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The Acute Toxicity of HEPA Filters on Reactive Oxygen Species (ROS) and the Heavy Metal Analysis in the Common Carp Fish (*Cyprinus carpio*)

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ABSTRACT

This study aimed to investigate the acute toxicity (LC50) of a HEPA filter and its impact on the levels of reactive oxygen species (ROS) and heavy metal accumulation in common carp fish. In this study, carps observing approximately 8-9 cm in length were subjected to various concentrations of HEPA filters, such as 5%, 10%, 20%, 30%, and 40%. The findings of the study indicated that the utilization of a HEPA filter had an obvious effect on the levels of reactive oxygen species (ROS) in common carp fish. especially it was observed that the ROS level experienced a significant increase at a concentration of 40% (40 pg/mL). The concentrations of heavy metals Ag and Cu in the gill tissue exhibited a significant increase at a concentration of 40%, with values of 0.04 ppm and 0.013 ppm, respectively. In the gastric tissue, there was a notable increase in the concentration of heavy metals at 40% (approximately 0.015 ppm and 0.010 ppm, respectively). The HEPA filter exhibits acute toxicity with an LC50 value of 39.65% concentration. In addition to this specific concentration, it has the potential to adversely impact the health of aquatic organisms at the tissue and organ levels.

INTRODUCTION

HEPA filters have gained widespread popularity in recent times as the predominant filtration method employed to effectively achieve and maintain a high standard of air quality. In recent years, there has been an increased recognition of the efficacy of this filter technology in effectively purifying the air and mitigating the transmission of COVID-19. Recent research has been conducted to assess the advantageous function of air filtration. The HEPA filter demonstrates a notable level of efficacy, as the filter media is capable of achieving an air filtration rate exceeding 99.97% for particles measuring 0.3 μm, which is considered the most penetrating particle size **(Mera *et al.*, 2022)**. HEPA

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filters are usually manufactured by pleating microfiber glass made with multiple layers that arranged randomly with diameters ranging from 2 to 500 nm[4] **(Christopherson *et al.*, 2020).** There are three mechanisms of HEPA filter, consist of: impaction, interception, and diffusion **(Tong *et al.*, 2020)**. Every mechanisms has its own ability to filtered up the air. For particle size >1 μm, the impaction and interception are the most significant mechanisms of filtration. The last mechanism, which is diffusion is more dominant mechanisms of trapping particles <0.1 μm[4]. HEPA filter as the filter media contain heavy metals in the form of nanoparticles (NPs). Metal NPs, referred to as nanobiotics, have been proposed as novel antimicrobial agents. They have the potential to reduce or eliminate the continuous emergence of bacterial resistance **(Fan *et al.*, 2021).** The metals used for these NPs are Ag and Cu. The heavy metals Ag and Cu have been widely used in the antimicrobial applications, especially in combatting antibiotic resistant bacteria and nosocomial infections **(Chaluopka *et al.*, 2010)**. HEPA filter needs to be analyzed for its toxicity before being used further. In general, the results of the toxicity test can provide information about the toxicity caused by HEPA filter that contain heavy metals and help identify possible side effects.

The utilization of HEPA filters has experienced a notable rise in recent times, leading to a corresponding increase in waste production. This escalation in waste generation has the potential to result in toxic effects. Engineered nanomaterials (ENs) are being manufactured and utilized on a significant scale, leading to their widespread presence in natural aqueous ecosystems. This phenomenon has emerged as a pressing concern in recent times. Silver nanoparticles (Ag NPs) are widely recognized as a significant class of heavy metals employed in nanoparticle form, boasting a substantial global annual production ranging from 135 to 420 tons **(Pulit-Prociak and Banach, 2016).** The acute toxicity of Ag NPs is 0.224mg L^{-1} and it's varied widely depends on the species of the fish **(Mahjoubian *et al.*, 2023)**. Several studies have been performed to detect the potential long term of toxicity of Ag NPs. In the acute of Ag NPs showed changes in behavior patterns and morphological, included movements generally, prolonged periods of inactivity with fish resting on the bottom of the tank and changes in shoaling behavior (fish swimming closer together). These findings are all consistent with the finding from other research for toxic efects for exposure to metal-based NPs **(Pirsaheb *et al.*, 2019; Sayed *et al.*, 2020; Haghighat *et al.*, 2021)**. In the other hand, copper (Cu) is a chemical element which soft, malleable, and ductil with very high thermal and electrical conductivity and also can be used as catalysts, antimicrobial, and colorimetric sensing **(Hyder *et al.*, 2022).** LC_{50} of Cu value in soft water and hard water are quite different. According to the researched by **Kim*, et al.* (2020)** LC_{50} value in soft water was 18.62 ± 4.5 mg/L, however in hard water the value was 28.62 ± 4.5 mg/L for zebra fish. The increased LC_{50} value was reported previously with various heavy metals. Thus, cautions must be excised when HEPA filter is taken as a preventive measure in action to stop the spreading of COVID-19. Furthermore, **Liu *et al.* (2021)** conducted a study that revealed the continuous release of F-, Cl-, NO3-, and SO42- ions, as well as other chemical substances like Zn, Pb, and As, from abandoned HEPA filters. Failure to implement effective treatment measures for these filters could potentially impact the water environment and hydrological cycle. However, despite broad spectrum of acute toxicity (LC_{50}) clinical evidences are still lack to support the effect of the waste of HEPA filter in the aquatic environment.

