The Effect of Zinc Speciation and Its Concentration on Bioaccumulation in Pomfret (*Colossoma macropomum*) and Sepat Fish (*Trichogaster Trichopterus*)

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Abstract

Heavy metal Zinc is still a pollutant from various industrial and domestic activities whose waste is directly dumped into the waters. The presence of biota in the waters can be used as a bioindicator to identify the presence of heavy metal contaminants. The kinetics of the Zinc bioaccumulation process through the freshwater route using pomfret (*Colossoma macropomum*) and sepat fish (*Trichogaster trichopterus*) as bioindicators have been investigated by analyzing the effect on variations in concentration and pH. This research was conducted by determining Zinc speciation's effect and concentration on the bioaccumulation process. The results showed an increase in concentration would also increase the rate of uptake and elimination rate of Zinc by pomfret and sepat, obtained concentration factors (CF) at variations of Zinc concentration in pomfret was 2.56 - 22.97 mL.g^{-1} and 2.14 - 56.87 mL.g^{-1} in sepat fish. While the value of the concentration factor (CF) in the variation of pH for pomfret is 0.65 - 13.15 mL.g^{-1} and for sepat fish is 0.93 - 15.29 mL.g^{-1}, and $ZnCl_4^{2-}$ was the dominant species containing Zn in most of the electrolytes in fresh water and from each concentration variation. This method is expected to provide information that can be used for environmental studies and against toxic effects for food safety.

Keywords: Bioaccumulation, Biokinetics, Pomfret, Sepat fish, Zinc

Introduction

Heavy metal pollutants have become a tremendous global due to their toxicity, intrinsic persistance, non-biodegradable, assertive accumulative behavior. Rapid industrialization, urbanization, populace growth, agriculture, and different human sports have resulted in excessive pollutants via heavy metals globally, particularly in growing countries [1]. Large quantities of heavy metals from these activities are discharged into rivers, accumulating along the water, sediments, and aquatic food chains, resulting in sublethal or lethal effects on sectional fish populations [2,3].

Zinc (Zn) is one of the maximum common pollution caused by natural and domestic activities. Zinc (Zn) is broadly utilized in diverse metal-associated industries, consisting of the smelting industry, the galvanized metallic industry, and the alloy utility industry [4]. Bioaccumulation studies through biokinetic approaches are still minimal. Moreover, this is related to aquatic biota, which is not yet known for sure capable of being a bioindicator of pollution in the waters. One marine biota that is often used as a bioindicator is fish. The use of fish as a bioindicator can be affected by water elements directly through water entering the gills or indirectly through food in the digestive tract [5].

Bioaccumulation is a process of accumulation of harmful substances or pollutants in an organism that occurs either through the abiotic environment around the organism, such as water, soil, air, or from the food or feed route [6]. The compartment model is a model that explains the process of taking and reducing pollutants in living things. In the single-compartment model, the bioaccumulation process can be analogized to balance 2 kinetics, uptake, and depuration. Accepting and releasing it is suspended between 2 kinetic processes [7,8].

Tracer methods have often been used in science and technology such as medicine, chemistry, biology, agriculture, physiology, instrumentation, toxicology, etc. A tracer is an atomic or molecular substance that provides identification, observation, and study of the behavior of more complex physical, chemical, or

biological processes, such as dispersion or concentration, kinetics, and dynamics, chemical reactions, physiological interactions, etc. that occur instantaneously or within a specific time interval. In this method, the object under study is traced or called a tracer, a substance, or a molecular component. Some radioactive tracers are ^{109}Cd, ^{210}Pb, ^{65}Zn, etc. The advantage of using this method is that it has a reasonably high detection sensitivity, and experiments can be carried out using a limited number of marine biota [9,10].

Pomfret (*C. macropomum*) and Sepat (*T. trichopterus*) are fish whose habitat is in 2 main rivers in the Bogor area, namely the Cisadane River and the Ciliwung River. Pomfret and sepat fish are aquatic biotas vulnerable to changes in environmental conditions and can live in freshwater, rivers, swamps, and even lakes. The community favors them for consumption because of their economic value, thick meat texture, and distinctive taste. In addition, pomfret is one of the fish that is easily cultivated and relatively susceptible to pests and diseases and is valuable as a fish that can be used to control cholesterol levels. Meanwhile, sepat fish can be grouped into foodstuffs that are often a source of protein for rural communities. These fish additionally occupy better trophic stages within the meals chain, so they are considered one of the maximum common bioindicators for pollutants.

