Microplastic Bioaccumulation by Tiger Snail (*Babylonia spirata*): Application of Nuclear Technique Capability using Polystyrene Labelled with Radiotracer ^{65}Zn

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Abstract

Plastic waste is a solid that is difficult to decompose but can turn into environmental microplastics. Microplastics are sizes between 0.1 µm–5 mm, highly prolific anthropogenic pollutants affecting terrestrial, freshwater, and marine ecosystems. The purpose of this study was to determine the ability of Babylonia spirata to accumulate and eliminate polystyrene microplastics. This experiment uses the nuclear applications technique. The experiment consists of two methods: manufacturing microplastics and biokinetics. The manufacture of microplastics labeled Zn-65 is based on the reaction of polystyrene sulfonate with zinc to form polystyrene sulfonate Zn resin. This microplastic labeled Zn-65 is very stable in the aquatic environment, so it is used for bioaccumulation experiments. Biokinetics includes acclimatization/adaptation of the organism for seven days, bioaccumulation consists of the organism accumulating microplastics labeled Zn-65 for seven days, and depuration for seven days that were placing the organism accumulating microplastics labeled Zn-65 in water free of contamination and biokinetics calculations include the BCF (Biocontration Factor), ku (uptake constant), ke (depuration constants), and t1/2 (half-life). The experimental results show that the ability to bioaccumulate polystyrene microplastics from seawater (BCF) is 79.2 to 304.31 $ml.g^{-1}$. This bioaccumulation is affected by the microplastic content in the water. The biological half-life of the microplastic is 14.54 to 41.78 d. There is a relationship between the concentration and the Ku, Ke and BCF. the polystyrene content. The experimental results show that microplastics bioaccumulate in a marine organism so that they can move through the food chain and are ultimately harmful to humans.

Keywords: *microplastic, polystyrene, bioaccumulation, labelling, radiotracer, Zn-65*

Introduction

Indonesia is predicted to become one of the most prominent pollutants-producing countries in the world. In the Indonesian sea, plastic pollutants originate from the domestic and foreign seas from several surrounding countries (Basri *et al*., 2021). It is estimated that 0.52 kg of waste per person generated daily, and 83% of that amount is mismanaged. Plastic waste management is only around 11%. Approximately 10.1% of this adequately managed, and the rest is untreated. With a population of 187.2 million people living within 50 km of the coastline, Indonesia produces around 5.4 million metric tons of plastic waste. As many as 3.22 million of them managed improperly, so 0.48–1.29 million metric tons end up as marine debris. Although these substances are not high enough to pose an immediate risk, plastic waste management in Indonesia is still inadequate. This acknowledges that Indonesia lives in a “one-use culture,”where Indonesia food and beverage packaging consists of plastic (Syakti *et al*., 2017).

Microplastics are microplastics with sizes between 0.1 µm–5 mm. Microplastics are highly prolific anthropogenic pollutants affecting worldwide terrestrial, freshwater, and marine ecosystems (Cole, 2016). Microplastic waste includes primary microplastics (such as scrubs in cosmetics), synthetic fibers, and secondary microplastics resulting from the photo-oxidative degradation of microplastics. Ecosystems can be polluted by microplastics from atmospheric precipitation, indiscriminate or improper isposal of plastic waste, and incomplete wastewater filtration. The risks posed by microplastic debris to

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aquatic life, ecological processes, and food security are critical considerations for environmental watchdogs and legislators. Microplastics are environmentally persistent, tend to absorb toxic persistent organic pollutants, and are bioavailable to various aquatic organism (Cole, 2016)

The Tiger snail, *Babylonia spirata (Linnaeus*) is one of the gastropods with high economic value and capable of accumulating various types of heavy metals and radionuclides contained in marine waters. *B. spirata* is utilized from its meat through its shell. Meat used as a source of food (seafood), as an operculum for medicinal materials, and shells for the manufacture of ornaments. Habitat *B. spirata* is a sandy or muddy bottom of a marine environment at 5-30 m depth with a relatively settled life and limited mobility. (Durai, 2018) *B. spirata* distribution is abundant and evenly distributed across several Indo-Pacific coastlines, including in some coastal areas of Indonesia. Based on its behavior, spread, and capability to accumulate contaminants (Budiawan *et al*., 2021). Tiger snails (*Babylonia spirata*) also have considerable potential for cultivation. Babylonia is a snail of the marine animals that have long been known to the public as a source of animal protein (Noordin *et al.*, 2014) rich in calcium and essential amino acids (arginine, leucine, lysine) (Nasution *et al.*, 2021).

