aquatic organism (marine and fresh water organisms). Apart from the field evidence, experiments in laboratories have also showed that species have highly variable of Cs-134 and Cd-109 bio-accumulation which are influenced by many factors such as genetic variation, physiological and biological factors, physic-chemical variables, geographical origin, exposure regime, species, environmental and biological behavior of interest elements and biota, etc. (Bustamante et al., 2002). In this case, a deep understanding regarding to mechanism of bio-accumulation is important especially to understand the interspecific difference of Cs-134 and Cd-109 in marine biota. This is very useful for choosing the best candidates species for contamination bio-monitoring of these radionuclides (Ke et al., 2016).

Bioaccumulation of Cs was consistently found to be slow and was potentially mediated by the ambient K^+ concentration due to their chemical similarity. While Cd is a very toxic metal often associated with and chemically similar to zinc (White and Rainbow, 1986). The ability of many edible marine organisms to accumulate Cd is potentially hazardous to human health (Joint FAO/WHO Expert Committee on Food Additives, 1972). It is known to accumulate in marine organisms, and cause rapid genetic changes (Nevo et al., 1986). Many laboratory studies have exposed the aquatic organisms to radionuclide such as Cs and Cd for a long period of time and a bio-concentration factor was calculated based on the assumption that equilibrium between the organisms and ambient radionuclides has been reached (Ke et al., 2000). In other word, predicting the radionuclide concentration in aquatic organisms is usually based on the steady-state approach, in which equilibrium is assumed between organism and ambient radionuclides (IAEA, 2004; Pinder et al., 2014; Tagami and Uchida, 2013).

In this study, we quantified the bio-kinetics of Cs and Cd in *Penaeus monodon* is one of marine organism which has been suggested to be useful bio-monitors for radionuclides. A trend kinetic model was then developed through identify and understand it accordingly to influx and efflux processes for parameter estimation. Finally, the model with the estimated parameters was used to predict Cs-134 and Cd-109 accumulation in the *Penaeus monodon* under waterborne exposure pathway scenarios. This study was conducted with an aim to quantify the uptake and loss/depuration of these two radionuclides in the *Penaeus monodon* and to understand the kinetic trend of their bio-accumulation via waterborne exposure pathway.

MATERIALS AND METHODS

Candidate Species for Experiment

Penaeus monodon is a scientific name and commonly known as the giant tiger prawn or Asian tiger shrimp, belongs to family of *Penaeidae.* The natural distribution covers the Indo-West Pacific oceans including East Africa, South Asia, Southeast Asia, the Philippines, Australia (Pérez-Farfante and Kensley, 1997; Benzei, 2000), southern Japan, Korea, China, Taiwan, Vietnam, Cambodia, Malaysia, Singapore, Indonesia, Papua New Guinea, Thailand, Myanmar, Bangladesh, Sri Lanka, India, Pakistan, Tanzania, Madagascar, and South Africa (FAO, 2012). *Penaeus monodon* is one of the marine crustaceans that are suitable to use as a bio-monitor because they are a very successful group of organisms, distributed in a number of different habitats including marine, terrestrial and freshwater environments. Not only they can accumulate heavy metals or radionuclides in their soft tissues but also can detoxification of potentially toxic metals like cadmium (Rinderhargen et al., 2000). Therefore, they are very useful as an indicator in predicting any contamination in marine environment. For experiment, these healthy *Penaeus monodon* (Figure 1) with the length of 10 to 12 cm were purchased from breeding and culturing of shrimp center in Bagan Lalang, Selangor in June 2016. These *Penaeus monodon* were stocked in aquaria set for a period time to maintain their healthy and to make them active as their normal life prior to perform further experiment. Then, Adult *Penaeus monodon* (weight: 14 – 17 g) were acclimated into seawater under experimental conditions (opened-circuit and static aquaria; constantly aerated; salinity: ~ 23 ppt; temperature: 24^oC; light/dark cycle: 11 h/13 h) for minimum two weeks prior to commencing the uptake and loss/depuration experiments. *Penaeus monodon* were fed daily with commercial feed pellets in an attempt to ensure good health and no mortality.