Research conducted by Takarina *et al.*, showed that the concentrations of Cd, Cu, and Zn in all fish ranged from 0.845 - 2.230 mg.kg^{-1} dry weight for Cd, 0 - 18.640 mg.kg^{-1} dry weight for Cu, and 21.540 - 105.520 mg.kg^{-1} dry weight for Zn [11]. Mapenzi *et al.*, also investigated the ability of African catfish and tilapia species to accumulate Zn, Cu, and Pb metals, which gave significant concentrations of Zn metal. It is because of seepage from agricultural land and mining sites in the Songe and Luika river basins [12]. Has-Schon *et al.*, who conducted a study on the distribution of heavy metals and their relationship to age in European carp and catfish, showed a tremendous full-size correlation among metallic accumulation concentrations (Pb, Hg, Cd, and As) on age and body mass in the flesh, liver, and kidneys of carp and catfish adults [13].

Based on the description above, a bioaccumulation study was carried out by observing the toxic effects, accumulation, and elimination of zinc metal in pomfret (*Colossoma macropomum*) and sepat fish (*Trichogaster trichopterus*) which have economic value and are widely consumed by the Indonesian people by using a ^{65}Zn radiotracer.

Materials and methods

The bioaccumulation of Zinc follows the principle of the entry of pollutants into living organisms. The approach in this research is based on the process of absorption and elimination of contaminants using a biokinetic model.

Materials

Radiotracer ^{65}Zn produced BATAN radioisotope with a concentration of 73.6×10^4 Bq, solids ZnSO$_4$, pomfret (*C. macropomum*), sepat fish (*T. trichopterus*), fish food, freshwater, and aquadest. The tools used are 10 L and 200 L aquariums, filter containers, hoses, water pumps, filter cotton, skimmers, aerators, aeration stones, 10 mL vials, statives, and gamma spectrometers.

Procedures
Aquatic biota
Samples of pomfret (C. macropomum) and sepat fish (*T. tricopterus*) were taken from freshwater fish farming in October 2021. The fish were then placed in a clean and leak-free aquarium with a capacity of 200 L that had been prepared beforehand and had been subjected to an aeration process before the biota was acclimatized at the Aquatic Laboratory of the Radiation Health and Safety Technology Research Center, Nuclear Technology Research Organization-National Agency for Research and Innovation (BRIN), Pasar Jumat, South Jakarta.

Acclimatization
The acclimatization process is carried out to provide adaptation time for the object of research in the form of living organisms in the research environment so that the biota is not stressed and can be used for experiments. Pomfret and Sepat fish were fed once a day for 7 days with the water temperature in the aquarium 27 - 29 °C.

Bioaccumulation through the freshwater path

After the acclimatization process was completed, 7 aquariums with a capacity of 10 L were prepared, each filled with 8 L of fresh aerated water. Furthermore, each 2 pomfret and 2 sepat fish with various masses were divided into 7 aquariums. The contaminant uptake process was carried out for 5 days.

Concentration variation

In 4 aquariums filled with 8 L of freshwater, 0.8 mL of 1000 ppm Zn solution was added so that the concentration was 0.1 ppm. In the other aquariums, 4 mL, 8 mL, and 12 mL of 1000 ppm Zn solution were added, respectively, so that the concentrations in the aquarium were 0,5 ppm, 1 ppm, and 1,5 ppm, respectively. Then each aquarium was added with a ^{65}Zn radiotracer with an activity concentration of 0.98 $Bq.mL^{-1}$.

Variation of pH

In the other 3 aquariums filled with 8 L of freshwater, each treatment was given with variations of pH 5, pH 6, and pH 7. Then 8 mL of 1000 ppm Zn solution was added so that all aquarium concentrations were 0.5 ppm, and a ^{65}Zn radiotracer was added with an activity concentration of 1.84 $Bq.mL^{-1}$.