The common carp fish as a test animal in a study is increasingly being used, especially for acute toxicity tests. Common carp is very popular in aquaculture industries, due to several specific features e.g., its feeding habits, high growth rate, reproduction in captivity, adaptation to commercial diets and density. Moreover, common carp is relatively insensitive, and can survive and accumulate contaminants at heavily polluted sites, which is why it is used as a freshwater bioindicator in environmental toxicology **(Stoyanova *et al.*, 2020).** The objective of this study was to analyze the potential toxic impact of waste from HEPA filters on aquatic organisms, with the aim of ensuring the safety of the resulting product for both the aquatic environment and human beings. The present investigation involved an assessment of the toxic effects of heavy metals derived from a HEPA filter on test subjects of common carp. The toxicity evaluation was conducted by determining the lethal concentration (LC50) over a duration of 96 hours. The research consists of two distinct stages. The first stage involves conducting a preliminary test to ascertain the critical concentration limit. This limit refers to the concentration level at which the mortality rate approaches 50% in both the highest and lowest death scenarios. Once the critical limit was established, the determination of the acute concentration was made using the logarithm series of concentrations as modified by **Rossiana (2006).** Subsequently, an investigation was conducted to examine the impact of toxic levels of heavy metals, specifically silver (Ag) and copper (Cu), on the morphology of common carp. This study will also include confirmation tests conducted on the gill and stomach tissue of common carp fish to determine the presence of reactive oxygen species (ROS) and heavy metals. Therefore, the obtained results provide support for the data regarding the acute toxicity test of HEPA filters and their impact on organisms.

MATERIALS AND METHODS

1. Fish preparation

A group of common carp fish (*Cyprinus carpio*) measuring 8-9 cm in length were kept in a freshwater aquarium with adequate aeration, at a temperature of 27°C. The carp fish were subjected to a one-week period of acclimatization prior to the commencement of the experiment. The fish were provided with a daily ration of commercial feed, which was administered twice a day..

2. Acute toxicity test (LC_{50}-96h)

Ten of carps were placed in 5 Lt of fiberglass tank clean and pathogen-free facilities in the laboratory of freshwater aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya. First, preliminary test and acute toxicity (LC_{50}-96h) were performed as described by **Rossiana (2006)** following modification. The contentrations are made by soaking the HEPA filter that has been cut into small pieces with 1.5 L DDH_2O in a 5 L erlenmeyer tube. Furthermore, to determine the critical concentration limit, the carps were exposed to HEPA filter waste in following concentrations (5%, 10%, 20%, 30%, and 40%) for 96h, respectively. Test medium was not renewed during the experiment and no food was provided to the animals. Furthermore, after 96h, The organ of fish (gill and stomach) and water were collected for heavy metals analysis, gills was also collected for the Reactive Oxygen Species (ROS) analysis. The study involved the observation of swimming behavior, operculum opening, and fish coloration. Each experiment was repeated three time respectively.

3. Heavy metal Analysis

The heavy metals were analyzed in water samples and fish tissues (specifically gill and stomach) obtained from each sample. In order to achieve a pH level below 2, the water sample was subjected to the addition of nitric acid. The gill and stomach preparation was conducted according to the methodology outlined in **Hertika et al. (2018)** study. The tissue samples were subsequently treated with 0.2 ml of 1 M HNO and incubated for 30 minutes. This step was performed to ensure the complete oxidation of the samples and the destruction of organic compounds at lower temperatures, thereby preventing the loss of minerals through evaporation. Subsequently, the samples were centrifugated at a speed of 12,000 revolutions per minute for 15 minutes. The supernatant was collected and subjected to heavy metals analysis using a Varian A220 Atomic Absorption Spectrophotometer (AAS).

4. Reactive Oxygen Species (ROS) Analysis

The ROS analysis was conducted in accordance with the procedure outlined in the MedikBio ROS ELISA Kit (E0347Fi). In a concise manner, the 40 microliter sample and 10 microliter Fish ROS antibody were introduced into the designated sample wells. Next, a volume of 50 microliters of streptavidin-HRP was added to the sample wells and thoroughly mixed. The well sample was subjected to incubation at a temperature of 37°C for a duration of 60 minutes. Subsequently, it was immersed in a wash buffer of 300 µl for a period ranging from 30 seconds to 1 minute for each washing step. In addition, 50 microliters of substrate solution A and substrate solution B were introduced into each respective sample well. Subsequently, the specimen well was subjected to an incubation period of 10 minutes at a temperature of 37°C, while being kept in a light-restricted environment. The Stop Solution was added to each well with a volume of 50 µL. The determination of reactive oxygen species (ROS) was conducted by measuring the optical density (OD value) of each well using a microplate reader set to a wavelength of 450 nm. This measurement was performed within 10 minutes after the addition of the stop solution.

5. Data analysis

Data are expressed as mean ± SD. Statistical significance of pairwise differences among three or more groups were determined using one-way analysis of variance (ANOVA) followed by LSD test. $P<0.05$ was considered statistically significant. Analysis was performed using SPSS for Windows (SPSS Inc., Version 20.0, Chicago, USA). Graph was performed using GraphPad Prism 7 (GraphPad Software, Inc. USA).