Uptake and Loss/Depuration Experiment

For uptake and loss/depuration experiment, three individuals of *Penaeus monodon* were randomly selected from the acclimatization aquaria and were placed in each six of rectangular polypropylene container (10 cm x 10 cm x 10 cm) containing 3 L of seawater (closed-circuit and static aquaria; constantly aerated; salinity: ~ 23 ppt; temperature: 24^oC; light/dark cycle: 11 h/13 h) and simultaneously exposed for 11 days to Cs-134 and Cd-109. There was no change in the measured pH of seawater after radionuclide addition. Experiment waters were renewed and spiked every two days to maintain constant radionuclide concentrations and minimize radionuclide recycling. They were also aerated to maintain constant levels of dissolved oxygen throughout the experimental exposure period.

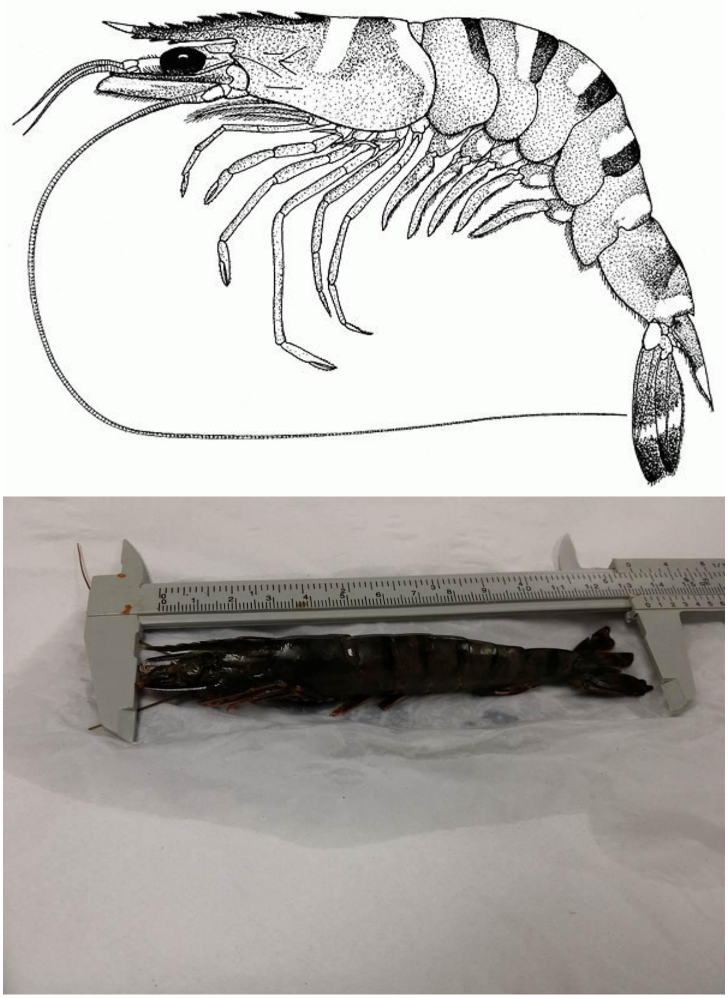

Figure1: Top - black and white drawing of *Penaeus monodon* (Source: FAO 2012); Bottom - measured total length 10 – 12 cm of *Penaeus monodon*

During the uptake experiment, single selected individuals of *Penaeus monodon* and water media from each three container were independently sampled daily for the first week and two days interval for the second week (except Saturday, Sunday and public holiday). While the rest three of containers were for loss/depuration experiment. The parameters i.e. temperature, pH, conductivity, salinity, turbidity, DO and alkalinity for experimental water were measured every uptake experiment day to assess the water quality. Then, *Penaeus monodon* were put in the 350 mL polyethylene counting container which are containing 100 mL of un-spiked seawater and weighed. This geometry was set to be the same with the standard counting geometry. Meanwhile, about 300 mL of water sample were put in the 350 mL polyethylene counting container and weighed. All weight of empty containers, water sample, un-spiked water and *Penaeus monodon* were recorded in the experiment logbook. The whole bodies of *Penaeus monodon* and water samples were counted using HpGe gamma spectrometer for 300 seconds.