Measurement of ^{65}Zn activity in biota

The ^{65}Zn activity measurement process was carried out every day during the contamination period. Pomfret and sepat fish to be measured are placed in a 10 mL vial filled with fresh water and placed in a holder. Each time measurement must be carried out under the same conditions, namely the distance of the container from the detector, the height of the water in the container, and the geometry of the container used. Measurements were carried out for 3 min on each pomfret and sepat fish.

Biota depuration

After the bioaccumulation process was carried out, the pomfret and sepat fish were transferred to a new aquarium containing 8 L of fresh water and free of contaminants and complete with a filtration and aeration system. Feeding the biota is still done every day.

Radiotracer ^{65}Zn activity measurement during depuration

Measurement of ^{65}Zn radionuclide activity in the depuration process was carried out every day for 5 days. Pomfret and sepat fish to be measured are placed in a 10 mL vial filled with fresh water and placed in a holder. Each time the measurement must be carried out under the same conditions, namely the distance of the container from the detector, the height of the water in the container, and the geometry of the container used. Measurements were carried out for 3 min on each pomfret and sepat fish.

Concentration factor value analysis

The value of the concentration factor (CF) is a ratio of the pollutant in the body of the biota to the concentration in the water. Formulated in the following equation:

$$CF_t = \frac{C_t}{C_w} \tag{1}$$

C_t is the concentration of the ^{65}Zn radiotracer in the organism against time or known as the concentration of biota activity. At the same time, C_w is the concentration of pollutants in the surrounding environment or the concentration of water activity.

Determination of zinc speciation using ChemEQL software

The content and behavior of Zn in water are conditioned by the interaction between the liquid and solid phases. Coprecipitation with carbonates can be an important mechanism for Zn deposition and will be a major component if the water contains excess carbonate concentrations. Some metals such as Zn in sediments and suspended particles will remobilize and diffuse to the surface [14].

Determination of Zn speciation is using ChemEQL software to calculate and describe the thermodynamic equilibrium concentration of species in complex chemical systems. This software performs the determination of homogeneous solutions, melting, precipitation, titration with acids or other components. The Zn concentration is inputted into the software by entering Ca^{2+}, CO_3, and H^+ concentration data, respectively. Then click Compile Matrix and continue by clicking Run Go so that each Zn speciation is formed with its concentration.

Results and discussion

Zinc metal (Zn), when it enters water bodies, will form a variety of chemical speciation, which is more in the form of Zn complexes. The distribution of chemical speciation on metals in the inorganic form, which is influenced by variations in concentration, was analyzed using ChemEQL software. This software can calculate and describe the thermodynamic equilibrium of complex chemical speciation by adding ions and their concentrations according to the conditions during the research. The concentration of each speciation uses the following equilibrium constant:

$$ZnCl_2 + 2Cl^- \rightleftharpoons ZnCl_4^{2-} \tag{2}$$

$$[ZnCl_4^{2-}] = K\,[ZnCl_2]\,[Cl^-]^2 \tag{3}$$

Based on the concentration of each speciation obtained using the above equation is shown in **Table 1**.

Table 1 Chemical speciation of Zn in various concentration.

No.	[Zn] ppm	Concentration (ppm)					
		Zn^{2+}	$ZnHCO_3^+$	$ZnCO_3$	$ZnCl_2$	$ZnCl_3^-$	$ZnCl_4^{2-}$
1	0.1	9.73×10^{-20}	3.53×10^{-5}	7.85×10^{-14}	6.52×10^{-11}	6.79×10^{-7}	0.099
2	0.5	4.87×10^{-19}	1.77×10^{-4}	3.92×10^{-13}	3.26×10^{-10}	3.39×10^{-6}	0.499
3	1	9.73×10^{-19}	3.53×10^{-4}	7.85×10^{-13}	6.52×10^{-10}	6.79×10^{-6}	0.999
4	1.5	1.46×10^{-19}	5.31×10^{-4}	1.18×10^{-12}	9.87×10^{-10}	1.02×10^{-5}	1.499

Zinc is one of the strongest metals as a chelating agent on the water's surface. The presence of Zn as a chelating agent makes the metal uptake rate controlled by the free metal concentration. The table above shows that the greater the concentration of Zn, the greater the attention of each speciation [15]. The availability of Zn^{2+} with chloride and carbonate also increased, and the concentration of Zn^{2+} complex was higher than that of free Zn^{2+}. It is indicated that the absorption of the Zn^{2+} complex becomes dominant in the Zn uptake process. The most dominant Zn^{2+} complex is the $ZnCl_4^{2-}$ species.