Nuclear techniques can be applied to study microplastic bioaccumulation using microplastic labeling with a radiotracer. This is to determine the biological effect of plastic particles at different levels of biological organization (Lanctôt *et al*., 2018). This study discusses the ability of *Babylonia spirata* to accumulate and eliminate microplastics.

Material and Methods

The experiment consists of two methods, namely the manufacture of microplastics (referring to the National Research and Innovation Agency Center Technology Radioisotope, Radiopharmaceutical and Biodosimetry) and biokinetics (acclimatization, bioaccumulation, depuration) referring to Budiawan *et al*. (2021), theoretical modelling the Bioaccumulation referring to Alava (2020) and various modifications.

Manufacture of Polystyrene Sulfonate (PSS)

Styrofoam materials was cut into small pieces and then weighed 1 g. The Styrofoam was dissolved in 20 ml of chloroform, put in a three-neck flask, and add 20 ml of 98% H_2SO_4. Using a magnetic stirrer, heated at 40°C and stirred at 700 rpm for 10 h. After the stirring process collided with the polystyrene sulfonate (PSS) solution, then 100 ml of distilled water was added for washing. Washing was carried out 4 times, during the process the pH was checked. The pH value formed was 5–6. The polystyrene sulfonate (PSS) solution was then filtered and the PSS powder obtained was stored at room temperature. Then the sonification results were processed for labeling using ^{65}Zn radioisotope.

Biokinetic experiment

Tiger snails (*Babylonia spirata*) were collected from Muara Kamal in Jakarta Bay on December 15, 2022. The sample was cleaned of mud adhering to its shell, then 4 ice gels were placed around the sack to maintain body temperature of *B. spirata*.

Acclimatization

Acclimatization provides adaptation time for organisms in the research environment to reduce death risk at the star of rearing. This helps to minimize stress on the organisms during bioaccumulation experiments. *B. spirata* was cleaned of attached animals and placed separately in a seawater aquarium. Acclimatization was carried out by raising *B. spirata,* for 7 d without contaminating the aquarium water. Replacement of seawater in the aquarium is done every day in the morning. During the acclimatization process, the experimental animals were given food twice a day in the form of detritus.

Bioaccumulation of ^{65}Zn

Babylonia spirata were transferred to 3 glass aquariums containing 10 L of seawater which had been filtered using a 0.2 μm filter at pH 7.8 then wi*thin were placed*. First aquarium was added the microplastics labeled with radioactive substance ^{65}Zn so that the concentration was 375 particles. ml^{-1}. The other two aquariums with microplastic concentration varied between 750 and 1125 particles.ml^{-1}. On day zero of bioaccumulation, the radioactivity of each microplastic labeled with ^{65}Zn was determined using a gamma spectrometer with High-Purity Germanium (HPGe) Gamma Spectrometer detector. During the bioaccumulation process, the experimental animals were fed twice a day in *Artemia sp*. *B. spirata* were taken every day from each aquarium to be weighed and analyzed for the content of labeled microplastics. Bioaccumulation ability is expressed in CF (Concentration Factor), namely the ratio of 65-Zn content in organism (Ct) and water (Cw).

$$CF_t = \frac{C_t}{C_w} \quad (1)$$

When the equilibrium condition (steady state) is expressed by the equation:

$$CF_t = CF_{ss}\,(1 - e^{-k_e.t}) \quad (2)$$

Depuration

B. spirata, which had accumulated microplastic substance ^{65}Zn, was depurated by placing three B. *spirata* individuals in an aquarium filled with contaminant-free seawater and running water equipped with filtration and aeration system (Budiawan *et al.,* 2021). Feeding *B. spirata* was carried out twice a day during depuration and the same type of feed as before. The microplastic content labeled radioactive substance ^{65}Zn was analyzed in the organism using a Gamma spectrometer. The value of the release rate constant (ke) is obtained from the slope of the percent (%) retained contaminant activity versus time. Then the BCF value is calculated to determine the bioaccumulation concentration factor, using the equation below.

