The Effect of Zinc Concentration on Bioaccumulation in Milkfish (Chanos chanos) Use of $^{65}$Zn as a Radioactive Tracer

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ABSTRACT

Bioaccumulation studies of zinc (Zn) in milkfish (Chanos chanos) have been carried out in the laboratory using a $^{65}$Zn radiotracer. The research was conducted to determine the effect of Zn concentration on the ability of Chanos chanos to accumulate and release these contaminants. Zn absorption follows the single-compartment model, and experiments were carried out until stable conditions were achieved. The stages of research were biota collection, acclimatization, bioaccumulation, and elimination. Acclimatization was carried out for biota adaptation in the experimental environment. Bioaccumulation was carried out for 7 days by placing the biota in an aquarium filled with seawater added with $^{65}$Zn radiotracer contaminants. The elimination process involves the release of contaminants from the biota by placing them in contaminant-free seawater for 7 days. The results of the bioaccumulation experiment showed that the concentration factor (CF) ranged from 0.67 - 18.18 mLgr$^{-1}$ at a concentration of ZnCl$_2$ of 1.5 ppm, and the absorption constant ($k_a$) 1.878 - 3.267d$^{-1}$ and R$^2$ was 0.0276. The release of contaminants in depuration experiments was highest at ZnCl$_2$: 2 ppm concentrations. The percentage of retention during depuration was between 14.61 - 79.91 %, the elimination constant ($k_e$) was 8.681, and R$^2$ was 0.7213.

INTRODUCTION

Milkfish is one of the most widely cultivated types of consumption fish in Indonesia's coastal waters because it is tolerant of changes in environmental conditions, resistant to various diseases [1], and can be cultivated in brackishwater, seawater, and freshwater. It is an important source of nutrients for the human survival process [2]. The meat is delicious and savory; the texture of the meat is not easily destroyed, and it can be used in various processed products at affordable prices for all levels of society [3]. Indonesia is one of the second largest fisheries producing countries after China, which accounted for 7 % of the total global catch in 2018 [4], however, the level of community fish consumption is still low and uneven between regions [5]. The fish consumption rate in Indonesia is 56.48 kg per capita in 2022, and it ranks 6th out of 8 ASEAN countries with the lowest amount of fish consumption [4].

The rapid development of industrialization, urbanization, human population growth, agriculture, fishing, etc. has led to excessive exposure to heavy metals, especially in developing countries [6]. Heavy metal pollution is a serious global environmental problem due to the severe health impacts due to the accumulated and persistent nature of heavy metals [7]. High concentrations of heavy metals in waters can...
Heavy metals can contaminate seawater and sediments and subsequently enter the food chain so that they accumulate in the bodies of living things [8]. Heavy metal contamination is one of the most pressing concerns for food safety and human health [9].

The heavy metal Zn is needed by living things in small amounts but can become harmful if present in an excessive amount. Excess and deficiency of Zn adversely affect human health. Heavy metals are natural elements that come from nature, and the largest ones come from anthropogenic sources because they release more pollutants in a form that is more toxic and easily moved [10]. The use of Zn in industry largely comes from its chemical and metallurgical properties, especially for galvanizing iron and steel items. It provides a corrosion-resistant coating that can be done with an electroplated metal coating or a natural coating and is used for construction, siding, equipment housings, office hardware, heating and ventilation ducts, vehicle and building roofing, vehicle door siding, and parts. Under the car body [11] and the industry battery [12]. Excess Zn due to the inhalation of Zn-containing smoke in the galvanizing industrial process will cause metal fume fever syndrome and can cause death [13]. Zn deficiency is observed in almost 17% of the global population and affects many organ systems, leading to disfunction of both humoral and cell-mediated immunity, thus increasing the susceptibility to infection [14].