Changes in pH will affect metal speciation in the aquatic environment. The results of the analysis of chemical speciation models in various pH using ChemEQL software with data adapted to the research conditions are shown in **Table 2**.

Table 2 Chemical Speciation of Zn in Various pH.

No.	pH	Concentration (ppm)					
		Zn^{2+}	$ZnHCO_3^+$	$ZnCO_3$	$ZnCl_2$	$ZnCl_3^-$	$ZnCl_4^{2-}$
1	5	7.71×10^{-19}	3.68×10^{-4}	6.04×10^{-13}	5.74×10^{-10}	4.72×10^{-6}	0.499
2	6	6.02×10^{-19}	2.72×10^{-4}	5.29×10^{-13}	4.43×10^{-10}	4.36×10^{-6}	0.499
3	7	4.87×10^{-19}	1.77×10^{-4}	3.92×10^{-13}	3.26×10^{-10}	3.39×10^{-6}	0.499

The change in pH showed that the most dominant chemical species Zn with the greatest concentration and tended not to change was $ZnCl_4^{2-}$, compared to other species, which decreased in concentration as the pH value increased. So based on various concentrations and pH values, the dominant speciation is $ZnCl_4^{2-}$.

One of the applications of voltammetry in environmental analysis is speciation studies. Speciation analysis is defined as the determination of the concentration of various physicochemical forms of elements that make up their total concentration in the sample. Metals can exist in various physicochemical forms in environmental samples, including hydrated metal ions, inorganic and organic complexes, and adsorbed on organic and inorganic colloidal particles. The toxicity of metal ions varies with the physicochemical form, and the main reason for speciation studies is to measure the toxic fraction of the metal.
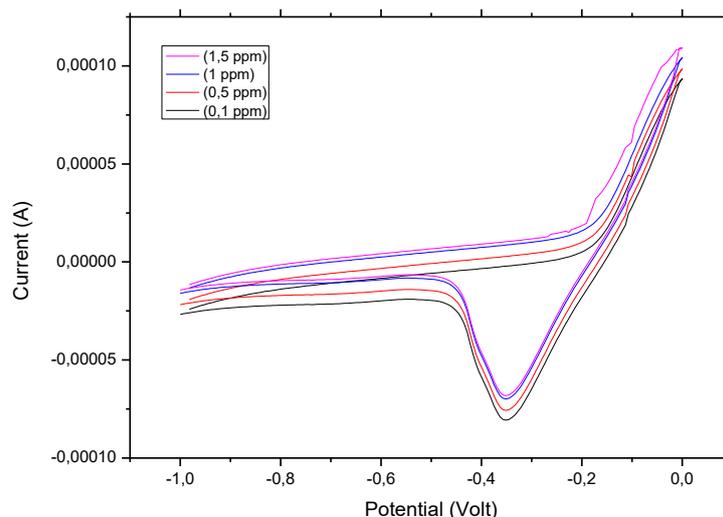
Figure 1 Cyclic voltammetry of $ZnCl_4^{2-}$ speciation at various concentrations.

Based on **Figure 1** there is an increase in peak oxidation current from the influence of Zn concentration. At a concentration of 0.1 ppm, it produces 9.157×10^{-5} A, a concentration of 0.5 ppm is 9.657×10^{-5} A, a concentration of 1 ppm produces 1.024×10^{-4} A, and for a concentration of 1.5 ppm it is 1.086×10^{-4} A. Zn speciation using voltammetry postulates that the species formed is the $ZnCl_4^{2-}$ ion derived from the main Zn species and there is a clear difference in the anodic charge in the potential region from 0.0 to -0.3 V and the analysis results based on this concentration variation show there is no difference in speciation formed from each concentration variation. This voltammetry characterization was carried out based on the reduction peaks with high absorption rates and these observations gave the results that $ZnCl_4^{2-}$ was the dominant species containing Zn in most of the electrolytes in fresh water and from each concentration variation was directly proportional to the metal concentration and the resulting current.