After 11 days exposed to waterborne Cs-134 and Cd-109, *Penaeus monodon* were transferred into three of rectangular polypropylene container (10 cm x 10 cm x 10 cm) which contained fresh un-spiked seawater. Single selected individuals of *Penaeus monodon* and water media from each container were independently sampled every day except Saturday, Sunday and public holiday until 14 days for assessment of loss/depuration experiment. Experiment waters were renewed with fresh un-spiked seawater every two days to maintain freshness of water. The whole experiments include water quality, sample preparation, counting and other assessment or measurement were carried out as such elaborated in uptake experiment.

In the end of experiment, all dead *Penaeus monodon* samples were put in radioactive waste dustbin and spiked seawater waste were taken by staff of Radioactive Waste Management Center, Malaysian Nuclear Agency for further radioactive waste treatment.

Data Analysis

Data of uptake and loss/depuration was calculated based on average from three replicates of Cs-134 or Cd-109 radioactivity measurements using HpGe gamma spectrometer. Uptake of the radioisotopes was expressed in terms of concentration factors (CF: ratio between the radioisotopes activity in *Penaeus monodon* (Bq/kg fw.) and time-intergrated activity in the seawater (Bq/L) over time for the seawater exposure of *Penaeus monodon* (Metian et al., 2011).

RESULTS AND DISCUSSION

Uptake of Cs-134 and Cd-109 in *Penaeus monodon*

Uptake kinetic in whole-body *Penaeus monodon* after exposed to Cs-134 for 11 days through water media are presented in Figure 2. In general, uptake of Cs-134 in whole body *Penaeus monodon* were expressed a simple double of linear and exponential kinetic model. This uptake displayed a linear pattern until four (4) days followed by exponential kinetics that seem to be reached a steady state after 11 days exposure (Figure 2). However, some fluctuations were observed in the kinetic trend of Cs-134 bioaccumulation by the *Penaeus monodon* probably due to considerable effect and modify by moulting at the stage in the mount cycle. This can be concluded the uptakes of Cs-134 in whole-body *Penaeus monodon* were greater after moulting i.e. at day 4 with uptake rate of 0.30 L/kg.d (Table 1). This may be due to the increased permeability of the soft cuticle (White and Rainbow, 1986). Changes in cuticle permeability after moulting were considered to account for variability in ceasium uptake rates in *Penaeus monodon.* Variability of Cs-134 uptake rates in *Penaeus monodon* can be considered in relation to changes in cuticle permeability after moulting. Thus, changes in cuticle permeability after moulting were considered to account for variability in Cs-134 uptake rates in *Penaeus monodon*.