Currently, industrial development in Indonesia has been very rapid, and not much has been done to treat the waste, so many waters receive the waste. One of the waters in the Indonesian capital that must be seriously considered is the waters in Jakarta Bay because it can cause food safety problems for fishery products originating from this region. Heavy metals, which are hazardous and toxic substances in Jakarta, come from human activities on land, such as various industrial activities, oil extraction, agriculture, transportation, hospitals, workplaces, and other domestic activities [15]. Heavy industry, improper agricultural practices, as well as urban development in turn become sources of soil heavy metal pollution [16]. Heavy metal pollution is a serious problem from a human health standpoint and a broader environmental perspective due to its non-biodegradable, harmful, and toxic nature [17]. Heavy metals can move from abiotic components to living things, accumulate at different trophic levels, and therefore contaminate the food chain [18]. In Jakarta Bay, there are only traditional fishing activities, shrimp, demersal, and small pelagic fisheries, which are caught using different fishing gear, both active and passive fishing gear [19], and cultivation of green muscle, milkfish, and shrimp.

The use of test biota in heavy metal bioaccumulation experiments generally uses the destruction method, in which the test animals are killed before destruction. Nuclear engineering can be used to study the bioaccumulation of heavy metals that have polluted the environment in marine organisms, such as fish, without killing them. This method can measure the biokinetics of contaminants over the long term in several individuals.

Based on the description above, bioaccumulation studies can be carried out using radiotracer [20]. Bioaccumulation refers to the absorption of contaminant concentrations from the surrounding environmental medium by aquatic organisms [21].

$^{65}$Zn has been successfully used by some researchers on metal bioaccumulation, such as research on zinc metal bioaccumulation in freshwater crustacean *Macrobrachium australians* decapod has been conducted, and the results showed that the process of zinc uptake is faster than cadmium and transports zinc metal accumulation internally to the antennal glands [22]. Research into the bioaccumulation of Zn in horse mussels (*Modiolus Micropterus*) occurs quickly and efficiently in shells [23]. The same study on pomfret (*Colossosoma macroporum*) and sepia fish (*Trichiogaster trichopterus*) states that increasing the concentration of Zn in aqueous media increased the absorption rate and elimination rate in both biotas [24].

This research is based on the fact that zinc (Zn) is an essential metal that can cause toxic effects on aquatic organisms if the amount is excessive, for example, in *Chanos chanos* which is an economically important commodity and is widely consumed by Indonesian people. Therefore, it is important to investigate how milkfish accumulate and eliminate Zn from their bodies.

This study aimed to analyze the ability of $^{65}$Zn accumulation by milkfish through seawater at various concentrations of contaminants. The characteristics of milkfish accumulation are represented in various bioaccumulation parameters as reference data for stakeholders in making policies related to the protection of aquaculture potential in Jakarta from the dangers of Zn heavy metal pollution.
METHODOLOGY

The bioaccumulation of zinc follows the same principle as the entry of pollutants into living organisms. The method used in this study is based on the absorption and release of contaminants using biokinetic models. The research method of Zn bioaccumulation on Chanos chanos with the isotope $^{65}$Zn refers to [24] with some modifications in the laboratory.

Location

Research samples of juvenile milkfish (Chanos chanos) were obtained from ponds in Tanjung Pasir Jakarta Bay waters. The research was conducted in October 2022, and bioaccumulation and depuration experiments were carried out at the Laboratory of the Research Center for Radioisotope Technology, Radiopharmaceuticals and Biodosimetry - BRIN, Serpong, South Tangerang.

Materials

The material used in this study was radiotracer $^{65}$Zn produced in the laboratory of the Research Center for Radioisotope Technology, Radiopharmaceuticals and Biodosimetry - BRIN Serpong, HCl solution, juvenile milkfish (Chanos chanos), fish feed, seawater, and aquadest. The tools used were 10 L and 200 L aquariums, filter containers, hoses, water pumps, filter cotton, skimmers, aerators, aerated stones, 10 mL vial bottles, plastic boxes, and spectrometry ORTEC Solid-State Photon Detector HPGE (High-Purity Germanium) Coaxial Detection System, which was linked to a multichannel analyzer and a computer running the Maestro software.