RESULTS

1. Acute toxicity of HEPA Filter

HEPA filter waste was shown the differences in lethal and toxicity in common carp (*Cyprinus carpio*) fish at each concentration (Table 1). This study also observed the behavior of swimming, operculum opening, and the color of carp fish. HEPA filter showed no toxicity at a dose of 5% and 10%. At this concentration, it was found that the mortality of common carp fish was low. The mortality was thought to be unaffected by HEPA Filter toxicity due to at this concentration common carps were observed to be normal in color, operculum opening, and swimming pattern behavior. For dose of 20% of HEPA filter waste, mortality increased a little more and it was known that the fish started to have different behaviour of swimming, operculum opening and the colors are starting to fade. On the other hand, the toxicity of HEPA Filter was shown at concentrations of 30% and 40% showed a fairly rapid death after exposure to HEPA filter waste. In this concentration range, several health problems occurred, such as faded colors, unstable operculum opening, and reversed swimming pattern behavior with the ventral facing upwards. This shows that concentrations above 30% of HEPA filter waste have a negative impact and are toxic to organisms. Furthermore, a probit test analysis was performed to determine the acute toxicity of LC_{50}. Moreover, the results of the 96-hour LC50 probit test are shown in Figure 1.

In the LC_{50} analysis, the probit test was carried out using the IBM SPSS Statistic 20 statistical program. The results showed that the lethal concentration that caused death toxicity up to 50% was obtained at a concentration of 39.65% with an R^2 of 0.882. Furthermore, the maximum concentration of HEPA filter waste toxicity, which is thought to be capable of causing death was obtained at 30% or more. Based on the results of the probit test, it shows that at concentrations above a dose of 39.65% it can be suspected that it causes physiological disturbances in the organism and it does not have a good effect on mortality, morphological disturbances, and fish behavior. To determine the disturbance that occurs in common carps caused by HEPA filter waste toxicity, a reactive oxygen species (ROS) analysis was carried out, as well as an analysis of heavy metal content in gill and stomach tissues.

Table 1. Results of LC50 HEPA filter acute toxicity test for 96 hours

Concentration (ppm)	Replication	∑ Fish	Mortality	∑ Mortality
Control	1	10	0	0
	2	10	0	
	3	10	0	
5%	1	10	1	1
	2	10	0	
	3	10	0	
10%	1	10	0	1
	2	10	1	
	3	10	0	
20%	1	10	2	4
	2	10	1	
	3	10	1	
30%	1	10	4	11
	2	10	4	
	3	10	3	
40%	1	10	6	15
	2	10	4	
	3	10	5	

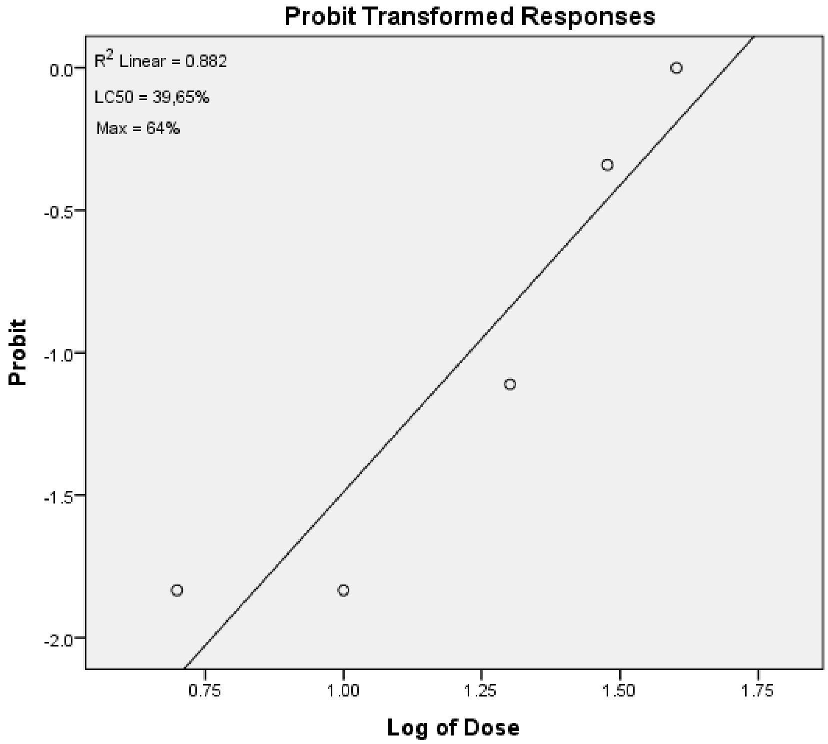

Fig. 1. Analysis of acute toxicity LC_{50} 96 H in probit analysis.

2. Heavy metal in water

The heavy metals content (Ag, Cu, Pb, and Cd) observed from each samples is shown in figure 2. The level of heavy metals Ag and Cu showed was higher as these are the NPs used in HEPA filter compared to Pb and Cd. The value of heavy metal Ag was significantly increased at 40% concentration of HEPA filter waste. This also happened to the value of heavy metal Cu at the same concentration, which was at levels >0.04 ppm. Based on the Indonesian Government Regulation No 22 year 2021 concerning Implementation of Environmental Protection and Management regarding National Water Quality Standards, Cu levels in the aquatic environment may not exceed 0.02 mg/L and Ag levels according to Minister of Health Regulation No 492 year 2010 may not exceed 1 mg/L. The value of Ag and Cu was significantly increased at the concentration of 40%. Heavy metal Ag showed a value <1 mg/L, in the other hand heavy metal Cu showed a high value >0.02 mg/L which was at level 0.04 and was categorized unsafe for freshwater aquatic organisms based on the Indonesian Government Regulation. The value of heavy metals Pb and Cd in the water was not exceeded which were ≤ 0.03 mg/L and ≤ 0.01mg/L, respectively.