$$BCF = \frac{k_u}{k_e} \qquad (3)$$

The biological half-life of 65-Zn in the tiger snail body is expressed by the equation:

$$t_{b1/2} = \frac{\ln 2}{k_e} \qquad (4)$$

Result and Discussion

Indonesia is a significant contributor to the global plastic waste problem. The entry of plastic waste into Indonesia's sea is the second largest contributor to the disposal of plastic waste worldwide after China (Sakti *et al*., 2021). Plastic waste pollution has become a significant threat to marine ecosystems with persistent characteristics that have been difficult to degrade and resistant for hundreds of years. Pollution plastics are included in the form of micro and nano particulates which decompose and are produced in a short time. Nano-plastic pollution can interfere with health through the food chain, causing deformation, disability, suffocation, and death. However, marine plastic pollution not only has a direct impact on marine life but also has a negative impact on local fishing, tourism and business industries.

One of the efforts to prove the accumulation of microplastics can be done through an aquaria experiment using marine organisms. Gastropods are facultative organisms, that is, organisms that can survive on a broader range of changes in environmental conditions. These organisms can survive in waters that contain various organic matter. However, animals cannot tolerate environmental stress and are quite sensitive to environmental degradation. In this study, tiger snails (*B. spirata*) were selected, which besides playing a role in marine ecosystems (movement of the food chain), this biota is a source of seafood.

The macro zoobenthos community, including tiger snail, can be used as a biological indicator because it lives relatively sedentary and has relatively slow mobility. It is directly influenced by materials that enter the environment. In addition, as a benthic organism, the tiger snail can recycle organic matter, an essential component in the 2nd and 3rd links in the aquatic community food chain. This mollusk belongs to the *Buccinidae* family, the *Neogastropod* order, which has significant economic value. Indonesia has started exporting tiger snails to several countries such as the PRC, Taiwan, Hong Kong, Malaysia, and Singapore; this is because the tiger snail meat has a high protein content, and the meat structure is chewy and has a delicious taste (Faizah *et al*., 2007; Rejeki *et al*., 2011).

The preferred ecosystem location for tiger snails is a sandy or muddy seabed at a depth of 5 to 20 m. Geographically, the distribution of tiger snails is abundant and evenly distributed on several Indo-Pacific coasts, including on several beaches in Indonesia. About 10% of products made of plastic are disposed of in rivers and end up in the sea. The Semarang region has two main rivers: the West Flood Canal and the East Flood Canal. The two rivers are transportation routes for almost all types of waste (including plastic waste) from land activities to sea waters (Yulina *et al*., 2021)

Plastic waste reaches the environment where exposure to ultraviolet (UV) radiation causes photo-oxidation of plastic and makes it brittle. Other environmental factors such as wind, waves, wave action, and abrasion lead to the degrading the plastic fragments into macro- (≥ 25 mm), meso- (<25 mm-5mm), micro- (<5 mm-1μm) plastics in a range of sizes and nano-plastic particles (<1 mm). One type of microplastic debris is the most common fibre observed in natural environments. These synthetic microfibers are usually made from polyethylene terephthalate (polyester, PET), nylon, or polypropylene (PP). The presence of these fibers is generally associated with the release of synthetic fibers from garments during laundering, degradation of cigarette butts leading to the release of cellulose acetate fibers, and fragmentation of marine equipment (*e.g.*, ropes and nets) (Cole, 2016). Microplastic debris risks aquatic life, and ecological processes and food security are crucial considerations for environmental watchdogs and legislators. Due to the persistent nature of microplastics in the environment, they tend to adsorb toxic, persistent organic pollutants and are bioavailable to a wide range of aquatic biota. Toxicity tests using representative microplastics have revealed that microplastics can cause adverse health impacts, including reduced feed quantity, egg size, and hatching success in marine copepods, reduced egg numbers number, egg size, and sperm motility in oysters, decreased feeding, digging, and energetic reserves in marine polychaetes and reduced motility, predator response, hatching success and growth (Cole, 2016; Printz and Korez, 2019)