Biota

As many as 50 juvenile milkfish were taken from ponds around Tanjung Pasir Teluk Naga Tangerang Banten. Milkfish samples were then put in plastic containers and transported to the laboratory of the Research Center for Radioisotope Technology, Radiopharmaceuticals, and Biodosimetry - BRIN Serpong. Milkfish were then acclimatized for 7 days in an aquarium of 200 liters of seawater. Feeding is done every day. The condition of the aquarium was visually checked twice a day, and the physical parameters of an aquarium seawater chemistry (temperature, salinity, pH) were maintained as follows: temperature 27± 2 °C; pH 8.2±0.2; salinity 30-33 %. The seawater of the aquarium is replaced only every day by 30 %.

Bioaccumulation of $^{65}$Zn through seawater

After the acclimatization stage of the biota was completed for 7 days, bioaccumulation experiments can be carried out. 4 aquariums with a capacity of 10 L were prepared, each filled with 8 L of seawater, and dissolved in ZnCl$_2$ which has been added to the $^{65}$Zn radiotracer so that the concentration of the aquarium becomes 0.5; 1; 1.5, and 2 ppm. Next, each aquarium inserted 3-tailed milkfish of uniform weight. The process of bioaccumulation of contaminants was carried out for 7 days.

Concentration variation

In 4 aquariums containing 8 L of seawater, 4 mL of ZnCl$_2$ solution of 1000 ppm was added, which had been spiked with a $^{65}$Zn radiotracer so that the aquarium concentration became 0.5 ppm. In other aquariums, 8 mL, 12 mL, and 16 mL of ZnCl$_2$ solution of 1000 ppm is added respectively, so that the concentrations in the aquarium are 1 ppm, 1.5 ppm, and 2 ppm respectively.

Measurement of $^{65}$Zn activity in biota

Activity measurements of $^{65}$Zn in juvenile milkfish and aquarium seawater were carried out daily during the contamination period. The milkfish to be measured was put into a 250 mL plastic container containing a small amount of contaminant-free seawater and then put in a detector. While aquarium water is taken and put into a 10 mL vial then measured its activity in a detector. Each time, the measurement must be made under the same conditions i.e., the geometry of the container, the distance traveled by the container from the detector, and the volume of water in the container. Measurements were taken for 3 minutes on each milkfish.

Biota depuration

After the contaminant absorption experiment was completed, milkfish were transferred into a new aquarium containing 8 L of contaminant-free seawater equipped with an aquarium filtration and aeration system. Feeding is done every day. Depuration experiments on milkfish were carried out for 7 days, and measurements of $^{65}$Zn activity were carried out every day using the same procedures measuring $^{65}$Zn activity at the time of bioaccumulation experiments.
Laboratory analysis

During the process of acclimatization experiments, bioaccumulation and depuration did not cause the death of the test biota. These experiments have determined the rate of Zn uptake and elimination in milkfish (Chanos chanos) using radiotracer techniques. The use of a radiotracer in a bioaccumulation experiment allows the test biota to not suffer damage or death.

The comparative value between the concentration of activity of $^{65}$Zn throughout the body of biota and the concentration of activity in seawater is called concentration factor ($CF$) absorption of $^{65}$Zn. When kinetics is measured at a steady state, the biokinetic model assumes the form of simple linear regression shown in Eq. (1) or the simple first-order exponential kinetics model shown in Eq. (2):

$$CF_t = k_a T$$

$$CF_t = CF_{ss}(1 - e^{-ket})$$

Where $CF_t$ (grmL$^{-1}$) a is the concentration factor at time t (d), $CF_{ss}$ (grmL$^{-1}$) is the concentration factor at stable conditions, $k_a$ (grmL$^{-1}$d$^{-1}$) is the absorption rate constant, and $k_e$ (d$^{-1}$) is the constant depuration [25].