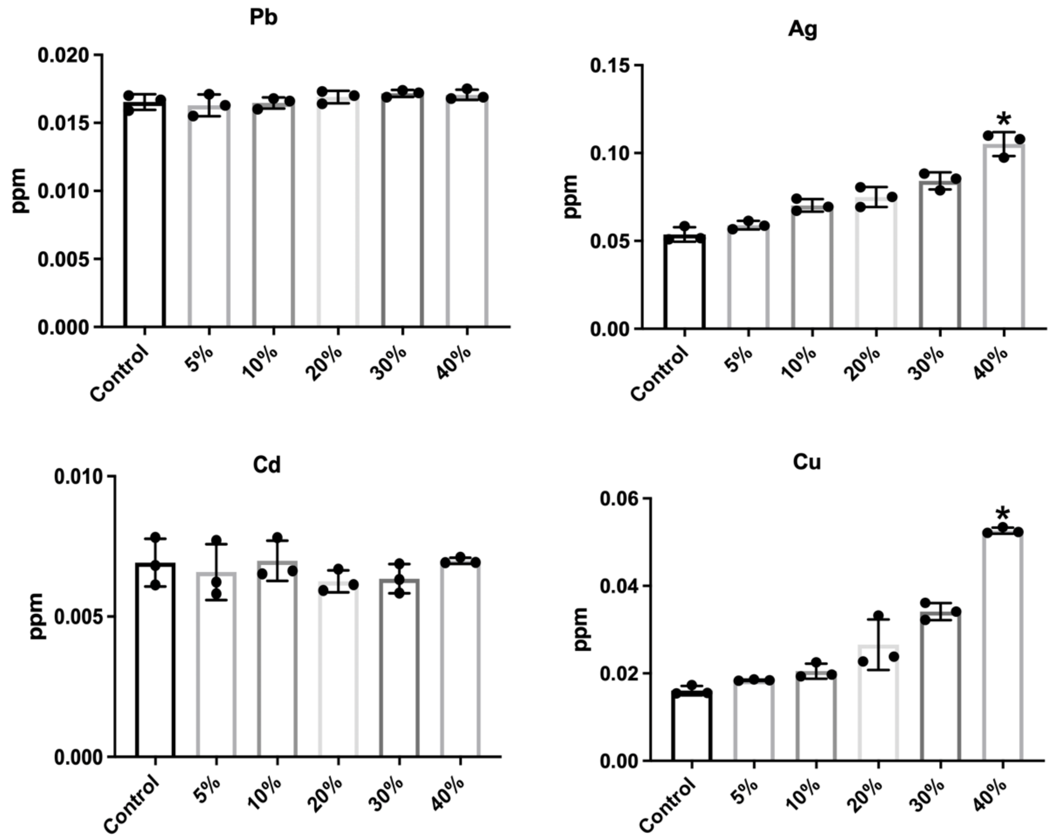

Fig. 2. Heavy metals level (Pb, Cd, Ag, and Cu) in the water sample. Results were the mean ± SD *P<0,05 was significant.

3. Heavy Metal in gill of *Cyprinus carpio*

HEPA filter waste has a toxic effect on the structure of the gill tissue of common carpso that there is an abnormality of respiratory activity through the opening speed of the operculum. The present study involves the analysis of heavy metal concentrations within the tissue samples, specifically from the gills to the stomach. The existence of heavy metal caused a damage in the organ tissue. This damage indicates that the heavy metal in the body has been accumulated and exceeded the limits that can be tolerated by fish. In the concentration of 5%, the amount of heavy metals Ag and Cu were 0.0273 ppm and 0.0295 ppm, respectively. This is the lowest concentrations of Ag and Cu value compared to the other concentrations. At this concentration, the observed fish did not experience symptoms expected from exposure to Ag and Cu metals. This is because the amount of these heavy metals can still be tolerated and excreted by the body so it wasn't accumulated in the gill organs. The value of heavy metals Ag and Cu were significantly increased at concentration of 40% which were 0.04 ppm and 0.013 ppm, respectively. The results of heavy metal value are shown in figure 3. The heavy metal of Ag and Cu in gill of common carp fish was significantly increased at the consentration of 40%. Heavy metals Pb and Cd were also measured and has the value around to 0.012 ppm and 0.004 ppm, respectively. Ag and Cu value as the NPs heavy metals used has significantly increased at the highest concentration, 40%. Ag and Cu value were at around to 0.04 ppm and 0.0135 ppm, respectively.