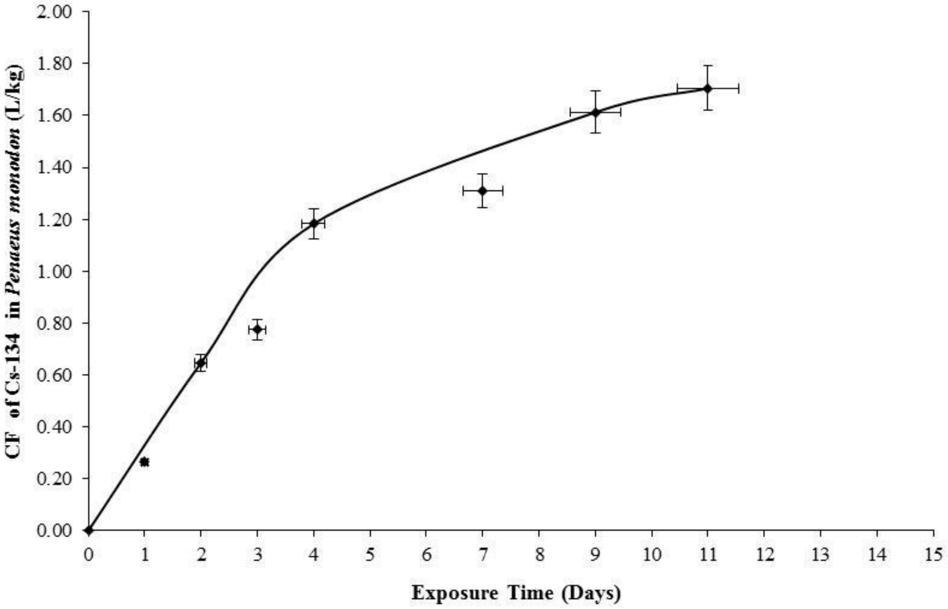

Figure 2: Uptake kinetic in whole-body *Penaeus monodon* after exposed to Cs-134 for 11 days through water media

Table 1: Accumulation rates of Cs and Cd in *Penaeus monodon*

Exposure Time (day)	Uptake Rate of Cs-134 (L/kg.d)	Uptake Rate of Cd-109 (L/kg.d)
1	0.26	0.38
2	0.32	0.49
3	0.26	0.45
4	0.30	0.48
7	0.19	0.47
9	0.18	0.40
11	0.16	0.32

There was a linear pattern within 1 – 4 days exposure with value for Cs-134 uptake of 0.26 – 1.18 L/kg, this presumably due to rapid sorption onto the *Penaeus monodon*’s tissues (Ke et al., 2000). Because Cs-134 is a soluble radionuclide thus it is tendency to associate onto soft part of *Penaeus monodon*. Thus, there was considered the uptake of Cs-134 measured for the whole individual *Penaeus monodon* representative of uptake by the tissues. Meanwhile, the exponential kinetics model for uptake pattern of Cs-134 in *Penaeus monodon* tended to reach a steady state (saturation or equilibrium CF) at day 11. The curve shape indicated that equilibrium CF for *Penaeus monodon* was approximately reached at 1.71 L/kg.

The uptake trend of Cd-109 in whole-body *Penaeus monodon* for 11 days through waterborne exposure is illustrated in Figure 3. This Cd-109 trend was also displayed a simple double kinetic model of linear and exponential. This exhibited a long zone of linear pattern within 1 – 7 days with CF range found to be 0.38 – 3.31 L/kg. Meanwhile, exponential kinetics model of Cd-109 uptake in *Penaeus monodon* was observed after 7 days exposure and reaching equilibrium CF (steady state) of 3.57 L/kg at 9 days exposure. This indicated that the time taken by *Penaeus monodon* to reach a maximum uptake level of Cd-109 was faster and presumably enough time for two weeks to implement this uptake experiment. In part of uptake rate range found to be 0.38 – 0.49 L/kg.d and 0.32 – 0.40 L/kg.d in respectively for 1 – 7 days and after 7 days to end of experiment periods (i.e. 11 days) (Table 1). There was reflected that significant rapid and efficient uptake of Cd-109 by *Penaeus monodon* at stage of 1 – 7 days exposure (linear kinetic) compared to 7 – 11 days exposure (exponential compartment kinetic). Somehow, the uptake kinetic of Cd-109 by *Penaeus monodon* was not significantly affected or modified by moulting.