The kinetics of the discharge of $^{65}$Zn was indicated by the percentage of radioactivity concentration (the activity concentration at time t divided by the activity concentration measured at the beginning of depuration multiplied by 100). Simple linear regression was used to calculate discharge kinetics with and without semi-log data transformations. The $^{65}$Zn radiotracer lost from the biota body over time can be described by an exponential curve using a single-component exponential model Eq. (3):

$$A_t = A_{ao}e^{-ket}$$

It can be explained that $A_t$ and $A_{ao}$ are the residual activity (%) at time t (day) and 0 days, while $k_e$ is the elimination rate constant (d$^{-1}$), which can be used to calculate the biological half-life of the radiotracer (t$_{1/2b}$) Eq. (4):

$$t_{1/2b} = \frac{ln2}{k_e}$$

The estimated average daily intake (EDI) of heavy metal Zn accumulated in Chanos chanos when consumed by people in the Jakarta Bay area can be calculated using the equation recommended by the U.S EPA [26] Eq. (5):

$$EDI = \frac{CI[RI][EF][ED]}{BW\times AT}$$

Where EDI is the average daily intake or dose through ingestion (µg/kg bw/day); C is the concentration of heavy metals in Chanos chanos as a result of bioaccumulation experiments (mg/L or mg/kg); IR is the consumption rate of Chanos chanos (L/day or kg/day) [27]; EF is the frequency of exposure (365 day/year); ED is the duration of exposure (70 years, equivalent to the average lifespan); BW is body weight (kg) 60 kg adults and 30 kg children. AT is the period during which the dose is averaged (365 days/year times the number of years of exposure, assumed to be 70 years in this study) [28].

The human health risk posed by Zn exposure to Chanos chanos is characterized by a target hazard quotient (THQ) [26] i.e., the ratio of the estimated average daily intake of Chanos chanos resulting from metal exposure in the medium compared to the reference dose (RfD) for the individual pathway using Eq. (6). The RfD is obtained from the Integrated Risk Information System [26]. The RfD value for Zn is 300 mg/kg/day.

$$THQ = \frac{EDI}{RfD}$$

If the THQ value is less than 1 then the risk of noncarcinogenic toxic effects is considered low and if the THQ exceeds 1 then there is a concern about potential health risks associated with over-exposure [28].

RESULTS AND DISCUSSION

The ability of Chanos chanos to accumulate zinc (Zn) is represented as a concentration factor (CF). The CF value is the ratio of the activity of the concentration of Zn metal in the body of Chanos chanos to the activity of the concentration of Zn in seawater. The kinetics of $^{65}$Zn absorption in juvenile Chanos chanos in a 7-day bioaccumulation experiment represented by a factor concentration value Fig. 1 were 0.67-18.18 mLgr$^{-1}$. Bioaccumulation of contaminants by an organism generally occurs when the absorption rate exceeds the excretion rate [29]. The bioaccumulation of metals is a very complicated process due to the different characteristics of metals, the properties and behavior of each organism, and environmental influences and exposure pathways [30]. The kinetic parameters and the statistics of related research results in Table 1 and Fig. 1 shows that with increasing time there is an increase in CF values, meaning that ZnCl$_2$ concentrations from 0.5-2 ppm when dissolved in the 8 L aquarium of seawater can accumulate rapidly in Chanos chanos. This shows
that the heavy metal Zn can accumulate in aquatic organisms indirectly through permeable membranes such as skin and gills, but not through the digestive system [31].

Based on the absorption constant \( (k_u) \) data in Table 1, the absorption speed of Zn by Chanos chanos was 1.878-3.267 \( \text{d}^{-1} \) with a non-sequential pattern of \( \text{ZnCl}_2 \) concentration of 1; 2; 0.5 and 1.5 ppm. The highest Zn absorption rate at 1.5 ppm \( \text{ZnCl}_2 \) concentration was 3.267 \( \text{d}^{-1} \) and the lowest at 1 ppm concentration was 1.878 \( \text{d}^{-1} \). The result of the linear regression model shown in Fig. 2 with \( R^2 \) was 0.0276. A small \( R^2 \) value indicates that there was no correlation between the \( ^{65}\text{Zn} \) absorption rate in Chanos chanos and the increase in Zn concentration in aquarium seawater media. This might be due to the different juvenile accumulation abilities of Chanos chanos caused by Zn metal contamination, which affects changes in behavior, physiology, blood flow patterns, ionic balance, cell structure, liver function, and carbohydrate metabolism in fish [32].

