Hexavalent chromium contamination and implications for human health risks related to fish consumption from small-scale aquaculture ponds in Bantul Regency, Yogyakarta, Indonesia

[1,2]Djoko Rahardjo, [3]Gatot Sasongko, [4]Suwarno Hadisusanto

[1] Development Studies Doctoral Program, Faculty of Interdisciplinary Satya Wacana Christian University, Jl. Diponegoro No.52-60, Salatiga, Indonesia; [2] Faculty of Biotechnology, Universitas Kristen Duta Wacana Jl. Dr. Wahidin 6–25 Yogyarkarta, Indonesia; [3] Interdisciplinary Faculty, Satya Wacana Christian University, Jl. Diponegoro No. 52-60, Salatiga, Indonesia; [4] Faculty of Biology, Gadjah Mada University, Jl. Teknika Selatan, Sekip Utara, Yogyakarta, Indonesia. Corresponding author: D. Rahardjo, djoko@staff.ukdw.ac.id

Abstract. The research aims to evaluate the level of contamination and health risks of using hexavalent chromium Cr(VI) polluted river water for small-scale aquaculture ponds. The research was carried out in the period April to November 2023, on a group of small-scale fish aquaculture ponds in five locations including 1 location as a control and 4 locations affected by the disposal of liquid waste from the leather tanning industry in the Piyungan Industrial Area. Samples were taken from aquaculture ponds of *Clarias gariepinus* and *Oreochromis niloticus*, and 4 samples were selected randomly from each pond. Measurement of chromium levels in samples was carried out using AAS (atomic absorption spectrophotometry) based on SNI 06-6989.17-2004 (SNI 2004). The analysis process was carried out with AAS PinAAcle 900T Perkin Elmer. The research results showed that the use of river water contaminated with chromium caused all small-scale aquaculture ponds in the affected locations to be contaminated with hexavalent chromium. Hexavalent chromium contamination in small-scale aquaculture ponds causes the accumulation of hexavalent chromium in fish with a range 0.2000-2.4000 mg/kg^{-1} in *C. gariepinus* and 0.2000-3.0000 mg/kg^{-1} in *O. niloticus*. The level of hexavalent chromium accumulation in fish is influenced by the location, type and age of fish and aquaculture system such as type of pond and water circulation system. Bioconcentration (BCF) and bioaccumulation (BCA) values differed significantly both based on location and fish type. Consumption of fish contaminated with hexavalent chromium has the potential to cause non-carcinogenic health risks (HQ ranges from 0.900-1.041) and the potential for cancer with a TR value of 1.4 x 10-4 to 6.3 x 10-4. The highest sequential HQ values were found at location 4 (1.041) > location 3 (0.900) > location 2 (0.513) > location 5 (0.207) > location 1 (0.014).

Key Words: accumulation, consumption, daily intake, polluted river, risk assessment.

Introduction. The leather tanning industry is among the four most polluting industries in the world after lead acid battery recycling, mining, ore processing and lead smelting (SMEP 2018). The leather tanning industry is part of the leather processing industry, namely processing raw leather into finished materials. This sector is considered the most polluting sector because it produces toxic pollutants at every step of the process (Suman et al 2021). In the leather tanning industry, processing 1 ton of raw leather requires around 20 to 80 cubic meters of waste water with a chromium concentration of 250 mg L^{-1} and a sulfide concentration of around 500 mg L^{-1} (Borrely et al 2018). Only 20% of raw materials are converted into usable leather products, while more than 60% of raw materials are converted into solid and liquid waste (Sivaram & Barik 2018). Even though leather tanning activities are very important for the economies of Asian countries, aspects of environmental pollution and health must remain a serious consideration (Borrely et al 2018). Hexavalent chromium Cr(VI) is a potential pollutant that can cause environmental and health problems (Costa & Klein 2006). According to Sharma et al

(2009) chromium can interact directly with intercellular proteins and nucleic acids, causing mutagenic, carcinogenic and teratogenic effects in animals and humans. Exposure to hexavalent chromium can cause chronic fatigue, infertility, nerve pain in the legs, hypothyroidism, dermatitis, ulcers, and lung cancer in humans.