Interestingly, our study confirmed that CF and uptake rate value for Cd-109 is relatively high compared to Cs-134 in *Penaeus monodon*, this suggested that *Penaeus monodon* has a high accumulation capacity for cadmium. Align with that, this was probably a large amount of cadmium is absorbed into internal tissues of *Penaeus monodon* such as muscle, while Cs-134 also absorbed onto soft tissue and young/new cuticle in particular during moulting cycle suggesting that Cs-134 sorption was a passive process. Moreover, *Penaeus monodon* was more efficiently concentrated of Cd-109 through waterborne exposure than Cs-134. This capability to concentrate and accumulate an elemental or radionuclide is often found to marine organism whenever exposed to radionuclide through seawater. It could be related to physico-chemicals properties of Cd-109 and there appeared to be considerable inter-species variation in the pattern of cadmium accumulation, particularly in the importance of the muscle in the storage of cadmium (White and Rainbow, 1986). Moreover, Cd-109 has a nutrient-like profile, thus it seems that Cd may replace Zn in some essential biochemical function (Lane and Morel, 2000) which would lead to direct adsorption in tissue of *Penaeus monodon*. Ideally, the explanation for the low CF and uptake rate of Cs may be competitive inhibition by the overwhelmingly high concentration of K^+ in the seawater (Bryan, 1963) and probably due to the metabolism of Cs in *Penaeus monodon* in a way similar to K^+. Furthermore, others findings reported elsewhere suggested that Cs uptake rate was much lower than the uptake rate of other trace elements in marine biota.

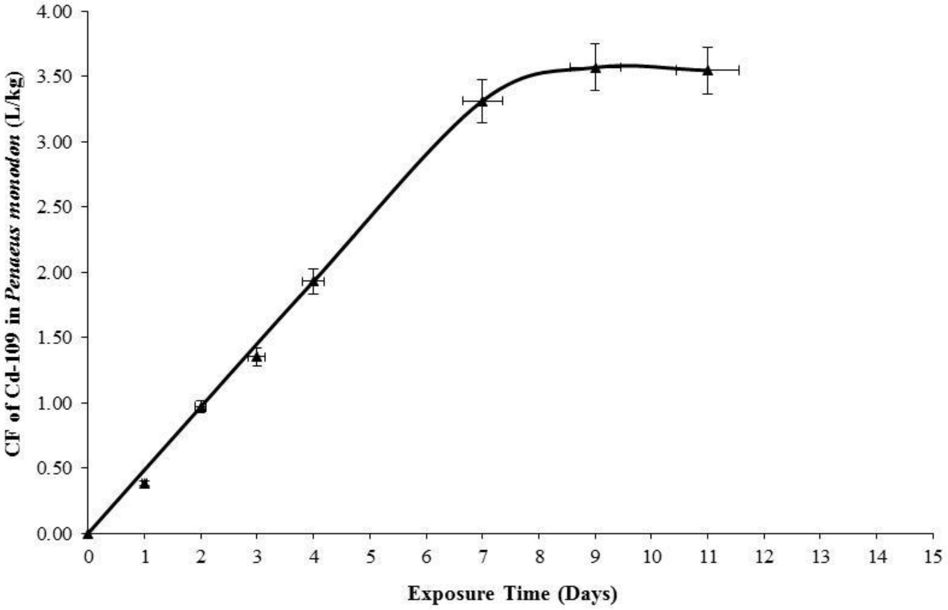

Figure 3: Uptake kinetic in whole-body *Penaeus monodon* after exposed to Cd-109 for 11 days through water media

Loss/Depuration of Cs-134 and Cd-109 in *Penaeus monodon*

In the end of the exposure time (11 days), non-contaminating conditions were restored and loss/depuration kinetics of Cs-134 and Cd-109 was followed in the laboratory experiment for 14 days. Retention/remaining of Cs-134 in *Penaeus monodon* are shown in Figure 4. The curve displayed that loss/depuration kinetics of Cs-134 from *Penaeus monodon* could be typically characterized at least a two (double)-compartmental exponential model. Day 4, 5, 6, 7, 11, 12 and 14 of loss/depuration experiment, remaining of Cs-134 in *Penaeus monodon* was found to be 66.6% 59.2%, 55.7%, 50.0%, 40.3%, 37.5% and 33.7%, respectively. This mean, a relatively large fraction i.e. 66.3% of Cs-134 was lost from the whole-body *Penaeus monodon* at the end of loss/depuration experiment. In other words, only 33.7% of the bioaccumulated Cs-134 was absorbed in *Penaeus monodon*. Caesium loss from *Penaeus monodon* showed considerable variation with some *Penaeus monodon* was loss Cs-134 more than 50% from the previously accumulated. The observation variability of loss for Cs-134 was probably due to intermittent excretion in *Penaeus monodon*.