Heavy metal pollution is one of the biggest environmental problems in the world because its distribution and toxins are harmful to human health. Improper management of landfills can lead to pollution of the ecosystem through vegetation blockage and environmental damage. The accumulation of heavy metals in the soil will lead to a decrease in crop productivity and safety, hindering the recycling and waste treatment processes [33]. Knowledge of the potential accumulation of chemicals in aquatic organisms is important in the identification of priority pollutants and the application of water quality standards for environmental protection. The experimental approach uses the concepts of bioconcentration, bioaccumulation, and trophic transfer to obtain data that can be adapted to either a kinetic model, where accumulation is expressed as a function of time or a partition equilibrium model that assumes thermodynamic equilibrium or at least a steady state has been reached. In aquatic organisms, dissolved pollutants are taken up through gills, body surfaces, and particulate pollutants through the digestive system. Once absorbed, these pollutants are diluted in the blood and channeled throughout the organism through the circulatory system, as shown in Fig. 3 [34].

In this study, based on model calculations, steady-state conditions were achieved on day 7 of the bioaccumulation experiment in Table 1. The concentration factor steady state \( (CF_{ss}) \) of \( ^{65}\text{Zn} \) in the experimental \( \text{ZnCl}_2 \) concentration of 0.5 ppm was 17.03 \( \text{mLgr}^{-1} \); 1 ppm: 12.24 \( \text{mLgr}^{-1} \); 1.5 ppm: 18.18 \( \text{mLgr}^{-1} \) and 2 ppm: 14.80 \( \text{mLgr}^{-1} \). A steady state is achieved when the process of absorption and elimination of pollutants becomes balanced.

![Fig. 1. The concentration factor of \( ^{65}\text{Zn} \) on Chanos chanos.](image1)

![Fig. 2. The linear constanta uptake \( (k_u) \).](image2)

### Table 1. Uptake kinetic \( ^{65}\text{Zn} \) on Chanos chanos.

<table>
<thead>
<tr>
<th>Zn (ppm)</th>
<th>Chanos chanos ( CF ) (mLgr(^{-1}))</th>
<th>( CF_{ss} ) (mLgr(^{-1}))</th>
<th>( K_u ) (d(^{-1}))</th>
<th>( K_s ) (d(^{-1}))</th>
<th>( T_{1/2} ) (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.23-4.46</td>
<td>4.00-16.36</td>
<td>2.317</td>
<td>5.912</td>
<td>11.72</td>
</tr>
<tr>
<td>1</td>
<td>3.78-5.42</td>
<td>0.87-10.02</td>
<td>10.02</td>
<td>1.878</td>
<td>4.983</td>
</tr>
<tr>
<td>1.5</td>
<td>3.19-3.59</td>
<td>0.60-18.18</td>
<td>18.18</td>
<td>3.267</td>
<td>7.421</td>
</tr>
<tr>
<td>2</td>
<td>4.25-4.46</td>
<td>1.06-14.12</td>
<td>14.12</td>
<td>2.115</td>
<td>8.681</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Zn (ppm)</th>
<th>Portunus pelagicus ( CF ) (gr)</th>
<th>( CF_{ss} ) (gr)</th>
<th>( K_u ) (d(^{-1}))</th>
<th>( K_s ) (d(^{-1}))</th>
<th>( T_{1/2} ) (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>118.5-123.7</td>
<td>19.54-60.62</td>
<td>0.784</td>
<td>0.089</td>
<td>6.183</td>
</tr>
<tr>
<td>1</td>
<td>106.7-114.4</td>
<td>13.45-39.93</td>
<td>0.420</td>
<td>0.075</td>
<td>5.212</td>
</tr>
<tr>
<td>1.5</td>
<td>122.5-124.2</td>
<td>11.15-35.46</td>
<td>0.393</td>
<td>0.073</td>
<td>5.000</td>
</tr>
<tr>
<td>2</td>
<td>120.5-122.7</td>
<td>7.82-25.55</td>
<td>0.303</td>
<td>0.065</td>
<td>4.533</td>
</tr>
</tbody>
</table>