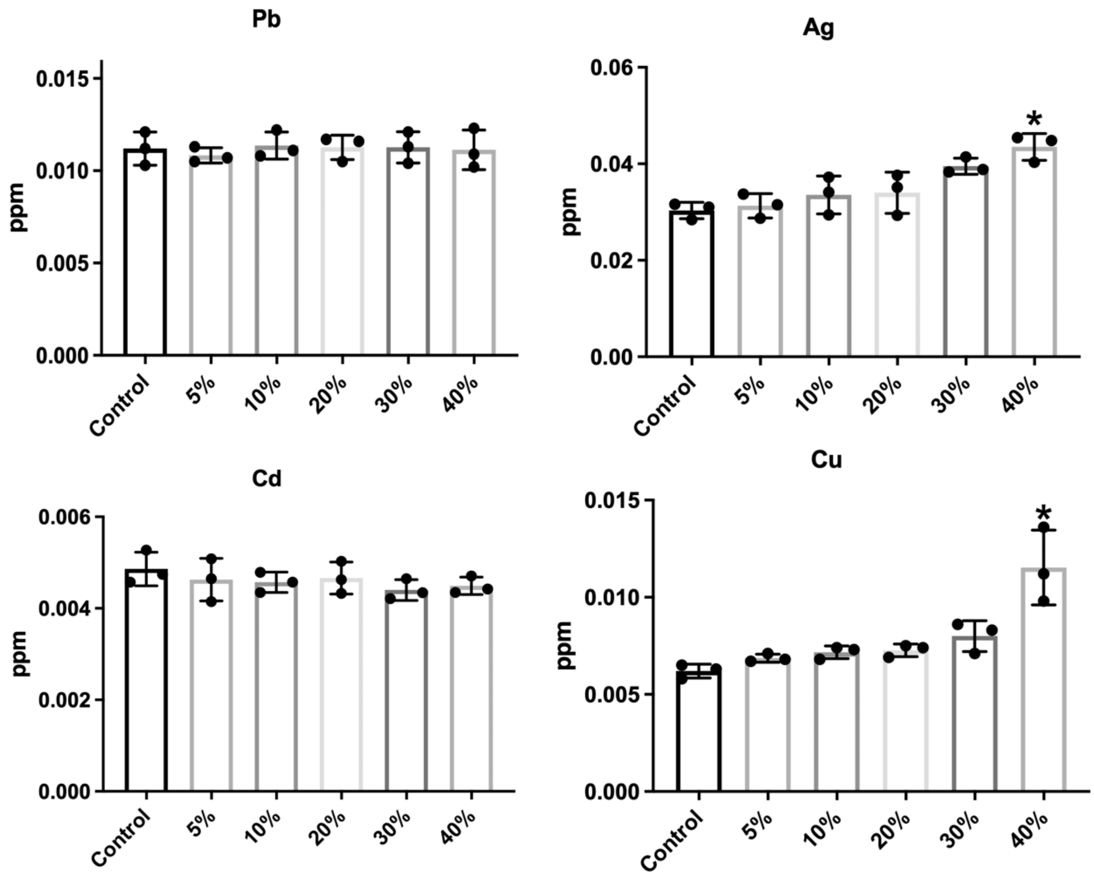

Fig. 3. Analysis of Heavy metals content in gill of common carp fish exposed by HEPA filter waste with concentration 5%, 10%, 20%, 30%, and 40%. Results were the mean ± SD *P<0,05 was significant.

4. Heavy Metal in Stomach of *Cyprinus carpio*

The heavy metals concentration (Pb, Cd, Ag, and Cu) in stomach tissue of *C. carpio* were shown at figure 4. The common carp fish were used as the object test because it is relatively insensitive, and can survive and accumulate contaminants at heavily polluted sites, which is why it is used as a freshwater bioindicator in environmental toxicology. Many studies have been conducted on the determination of the heavy metals level in common carp fish stomach tissue. Figure 4 shows that heavy metals level was significantly increased at the concentration of 40% followed by the other lower concentration. The value of heavy metals Pb and Cd were found to be < 0.01 ppm and ≤ 0.002 ppm, respectively. Heavy metals Ag and Cu at the concentration of 40% were valued at around 0.015 ppm, and 0.010 ppm, respectively. The accumulation of heavy metals in the stomach tissue can be happen through three routes. The heavy metal of Pb was showed a stable value at around 0.006 – 0.007 ppm. Cd concentration in stomach tissue of *C. carpio* were also stable, the value were at around 0.0018 – 0.0019 ppm. Meanwhile, the heavy metals Ag and Cu value were significantly increased at around 0.015 ppm, and 0.010 ppm, respectively. The higher concentration given, the higher heavy metals accumulated in the stomach of *C. carpio*.