The ability of pomfret (*C. Macropomum*) to accumulate Zn is represented as concentration factors (CF). The CF value is the ratio of the attention of Zn in pomfret to the concentration in water. Based on **Figure 2(a)**, data is obtained that increasing the attention of Zn in water media can also increase the value of CF. At 0.1 ppm Zn concentration, the CF value on the primary day was 2.90 mL.g^{-1} until the previous day was 17.17 mL.g^{-1}. The concentration of Zn 0.5 ppm on the 1st day was 2.79 mL.g^{-1}, and until the previous day was 19.86 mL.g^{-1}. At a concentration of 1 ppm, the CF value on the 1st day was 4.51 mL.g^{-1} and the CF on the previous day was 20.07 mL.g^{-1}. Meanwhile, the Zn concentration of 1.5 ppm on the primary day was 2.56 mL.g^{-1} and the previous day was 22.97 mL.g^{-1}. These data indicate that the higher concentration of Zn in the waters, the higher exposure to Zn accumulates in biota.
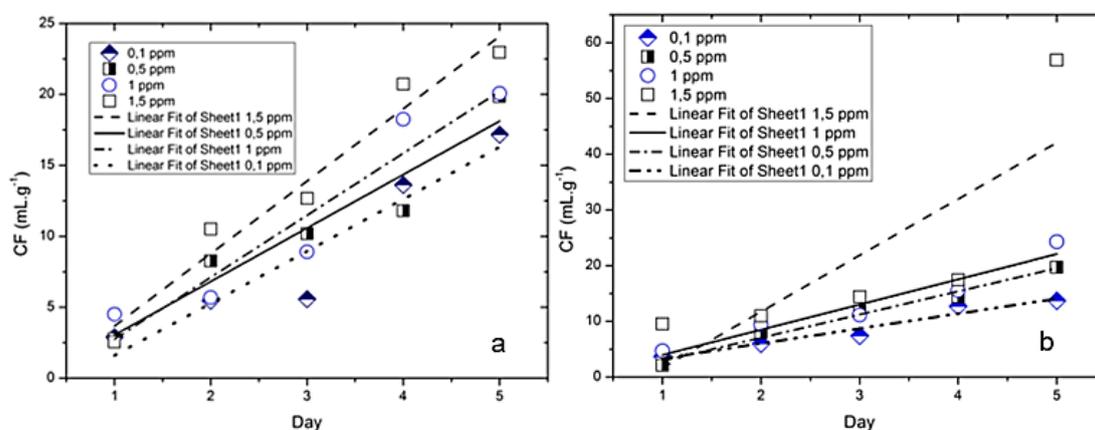
Figure 2 Uptake of various Zn Concentration Variations on Bioaccumulation; (a) Pomfret (*C. macropomum*); (b) Sepat Fish (*T. trichopterus*).

Meanwhile, after carrying out the uptake process for the sepat fish, the CF value is shown in **Figure 2(b)** for the 0.1 ppm Zn concentration on the primary day of 3.71 mL.g^{-1} and the last day of 13.72 mL.g^{-1}. The concentration of Zn at 0.5 ppm on the 1st day was 2.14 mL.g^{-1} and the previous day was 19.72 mL.g^{-1}. Then the Zn concentration at a concentration of 1 ppm on the primary day was 4.72 mL.g^{-1} and the last day was 24.29 mL.g^{-1}. And for the highest variation of Zn concentration, it is 1.5 ppm on the first day was 9.57 mL.g^{-1} and the previous day was 56.87 mL.g^{-1}. In the metabolic process, these aquatic biotas will process any toxicant that enters their body to accumulate and affect the toxicity of the metal itself. Of course, various species of marine biota have different abilities to accumulate Zn. The other metabolic systems of biota cause it to Zn in the body. The difference is related to the power of Zn adsorption to the cell wall, which then passes through the cell membrane to detoxify out of the cell. Internalization of Zn from water media into the body also depends on the organs of each biota to transport it into the circulatory system [16].

Budiawan *et al.*, suggested the effect of Cs and Zn concentrations as well as the impact of seawater at the capacity of *M. Micropterus* in accumulating these 2 pollutants, which resulted in the absorption of Zn and Cs according to a single-compartment model and the test becomes executed till constant situations have been reached. The concentration factor value was at the steady-state conditions for Zn were 31.94 - 45.54 mL.g^{-1} and 23.22 - 33.26 mL.g^{-1}, respectively, were affected by the concentration and salinity of seawater [17]. The following are some CF values from research results of Zn bioaccumulation through water in various biota.