Phytoplankton, as primary producers, can absorb Microplastics. The microplastic then moves through the food web. The bioaccumulation process is more complex for secondary and tertiary consumers because it is absorbed directly from the water through respiration, digested through food, and eliminated from marine organisms as feces (Alava, 2020). Considering its lower density than water, microplastic will still float in seawater (Choong *et al*. 2021). Bioaccumulation of microplastics and related chemical additives was estimated across all species at each level. Biomagnification occurs after the accumulation of microplastics by particular biota and being eaten by predators. Costa *et al*. (2020) proved that microplastic biomagnification occurs from *copepods* to *ephyrae* after 24 h.

This accumulation occurs at five trophic levels resulting in a biomagnification process in which microplastics move in the food web. Like marine organisms, accumulations in *B. spirata* can be exposed through ingestion, through inhalation. However, the accumulation of microplastics can cause physical harm and chemical effects on other organisms. Bioaccumulation and elimination of polystyrene microplastics through the waterways are shown in Figures 1 and 2. As inputs of polystyrene microplastics in seawater were concentrations of 375, 750, and 1125 particulates per ml. The particulate matter made of polystyrene labelled with radiotracer ^{65}Zn.

The bioaccumulation ability of polystyrene microplastics up to the 7th day was 32.54 to 54.50 ml.g^{-1}. On the other hand, in the microplastic elimination process, *B. spirata* on day 1 to 6 are able to retain 58.17 to 83.89% of microplastic contaminants in its body tissues. The results of biokinetic calculations are shown in Table 1. Based on the biokinetics contained in Table 1, the ability to bioaccumulate polystyrene microplastics from seawater (BCF) is 79.2 to 304.31 ml.g^{-1}. The biological half-life of the microplastic is 14.54 to 41.78 d. The relationship between particulate concentration and Ku, Ke, BCF and $t1_{/2}$ is shown in Figures 3 to 7

There is a relationship between the concentration and the uptake constant and BCF. This is shown in the adj R square above 0.5. Conversion of ^{65}Zn radiotracer labeled polystyrene micro plastic where the particulate content in the input seawater is 375, 750, 1125 particulate.ml^{-1}; the content of polystyrene plastic microparticles in *B. spirata* is 10403 to 17651 particles.g^{-1} biota.

Its accumulation capacity is 32.53 to 54.46 ml.g^{-1}, while its ability to retain microplastics in its body is 58.17 to 83.89%. As a comparison, according to Pagter *et al*. (2021), there is a 95% positive relationship between bioaccumulation ability and location (representing microplastic concentration). Related to marine plastic pollution is the ability of

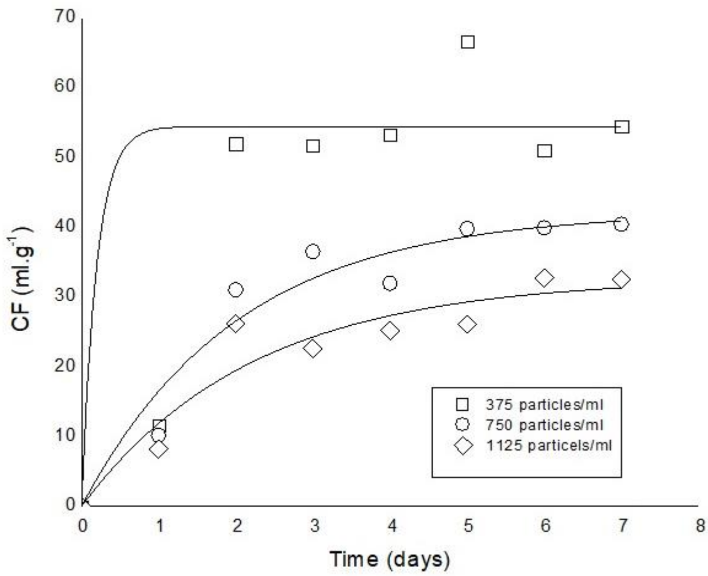

Figure 1. Bioaccumulation microplastic polystyrene that labelled ^{65}Zn from sea water