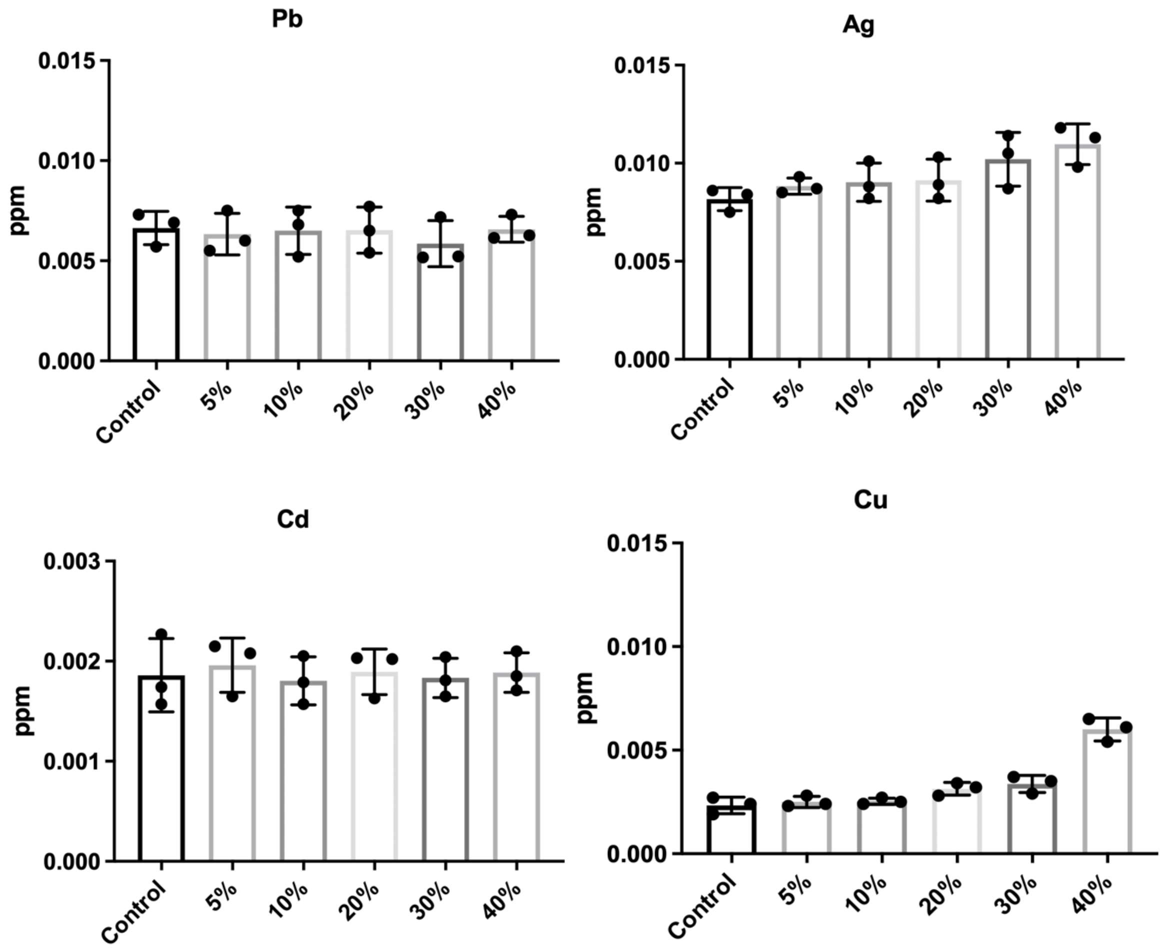

Fig. 4. Analysis Heavy metal of Stomach of fish in the structure of stomach tissue in *C. carpio*. Results were the mean ± SD *P<0,05 was significant.

5. Reactive Oxygen Species (ROS) analysis of Cyprinus carpio

Induction of Reactive Oxygen Species after HEPA Filters exposure was shown in Fig 5. The result showed that at the concentration control, 5%; 10% and 20% of HEPA Filters in the gill tissue of *C. carpio* were below than 20 pg/mL which is half of the highest concentration. Furthermore, at the concentration of 30%, the ROS level was increased at around 25 pg/mL not significantly. The ROS level was significantly increased than other contetration and at the highest concentration which was 40% with value at around 40 pg/mL. Based on heavy metal analysis, the highest accumulating heavy metal in gill of Carp were Ag and Cu. Then, the Elevating ROS in gill of Carp might induced by heavy metal Ag and Cu.

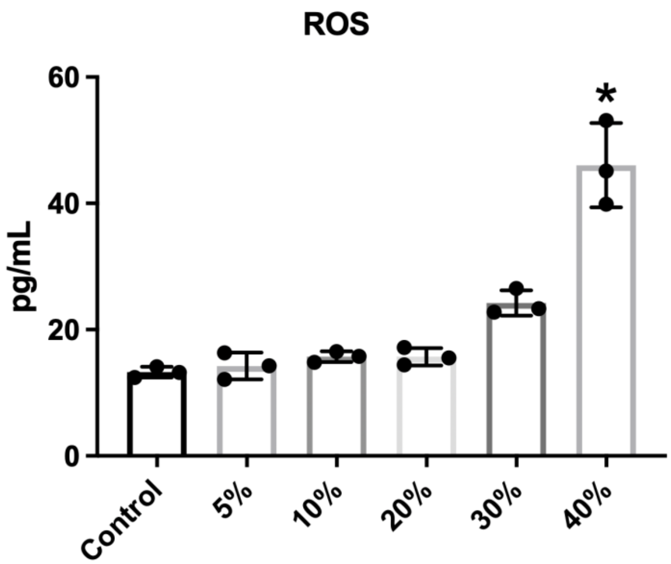

Fig. 5. Effects of Ag and Cu in HEPA filter waste on reactive oxygen species (ROS) level in gill tissue of *C. carpio*.

DISCUSSION

The toxicity of HEPA filter was still lack of information and needs to be analyzed further. HEPA filter was selected for toxicity studies because of its widespread use as an air filter and its NPs heavy metal material that can harm the environment and aquatic organisms **(Authman *et al.*, 2015)** if thrown without going through any processes to reduce its compounds. In order to preserve the aquatic ecosystem from the potential effects of HEPA filter toxicity, it is necessary to conduct the toxicity test on this air filter tools study on aquatic organisms. In this study showed that HEPA filter toxicity in water bodies that exceeded the limit starting at a concentration of 30%, which was indicated by changes in swimming habits, fading color, and abnormal breathing of the operculum. However, this concentration is not a lethal dose due to the mortality rate does not reach 50%. Furthermore, based on the results on this study, HEPA filter has a lethal dose of acute toxicity at a concentration of 39.65% with a maximum lethal limit of 40%. Acute toxicity of HEPA filter has been reported in the LD_{50} assay.