**Table 3** The CF Value of Zn bioaccumulation research results in various biota.

Biota	CF (mL.g^{-1})	Refrences
Litopenaeus schmitti	16.71 - 53.27	[11]
Streptomyces zinciresistens	90.00 - 151.55	[12]
Malleus regula	26.60 - 98.00	[13]
Isognomon isognomon	72.20 - 121.20	[13]
Mactra veneriformis	52.00 - 70.90	[14]

Zinc is one of the metals that are widely available in the free form and can form complex compounds with chlorides or carbonates, so it is estimated that the absorption of Zn in addition to the Zn^{2+} is also absorbed in its complicated form. **Figure 3** shows the influence between the concentrations of various chemical species Zn and the concentration factors (CF) of the bioaccumulation of pomfret and sepat fish. Based on the results of the speciation calculation, it appears that the $ZnCl_4^{2-}$ species is more dominant because it shows a higher concentration than other Zn chemical species. It allows the absorption of Zn-chloride complexes due to weak coordination covalent bonds and is the dominant complex formed based on ChemEQL software modeling. Weak complex bonds are preferred because, through chemical equilibrium, free metal ions are released into forms that are more available for bonding to the ligand chain. Compared with Zn-carbonate, which has a stronger complex bond.

In this study, freshwater acidification conditions were treated with pH variations of 5, 6, and 7. The effect of pH on the bioaccumulation ability of Zn contaminants by pomfret with CF values is shown in **Figure 4(a)**. At the uptake stage, which was carried out for 5 days, it was seen that the pomfret was only able to accumulate Zn metal the best at pH 5 conditions compared to other pH conditions. The high value of CF evidences it at pH 5 compared to other pH conditions. The CF value, which is the ratio between the concentration of contaminants in the body of the biota and the concentration of contaminants in the water for pH 5 on the first day, is 1.45 mL.g^{-1} and the previous day is 13.15 mL.g^{-1}. As for pH 6, the CF value on the primary day was 2.90 mL.g^{-1} and the last day was 3.65 mL.g^{-1}. At pH 7, the CF value on the first day was 0.64 to 3.54 mL.g^{-1} on the previous day. This change in pH is used as a research parameter adjusted to changes in conditions in the freshwater environment.
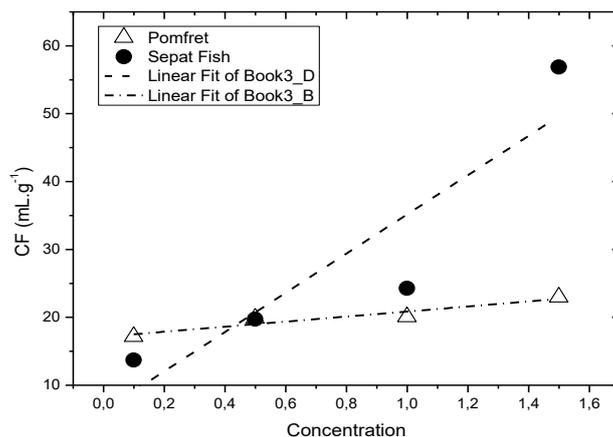
Figure 3 Chemical species of $ZnCl_4^{2-}$ on CF values based on concentration variations.
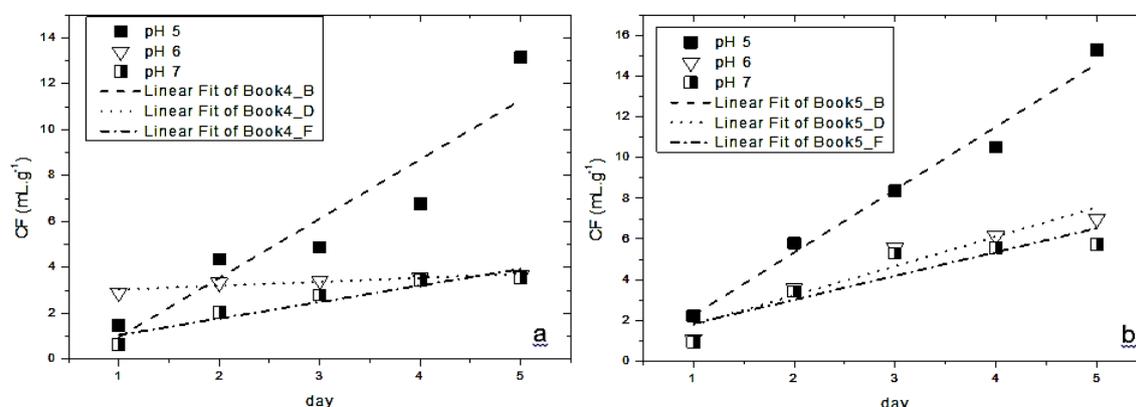
Figure 4 Uptake at various pH variations of Zn on Bioaccumulation; (a) Pomfret (*C. macropomum*); (b) Sepat Fish (*T. trichopterus*).