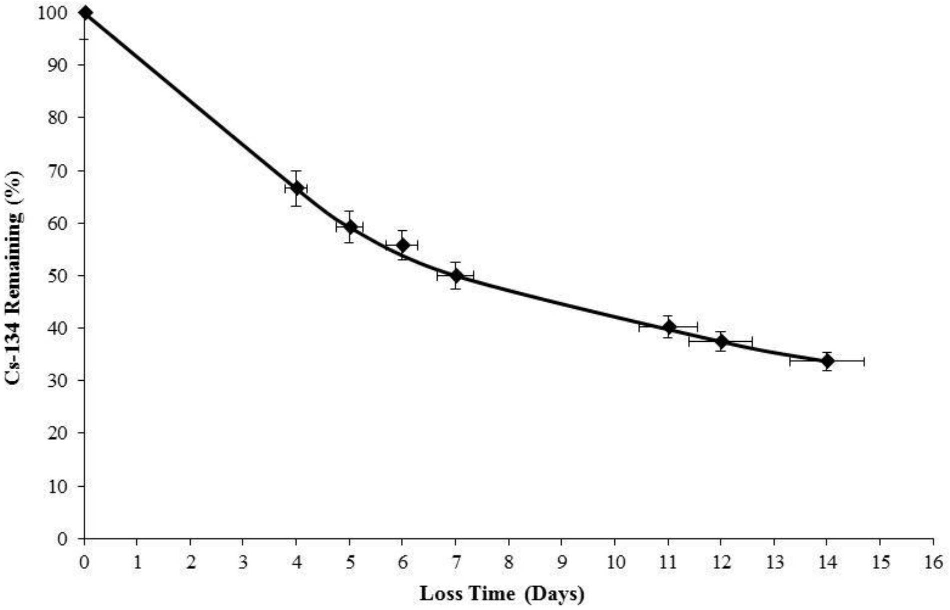

Figure 4: Loss/depuration kinetic of Cs-134 in *Penaeus monodon* after exposed through water media for 11 days

Retention/remaining of Cd-109 in *Penaeus monodon* expressed as a percentage are presented in Figure 5. All *Penaeus monodon* exhibited significant loss of Cd-109 which was typically characterized at least a double-compartmental exponential model. Remaining of Cd-109 in *Penaeus monodon* at 4, 5, 6, 7, 11, 12 and 14 days of loss/depuration experiment was found to be 88.6% 80.7%, 81.7%, 83.6%, 81.1%, 82.9% and 74.3%, respectively. A feature of the Cd-109 remaining in Figure 4 indicated that Cd-109 was not systematically remained in the whole-body *Penaeus monodon,* this considerable effect of moulting. This was supported by White and Rainbow (1986) that moults take up large amounts of cadmium after being shed into water with elevated Cd concentration. Clearly, the loss/depuration of Cd-109 from *Penaeus monodon* found to be a relatively small amount with the ranges of 11.4% to 25.7% of previously accumulated total during 14 days of loss/depuration experiment. This mean, the majority of the bioaccumulated Cd-109 was efficiently absorbed (74.3%) in *Penaeus monodon*. This could be related to the mechanisms of cadmium loss, other than by moulting, whereby the loss of adsorbed metal is not significant to all shrimp that would be expected to show a similar losses magnitude and rate. Moreover, this is further supported by the fact that cadmium loss was independent to the external cadmium concentration (White and Rainbow, 1986). This slow rate of loss and small initial losses of cadmium might be explained, these were probably attributed to the loss of surface-adsorbed metal and any further loss of cadmium was too slow to be detectable (Ray et al., 1980).