Immediate water absorption is the first path of exposure to these toxic compunds. Sediment as the primary trace element repository in marine settings provide a second route. The dietary intakes provide the third route of the accumulation of heavy metals in aquatic organisms **(Zaynab *et al.*, 2022).** The accumulation of heavy metals in exceeded conditions can be seen by the fish development that is hampered. Heavy metals concentrations in the stomach tissue cause a variety of metabolic, physiological, and histological changes by altering various enzymes and metabolites.

In this study was also measured the value of heavy metals contain within the HEPA filter in the tissue of gill and stomach. It is found that the value of Ag was higher in both the gill and stomach tissue of *C. carpio* at level around 0.04 ppm and 0.015 ppm, respectively. This value has significantly increased at concentration of 40%. The presence of metals in fish species depends on the age and development fishes, and other physiological factors **(Govind and Madhuri, 2014)**. The heavy metals can have toxic effects on different organs. They can enter into water via drainage, atmosphere, soil erosion and all human activities by different ways. With increasing heavy metals in the environment, these elements enter the biogeochemical cycle leading to toxicity in animals, including fishes. Absorption of heavy metal Cu through respiration and food into the blood can occur in acidic conditions in gill and the stomach. During the process of absorption of Cu have been processed primarily in the gill and stomach, the existing Cu is also absorbed. In blood, Cu exists in two ionized forms, there are Cu+ and Cu++. Cu also binds to red blood cells as erythrocuprein, which is about 60% of erythrocytes-Cu, while the rest are labile fractions. The next blood will carry Cu into the liver. From the liver, Cu is sent to the gallbladder. From the bile, Cu is excreted back into the intestine for further excretion through the feces **(Sekarwati *et al.*, 2015).** The toxicity of copper to fish and its availability in water are influenced by the physicochemical characteristics of the water, such as pH, alkalinity, suspended solids, organic compound content, and hardness **(Di Giulio and Meyer, 2008)**. The cellular toxicity of copper can be elucidated by its involvement in the Fenton reaction. The cuprous (I) ion has the ability to catalyze the generation of hydroxyl radicals.

The increasing of ROS will not only cause lipid oxidation but also protein oxidation in fish **(Hematyar *et al.*, 2019)**. Free radicals are highly ROS that can cause damage to biological materials. Heavy metals Ag and Cu in HEPA filter has the potential as an immunotoxicity and the specific related mechanisms in the common carp (*C. carpio*) gill tissue. The oxidative damage caused by copper can be enhanced by a variety of substances. In their study, **Gravato et al. (2006)** reported the observation of heightened levels of copper-associated lipid peroxidation (LPO) and DNA damage in the European eel. Anguilla anguilla specimens were subjected to pre-exposure with β naphthoflavone (BNF), a compound resembling polynuclear aromatic hydrocarbons. This study proposes the existence of a synergistic association between copper and biological nitrogen fixation (BNF). The experimental findings demonstrated that the administration of β-naphthoflavone resulted in an elevation in the enzymatic activity of ethoxyresorufinO-deethylase within the liver, consequently leading to a decrease in copper levels. This mechanism enables the process of copper redox cycling, resulting in heightened levels of reactive oxygen species (ROS).

HEPA filter has also toxic effect on the level of oxidative stress that triggered by the reactive oxygen species (ROS) as its increasing of which results in elevated superoxide production that may directly contribute to cell damage **(Brownlee, 2001)**. The value of ROS in gills of common carp fish was significantly increased at the concentration of 40% with level at around 40 pg/mL followed by the lower concentrations. Free radicals are highly ROS that can cause damage to biological materials. Heavy metals Ag and Cu in HEPA filter has the potential as an immunotoxicity and the specific related mechanisms in the common carp. As excess of ROS generation damages cellular lipids, proteins, nucleic acids, membranes and organelles which in turn can lead to activation of cell death processes such as apoptosis **(Chowdhuri and Saikia, 2020).** The induces of copper (Cu) may have different effect on every species. Exposure of freshwater rainbow trout to copper reduces the plasma concentration of sodium, potassium, calcium ions in fish also results in increased secretion of mucus in gill, liver, and kidney tissue.

CONCLUSION

In conclusion, HEPA filter has acute toxicity (LC_{50}) at concentration of 39.65% in common carp (*C. carpio*) after exposed for 96h. The highest concentration promoted some accumulated heavy metals content in both gill and stomach tissue in carps. ROS level was also significantly increased at the highest concentration which damages the biological materials in fish.

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REFERENCES

Authman, M. M.; Zaki, M. S.; Khallaf, E. A. and Abbas, H. H. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development.*, *6*(4) **:** 1-13.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813–820.

Chaloupka, K.; Malam, Y. and Seifalian, A.M. (2010). Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol., *28*(11): 580–588.

Chowdhury, S. and Saikia, S. K. (2020). Oxidative Stress in Fish: A Review. *Journal of Scientific Research*, *12*(1).

Christopherson, D. A.; Yao, W. C.; Lu, M.; Vijayakumar, R. and Sedaghat, A. R. (2020). High-efficiency particulate air filters in the era of COVID-19: function and and efficacy. *Otolaryngology–Head and Neck Surgery.*, *163*(6): 1153-1155.

Di Giulio, R. T. and Meyer J. N. (2008). Reactive oxygen species and oxidative stress. In: Di Giulio, R.T. and Hinton, D. E. (eds.): The Toxicology of Fishes. CRC Press, Taylor and Francis Group. 273–324.