Based on the concentration factor \( CF_{ss} \) in Table 1, the concentration of Zn in aquarium seawater media. The different juvenile accumulation abilities of Chanos chanos might be due to the different juvenile accumulation abilities of Chanos chanos caused by Zn metal contamination, which affects changes in behavior, physiology, blood flow patterns, ionic balance, cell structure, liver function, and carbohydrate metabolism in fish [32].
In this study, based on model calculations, steady-state conditions were achieved on day 7 of the bioaccumulation experiment in Table 1. The concentration factor steady state (CFs) of $^{65}$Zn in the experimental ZnCl$_2$ concentration of 0.5 ppm was 17.03 mLgr$^{-1}$; 1 ppm: 12.24 mLgr$^{-1}$; 1.5 ppm: 18.18 mLgr$^{-1}$ and 2 ppm: 14.80 mLgr$^{-1}$. A steady state is achieved when the process of absorption and elimination of pollutants becomes balanced.

Several studies of pollutant bioaccumulation in aquatic organisms explain the complexity of the process of bioaccumulation and the distribution of pollutants. The results of research by Cresswell [22] on the kinetics of bioaccumulation and distribution of zinc ($^{65}$Zn) in freshwater decapod crustaceans Macrobrachium australiense explained that metal absorption and elimination rates are the same when exposed to individual metals, mixtures, and depletion rates are close to zero. Most of the uptake of cadmium is localized in the gills and hepatopancreas, while zinc is in the antennal glands (concentration is greater than in other organs). While research on the bioaccumulation of plutonium (Pu) in Babilonia spirata from Jakarta Bay using a $^{240}$Pu radiotracer shows that the bioavailability of $^{240}$Pu in the form of Pu$^{4+}$ oxide has the potential to accumulate in more significant values than Pu$^{3+}$ oxide by Babilonia spirata, where Pu is more quickly distributed and lasts longer in the proboscis and gastrointestinal tract [23]. Other studies have explained that the bioaccumulation of dissolved essential trace elements Co, Mn, and Zn in blue mussels (Mytilus edulis) is not significantly affected by pre-exposure to the algae poison Karenia brevis. In contrast, the Cd uptake rate constant was significantly higher in the pre-exposure group, likely due to a decrease in the rate of shellfish clearance after exposure to Karenia brevis algae. These results suggest that the effect of algae toxin exposure on the bioaccumulation of trace elements in shellfish may depend on trace elements [35]. While bioaccumulation modeling and determination of kinetic parameters conducted by Urien [36] explained the first-order kinetic model [37], bioaccumulation of clean water-borne metals can be described with simple biodynamic models and the results of statistical analysis of nonlinear least squares regression following bioaccumulation data. In addition, measurements of variability between natural populations can be carried out using the kinetic parameters of bioaccumulation.

After completing the bioaccumulation experiment, Zn depuration was followed by a 7-day contaminant-free seawater experiment. The depuration result is shown in the retention percentage in Fig. 4.

The results of the 7-day depuration experiment for the release of $^{65}$Zn (elimination) from the body of Chanos chanos are shown by the percentage of retention in Fig. 4 and Table 1 along with the elimination constant depicted ($k_e$) in Fig. 5. During the depuration phase, the concentration of $^{65}$Zn decreases in the same pattern. The most rapid release of $^{65}$Zn from the body of Chanos chanos occurs in biota treated with ZnCl$_2$ concentration of 2 ppm, followed by concentrations of 1.5, 0.5, and 1 ppm, with corresponding elimination Constanta values ($k_o$) of 8.681, 7.421, 5.912, and 4.983, respectively, as shown in Table 1. The elimination rate of $^{65}$Zn at the end of the depuration period was 38.21 % for 0.5 ppm, 44.82 % for 1 ppm, 14.61 % for 1.5 ppm, and 17.54 % for 2 ppm exposure concentration. The linear model of elimination Constanta ($k_o$) in Fig. 5 shows that the value of R$^2$ is 0.7213, which indicates that there is a significant
correlation between the concentration of pollutants in the aquarium seawater media and the seed release of pollutants. The biological residence time of zinc in the tissues of Chanos chanos, up to half the initial concentration, ranges from 7.98 to 13.91 days. Generally, it can be observed that the smaller the size of Chanos chanos, the higher the value of $CF_{z_{2}}$, $k_{eq}$, and $k_{n}$, leading to the shorter residence time.