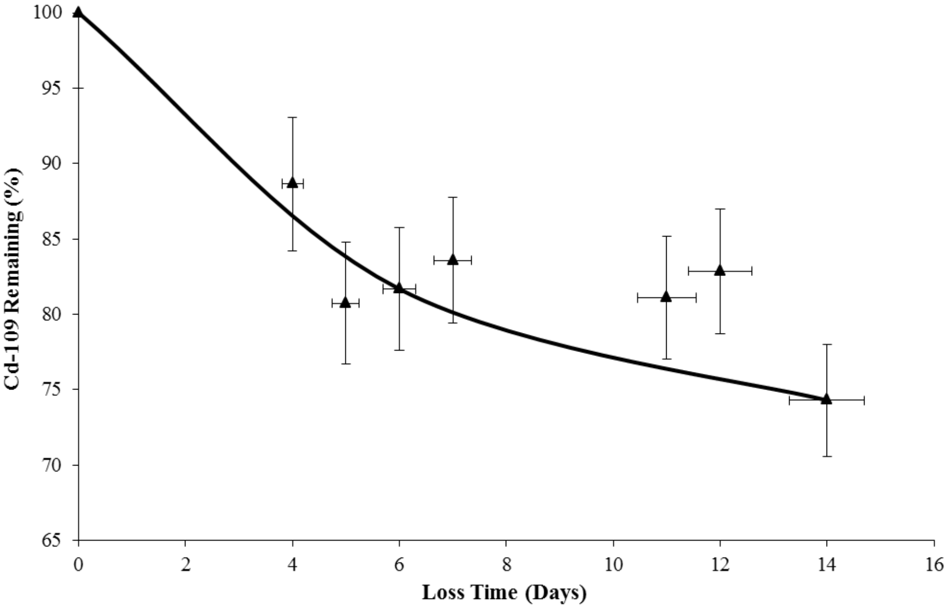

Figure 5: Loss/depuration kinetic of Cd-109 in *Penaeus monodon* after exposed through water media for 11 days

This indicated that the both estimated loss rate were significantly different depending on type of elements. In our study showed that the loss/depuration of Cs-134 in *Penaeus monodon* was so comparatively rapid and high than Cd-109. One of the reasons for the discrepancy between Cs-134 and Cd-109 loss/depuration kinetic could be due to extremely higher dissolved Cs-134 activity concentration used in the uptake experiment. This possibly overwhelmed the biological mechanism of *Penaeus monodon* and their internal organ or cell to bind the Cs-134, consequently the rapidly excretion of Cs-134 from the body of *Penaeus monodon*. However, in this study we did not test of the temperature, salinity and body size dependence on Cs loss/depuration in *Penaeus monodon* but our presumably that both environmental conditions (e.g. temperature and salinity) and biological conditions (e.g. body size) considerably direct effect to loss/depuration of Cs in *Penaeus monodon*. Furthermore, variation of contaminants depurate or loss or excrete from organisms depends on their system to store either short or long term storage at different part in the body. The other factors i.e. species, taxa and elements also might be influenced the depuration kinetic (Othman et al., 2012).

CONCLUSIONS

Overall, the results of the current study mainly found that CF and uptake rate of Cd-109 in *Penaeus monodon* is relatively high compared to Cs-134. Inversely, the loss/depuration kinetic of Cs-134 in *Penaeus monodon* is comparatively rapid and high than Cd-109, this mean the majority of the bioaccumulated Cd-109 was efficiently absorbed (74.3%) in tissue of *Penaeus monodon.* However, Cs-134 also absorbed onto soft tissue and young/new cuticle in particular during moulting cycle suggesting that Cs-134 sorption was a passive process. Therefore, the variation of Cs-134 and Cd-109 bio-concentration factor considerably influence by moulting cycle, environmental and biological condition as well as physico-chemical that direct effects on their uptake and loss/depuration kinetic.

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