Fan, X.; Yahia, L. H. and Sacher, E. (2021). Antimicrobial properties of the Ag, Cu nanoparticle system. *Biology*, *10*(2), 137. efficacy. *Otolaryngology–Head and Neck Surgery.*, *163*(6): 1153-1155.

Fernández de Mera, I. G.; Granda, C.; Villanueva, F.; Sánchez-Sánchez, M.; Moraga- Fernández, A.; Gortázar, C. and de la Fuente, J. (2022). HEPA filters of portable air cleaners as a tool for the surveillance of SARS- CoV- 2. *Indoor air.*, *32*(9): 1-9.

Garai, P.; Banerjee, P.; Mondal, P. and Saha, N. C. (2021). Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J Clin Toxicol. S*, *18*.

Gravato C.; Teles M.; Oliveira M. and Santos, M. A. (2006). Oxidative stress, liver biotransformation and genotoxic effects induced by copper in Anguilla anguilla L. – the influence of pre-exposure to β-naphthoflavone. Chemosphere 65: 1821–1830.

Haghighat F.; Kim Y.; Sourinejad I.; Yu I. J. and Johari S. A. (2021). Titanium dioxide nanoparticles afect the toxicity of silver nanoparticles in common carp (*Cyprinus carpio*). Chemosphere 262:127805.

Hematyar, N.; Rustad, T.; Sampels, S. and Kastrup Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. *Aquaculture Research*, *50*(5): 1393-1403.

Hertika, A. M. S.; Kusriani, K.; Indrayani, E.; Nurdiani, R. and Putra, R. B. (2018). Relationship between levels of the heavy metals lead, cadmium and mercury, and metallothionein in the gills and stomach of Crassostrea iredalei and Crassostrea glomerata. *F1000Research*, *7*.

Hyder, A.; Buledi, J. A.; Nawaz, M.; Rajpar, D. B.; Orooji, Y.; Yola, M. L. and Solangi, A. R. (2022). Identification of heavy metal ions from aqueous environment through gold, Silver and Copper Nanoparticles: An excellent colorimetric approach. *Environmental research*, *205*: 112475.

Jing, H.; Zhang, Q.; Li, S. and Gao, X. J. (2020). Pb exposure triggers MAPK-dependent inflammation by activating oxidative stress and miRNA-155 expression in carp head kidney. *Fish & shellfish immunology*, *106*: 219-227.

Kim, H. J.; Mahboob, S.; Viayaraghavan, P.; Al-Ghanim, K. A.; Al-Misned, F.; Kim, Y. O. and Ahmed, Z. (2020). Determination of toxic effects of lead acetate on different sizes of zebra fish (Danio rerio) in soft and hard water. *Journal of King Saud University-Science*, *32*(2), 1390-1394.

Liu, Y.; Peng, S.; Shan, H. and Zhao, Y. (2021). Experimental study on the effects of used HEPA filters on water environment. In *E3S Web of Conferences* (Vol. 269, p. 01003). EDP Sciences.

Mahjoubian, M.; Naeemi, A. S.; Moradi-Shoeili, Z.; Tyler, C. R. and Mansouri, B. (2023). Toxicity of silver nanoparticles in the presence of zinc oxide nanoparticles differs for acute and chronic exposures in zebrafish. *Archives of environmental contamination and toxicology*., *84*(1): 1-17.

Pandey, G. and Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*., *2*(2): 17-23.

Pirsaheb, M.; Azadi, N.A.; Miglietta, M.L.; Sayadi, M.H.; Blahova, J.; Fathi M. and Mansouri B (2019). Toxicological efects of transition metal-doped titanium dioxide nanoparticles on goldfsh (Carassius auratus) and common carp (Cyprinus carpio). Chemosphere 215: 904–915.

Pulit-Prociak, J. and Banach, M. (2016). Silver nanoparticles a material of the future? Open Chem., 14: 76–91. https://doi.org/10.1515/ chem-2016-0005

Rossiana and Nia. (2006). Toxicity Test of Sumedang Tofu Liquid Waste Against Reproduction of Daphnia carinata KING. Journal of Biology. Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University: Bandung.

Sayed, A.E.D.H.; Mekkawy, I.A.; Mahmoud, U.M. and Nagiub M (2020). Histopathological and histochemical ffects of silver nanoparticles on the gills and muscles of African catfsh (Clarias garepinus). Sci Afr 7: e00230.

Stoyanova, S.; Georgieva, E.; Velcheva, I.; Iliev, I.; Vasileva, T.; Bivolarski, V. and Yancheva, V. (2020). Multi-biomarker assessment in common carp (Cyprinus carpio, Linnaeus 1758) liver after acute chlorpyrifos exposure. *Water*, *12*(6): 1837.

Tong, Z.; Li, Y.; Westerdahl, D. and Freeman, R. B. (2020). The impact of air filtration units on primary school students' indoor exposure to particulate matter in China. *Environmental Pollution*, *266*, 115107.

Zaynab, M.; Al-Yahyai, R.; Ameen, A.; Sharif, Y.; Ali, L.; Fatima, M. and Li, S. (2022). Health and environmental effects of heavy metals. *Journal of King Saud University-Science*., *34*(1): 101653.

Zhang, X.; Yang, F.; Zhang, X.; Xu, Y.; Liao, T.; Song, S. and Wang, J. (2008). Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (Gobiocypris rarus) exposed to waterborne hexabromocyclododecane (HBCDD). *Aquatic Toxicology*., 86 (1): 4–11.