The pH variations were also carried out on sepat fish (*T. trichopterus*) with the same pH variations, namely pH 5, 6, and 7. **Figure 4(b)** describes the pattern of Zn bioaccumulation of pH variations in sepat fish. Variations in pH are carried out to see the effect of acidity levels in influencing metabolic processes in the body of biota. The ability of sepat fish to accumulate Zn is shown in **Figure 4(a)** with a representation of the concentration factor (CF) value. The power of sepat fish to absorb Zn at pH 5 was better than at pH 6 and pH 7. The CF value at pH 5 for the primary day was 2.21 mL.g^{-1} and the last day was 15.29 mL.g^{-1}. At pH 6 the CF value on the first day was 1.07 mL.g^{-1} and the previous day was 7.00 mL.g^{-1}. Meanwhile, for pH 7 the CF value for the primary day was 0.93 mL.g^{-1} and the last day was 5.72 mL.g^{-1}. Although the CF value increases every day, pH 5 is considered the best condition for sepat fish to absorb Zn metal contaminants.

The pH value is related to the bioaccumulation of contaminants in the body's biota. It is because pH is one of the essential factors in the metabolic process of aquatic biota. Millero *et al.*, estimated that the absorption of Zn metal is not only available in the form of Zn^{2+} ions but also its complex form [18]. The availability of Zn^{2+} increases when the pH decreases because most metals, including Zn, are more soluble in more acidic waters. The increase in pH in the living environment of biota reduces the accumulation of Zn. There may be a decrease in the biological function that absorbs contaminants due to an increase in pH that is not by the habitat biota and a decrease in pH, increasing the availability of Zn^{2+} metal ions.

Changes in the distribution of chemical species were analyzed using ChemEQL software. The concentration of stable Zn used was 0.5 ppm. Each Zn species in **Table 2** experienced a decrease in concentration, although not significantly, except for $ZnCl_4^{2-}$ which had a constant concentration trend and had the highest concentration. So, it is considered that the $ZnCl_4^{2-}$ species is the dominant species at pH variations.

Metal complexes with organic and inorganic ligands tend to dissociate when the pH decreases, increasing the concentration of free ions. The water's acidity level can affect the speciation of metals in solution and the biological sensitivity at the cell surface level. Certain metal species are also affected by a decrease in pH depending on the acid-base properties of the ligand and the stability of the metal species in the ligand. Zn^{2+} ions are widely available in the free form and can quickly form complexes with Cl^- ions. Changes in the concentration of various chemical species of Zn with this pH variation tend to experience an insignificant decrease as the pH value increases. So, there may be no effect of pH variations on the chemical speciation of Zn because the bond strength of organic ligands is not affected by changes in pH. The difference in pH on the speciation of organic metal complexes in the environment cannot be well characterized compared to the speciation of inorganic metal complexes due to the nonhomogeneous composition and unknown structure of the organic ligand bonds.

Conclusions

The kinetics of the zinc bioaccumulation process in biota, especially in aquatic biota, is fundamental in determining the characteristics of biota as a pollutant bioindicator in the environment. The results obtained showed the ability of Pomfret (*C. macropumum*) and Sepat Fish (*T. trichopterus*) to accumulate Zn in the environment and the effect of zinc speciation and its concentration in the environment on the bioaccumulation process. It shows the importance of bioaccumulation studies that are not only focused on the concentration ratio but must carry out comprehensive research by determining the bioaccumulation ability of contaminants by observing their ecotoxicology.

Acknowledgements

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