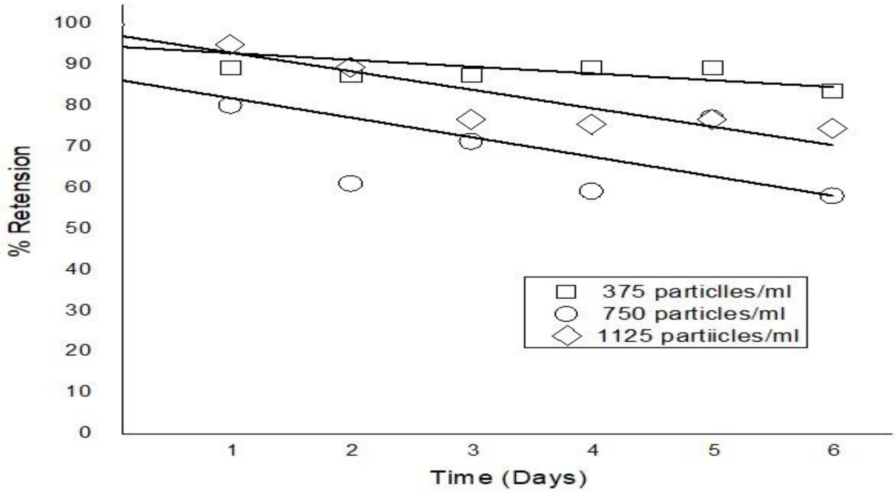

Figure 2. Elimination microplastic polystyrene that labelled ^{65}Zn from sea water

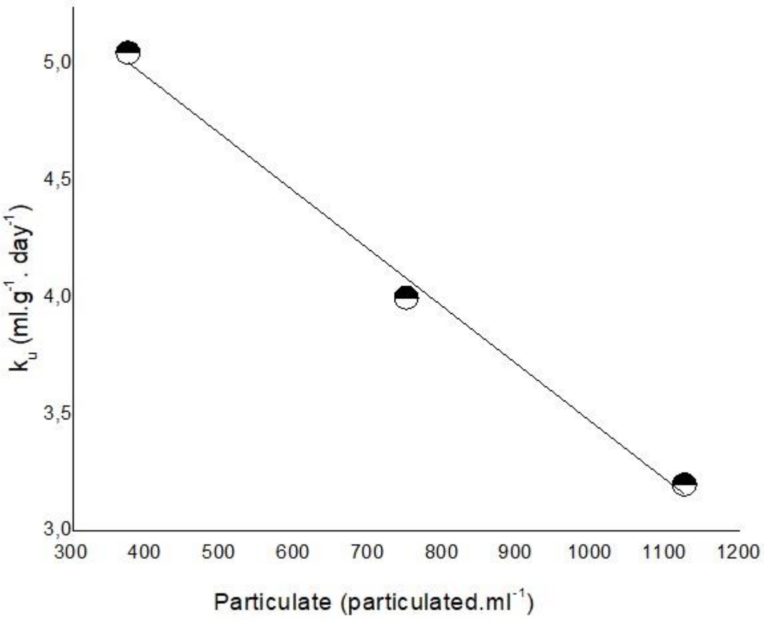

Figure 3. Relationship particulate concentration and uptake constant

Table 1. Biokinetic Parameter *Babylonia spirata*

Particulate Concentration of Microplastic (Particle.ml^{-1})	Ku (ml.g^{-1}.day^{-1})	Ke (day^{-1})	BCF (ml.g^{-1})	$t_{1/2}$ (day)
375	5,0481	0.016588	304.32	41,786
750	3,9943	0.04766	83.80	14,542
1125	3,1966	0.040336	79.20	17,184