![Fig. 5. The correlation of Constanta Elimination ($k_{n}$) with Zn concentration.](image)

In this study, the use of $^{65}$Zn radioisotope tracers has increased our knowledge of contaminant transfer in Chanos chanos and comprehended the biogeochemical cycles of organic and inorganic particles, as well as dissolved species in global environmental changes. The ability to analyze living radio-organisms and the increased sensitivity of radiotracer detection allow for (1) assessment of biological variation among individual organisms; (2) measurement of the biokinetics of contaminants over the long term; and (3) distinction between foods from sources of contaminants that are difficult to investigate using standard analytical techniques [38]. Additionally, nuclear engineering facilitates experiments using concentrations of contaminants that are comparable to those found in natural waters [39].

Gills have a complex morphological and physiological structure, including one of the first fish organs exposed to heavy metals in the aquatic environment. Exposure to heavy metals in Oreochromis mossambicus showed adverse effects on normal fish behavior and varying degrees of gill damage, such as nuclear deformity, cell swelling, deposition of lipid droplets, and increased vacuolation in affected gills [40]. Physiological changes in gill tissue are important biological signs in the biomonitoring of water quality due to pollutant exposure [41].

Research into the kinetics of bioaccumulation and distribution of metals in Macrobrachium australiense shrimp showed that metals were distributed into gills, hepatopancreas, and antenna glands [22]. The kinetic experiments of bioaccumulation and radioactive distribution of $^{137}$Cs on Chanos chanos were distributed into the gills, skin, head, and internal organs [42]. Bioaccumulation of metals in fish tissue depends on the rate of absorption, storage, and elimination, in addition, there are other influencing factors, namely temperature, age of fish, interaction with other metals, water chemistry, and metabolic activity of tissues and fish [43].

A better understanding of metal accumulation and internal location will improve our understanding of exposure history and potential toxic effects on affected organisms. After accumulation, metals can remain in metabolically available forms, which can result in toxicity to organisms, be processed internally and excreted from the body, or be stored in biologically inactive forms [44,45].

The general metal elimination pathway for fish is through gills, bile, urine, and mucus [43]. The factors affecting the elimination of metals from fish tissues are the concentration of pollutants in the target organ, time duration, temperature, interacting agents, age of fish, the metabolic activity of fish, organ abnormalities, and the biological half-life of metals [46,47]. The results of the study stated that the presence of lag-binding proteins in tissues causes metal accumulation faster than metal elimination [48].

Organisms process contaminants either through detoxification or release through excretory products back into the environment; when the process is ineffective, side effects/toxicity may occur. Contaminant loss data is important in biodynamic modeling and can be measured with radiotracers. The degree of complete depuration in the organism's body (specific tissues and organs) has been evaluated using radiotracers [49] and definitive predictions for specific contaminant loss rates from different animals remain elusive. Metian describes a more comprehensive assessment to explain the extent of contaminant loss, using a radiotracer [50].

Assessment of depuration rates in commercial seafood species can reduce the risk of consumer exposure to contamination by maintaining the organisms for a period in clean, open-circuit water systems after the harvesting phase. Chemical and physical speciation of dissolved metal contaminants in fresh and seawater can affect their bioavailability to aquatic organisms, including plants (phytoplankton) and animals [30].

Research on the bioavailability of $^{137}$Cs from sediments off the coast of Fukushima, Japan, after the accident at the Fukushima-Daiichi nuclear power plant, showed that contaminant levels were higher in
benthic fish than pelagic fish in Japanese coastal waters [51]. This is evidence that Cs can form in benthic food chains through the assimilation of Cs in detritus-eating worms that assimilate it from contaminated sediments and transfer it to fish or macroinvertebrates [52]. In addition, physical factors (temperature, and pH of the seawater) can also affect the bioaccumulation of metals and radionuclides.

The estimated average daily intake (EDI) of heavy metal Zn accumulated in *Chanos chanos* when consumed by people in the Jakarta Bay area can be calculated using the equation recommended by the US EPA [26]. The calculation of the average estimated daily intake (EDI) of heavy metal Zn in *Chanos chanos* based on data on Zn metal bioaccumulation during the experiment and the amount of human health risk posed by exposure to heavy metal Zn marked with the target hazard quotient (THQ) is shown in Table 2.

Table 2. Calculation of EDI (Estimated Daily Intake) and THQ (Target Hazard Quotient).

| No | C IR EF ED BW AT EDI RDI THQ |
|----|------------------|------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| C  | IR, EF, ED, BW, AT, EDI, RDI, THQ | Children, Adult, Children, Adult, | 1 | 6.36 | 0.0185 | 365 | 70 | 30 | 60 | 70 | 3.68 | 1.84 | 300 | 0.012 | 0.006 |
| 2  | 10.02 | 0.0185 | 365 | 70 | 30 | 60 | 70 | 2.26 | 1.13 | 300 | 0.008 | 0.004 |
| 3  | 18.18 | 0.0185 | 365 | 70 | 30 | 60 | 70 | 4.09 | 2.03 | 300 | 0.014 | 0.007 |
| 4  | 14.12 | 0.0185 | 365 | 70 | 30 | 60 | 70 | 3.18 | 1.59 | 300 | 0.011 | 0.005 |

Table 2 explains that the estimated daily intake (EDI) of *Chanos chanos* accumulated Zn metal (18.18 mg/L) in Jakarta Bay for children was 4.09 (µg/kg bw/day) and for adults was 2.05 (µg/kg bw/day). Then the value of the magnitude of the human health risk posed by exposure to heavy metal Zn characterized by target hazard quotient (THQ) in children was 0.014 and adults 0.007, this indicates that the risk of noncarcinogenic Zn toxic effects is considered low (THQ value less than 1). The results of this study suggest that *Chanos chanos* can develop a zinc detoxification system by converting the metal into a relatively inactive and non-toxic organism, as indicated by the absence of deaths of *Chanos chanos* during the experiment, but more research is needed.

**CONCLUSION**

Biokinetic research on the absorption and depuration of Zn into cultivated organisms, which become part of community’s consumption, is important for monitoring the concentration of contaminants in biota body tissues. Milkfish (*Chanos chanos*) is an economically important fish commonly consumed by the public. Bioaccumulation and depuration studies of $^{65}$Zn in juvenile *Chanos chanos* have showed that the concentration factor (CF), uptake constant ($k_u$), and elimination constant ($k_e$) vary with Zn concentration. Furthermore, the relative retention percentage is inversely proportional to the pollutant concentration of ZnCl$_2$. The highest uptake constant ($k_u$) value was observed at a ZnCl$_2$ concentration of 1.5 ppm, reaching 3,267 d$^{-1}$, while the highest elimination constant value ($k_e$) was obtained at a ZnCl$_2$ of 2 ppm, reaching 8,681 d$^{-1}$.

The estimated daily intake (EDI) of Zn accumulated in *Chanos chanos* at 18.18 mg/L in Jakarta Bay is 4.09 (µg/kg bw/day) for children and is 2.05 (µg/kg bw/day) for adults. Additionally, the target hazard quotient (THQ) for children is 0.014, and for adults, it is 0.007. These values indicate that the risk of noncarcinogenic Zn toxic effects is considered low.

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**AUTHOR CONTRIBUTION**

Ikhsan Budi Wahyono, Heny Suseno, Muslim, and Chrisna Adhi Suryono were the main contributors to the idea of research and writing this manuscript.

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