microplastics to absorb other pollutants from the environment and transfer them to aquatic biota through consumption or contact. The hydrophobic surface nature of microplastics means that microplastics can easily absorb and concentrate various chemical contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and trace metals, and so on (Lanctôt *et al*., 2018). In other words, bioaccumulation occurs when a contaminant's absorption is greater than an organism's ability to absorb the contaminant. Bioaccumulation and trophic transfer lead to biomagnification of these contaminants at higher trophic levels. Biomagnification in a food web is defined as an increase in the concentration of contaminants (i.e., microplastics or additives) in an organism compared to the concentration of its prey (Miller, 2020). Microplastic contamination in ecological risk is the adverse environmental effects of exposure. Microplastic contamination in ecological risk is the adverse environmental effects of exposure. Marine organisms are exposed through direct and indirect ingestion of MP or inhalation (Hidalgo Ruz, *et al.*, 2012; Kaposi *et al.*, 2013; Neves *et al.*, 2015; Ogunola and Palanisami, 2016; Auta *et al.*, 2017; Gallo *et al.*, 2018; Wibowo *et al.*, 2019; Ugwu *et al.*, 2021; Wootton *et al.*, 2021; Harqu and Fan, 2022).

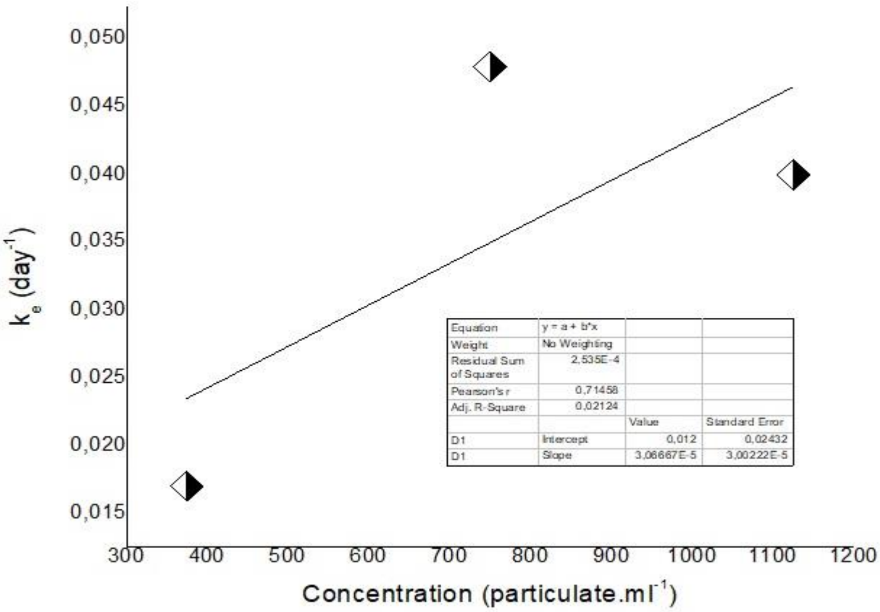

Figure 4. Relationship particulate concentration and elimination constant

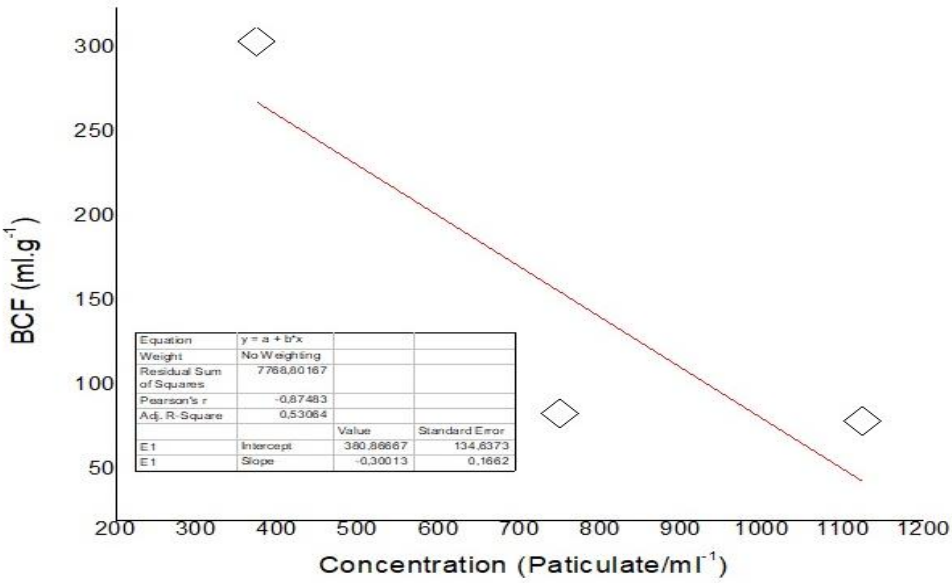

Figure 5. Correlation particulate concentration and Biocontration Factor

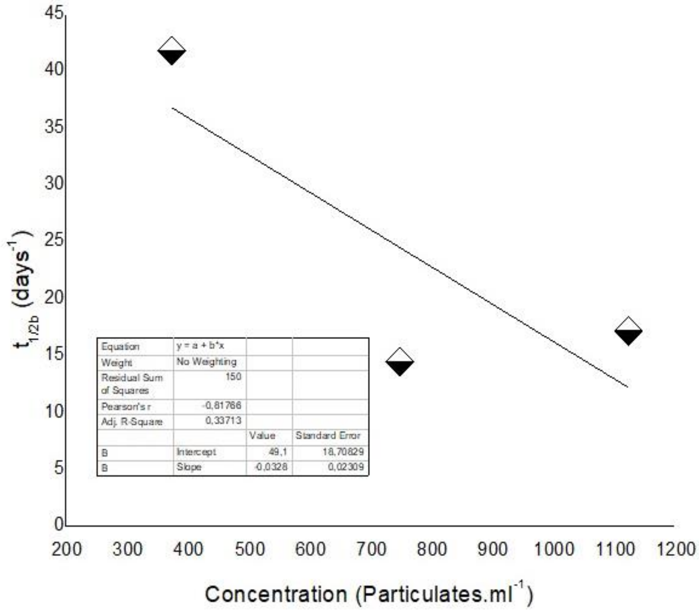

Figure 6. Correlation particulate concentration and biological half live

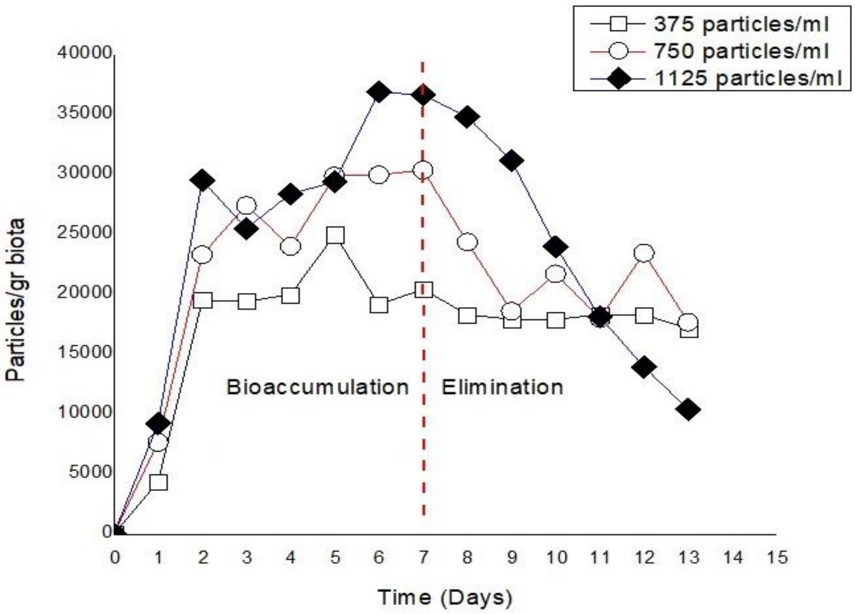

Figure 7. Accumulation and Elimination Particulate microplastic polystyrene

Conclusion

The ability to bioaccumulate polystyrene microplastics from seawater (BCF) is 79.2 to 304.31 ml.g^{-1}. The biological half-life of the microplastic is 14.54 to 41.78 d. There is a relationship between the concentration and the uptake constant and BCF. The content of polystyrene plastic microparticles in *Babylonia spirata* is 10403 to 17651 particles. gram^{-1} biota.

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