The activity of disposing of liquid waste from the leather tanning industry into river waters has caused a decrease in water quality, widespread distribution of chromium pollutants in various environmental compartments (Rahardjo & Prasetyaningsih 2017; Rahardjo et al 2021a; Rahardjo et al 2021b). Water pollution poses a significant threat to the aquatic environment, human health and human productive activities (UNEP 2016; Mateo-Sagasta et al 2017). The widespread distribution and increasing concentration and accumulation of chromium in various components in the environment pose a potential threat to the environment, fisheries and public health along the Opak River (Rahardjo et al 2023). Environmental pollution by hexavalent chromium is receiving increasing attention because it is widespread throughout the world (Kazakis et al 2017; Brasili et al 2020). Many studies related to chromium pollution have been carried out by various researchers, both for: 1) monitoring chromium concentrations in waters (Poshtegal & Mirbagheri 2019; Baizura et al 2020; Siddiqua et al 2021; Rahardjo et al 2021a), 2) determining the effect of chromium pollution on various aquatic biota (Leblebici et al 2020; Srivastava et al 2021; López-Bucio et al 2022; Chen et al 2022), and 3) determining the level of chromium accumulation in various foodstuffs such as rice, soybeans, vegetables, fruit, fish, shrimp and health risks, especially in water areas affected by industrial and mining activities (Gomah et al 2019; Ezeofor et al 2019; Leblebici et al 2020; Dimuthu Nilmini Wijeyaratne & Charuni Sewwandi Kumar et al 2021; Bayissa et al 2021; Salihović et al 2021; Alsafran et al 2021; Srivastava et al 2021; Sibuar et al 2022; López-Bucio et al 2022; Chen et al 2022; Saud et al 2022).

Meanwhile, monitoring of the level of contamination, accumulation and health risks of small-scale aquaculture that use chromium-polluted river water has not been carried out much. For communities around the river area, river water is a source of life and livelihood, both as a source of clean water and for food production processes such as growing rice, vegetables and aquaculture. The use of chromium-contaminated water for aquaculture provides opportunities for contamination of aquaculture ponds and accumulation of chromium in the fish produced. Varol and Sünbül (2020), stated that consumption of fish contaminated with heavy metals such as chromium is a risk factor for public health. Fish consumption poses a dietary exposure risk for humans because it has the potential to accumulate heavy metals (Rakocevic et al 2018). Even in low concentrations it remains dangerous because it can accumulate in the body and reach toxic levels (Chen & Chau 2019; Ustaoğlu & Tepe 2019). The accumulation of heavy metals in fish can cause health problems for consumers through food intake. Therefore, it is important to assess the concentration of the heavy metal chromium in fishery products from various environments to guarantee that they are safe for consumption. Efforts to mitigate food safety risks can be carried out through a risk-based food safety approach that is applied along the value chain through food safety programs. The risk-based approach is a science-based strategy to reduce food safety risks for consumers which consists of three stages, namely risk assessment, risk management and risk communication (FAO & WHO 2006).

Health risk assessments from chromium contamination in various food commodities have been carried out. Research focused on vegetable plants such as tomatoes, cabbage, radishes, parsley, coriander, pakchoi (Ahmed et al 2016; Gaurav et al 2018; Alsafran et al 2021; Bayissa et al 2021; Dimuthu Nilmini Wijeyaratne & Charuni Sewwandi Kumar et al 2021). Contamination of paddy and rice (Gomah et al 2019; Ezeofor et al 2019). Contamination of fish and shrimp (Huang et al 2019; Kanda et al 2020; Tore et al 2021; Leonard et al 2022). From these studies, various results were obtained regarding the level of contamination and health risks for consumers. The level of chromium contamination in food products (fish, shrimp, vegetables, rice, etc.) is influenced by environmental factors such as location, concentration levels in water and sediment as well as the type of food studied. This is confirmed by research by Rajeshkumar and Li (2018), that the accumulation of heavy metals in fish is influenced by extrinsic factors (water, sediment and chemical characteristics of heavy metals, spatial and temporal variables, and the environment) and intrinsic factors (fish characteristics).

However, data on the level of hexavalent chromium contamination in aquaculture ponds and assessment of health risks from small-scale aquaculture in the community are not yet available. Small-scale aquaculture is widely practiced by the community and is useful for increasing income, food availability and opening up employment opportunities. Therefore, monitoring and controlling heavy metal pollutants as well as assessing health risks from consumption of fish contaminated with hexavalent chromium are very important. Through this research, it is hoped that we will get an overview of the level of fish contamination and potential health risks, so that it can strengthen parties in taking real action to prevent pollution, food safety and health risk management.

Material and Method

Time frame and site description. The research was carried out from April to November 2023, on groups of fish farmers in five locations (districts) who used the Opak River as a water source for aquaculture. These locations include location 1 as a control and four other locations as locations affected by wastewater discharge from the leather tanning industry in the Piyungan Industrial Area. Communities along the Opak River generally develop small-scale aquaculture businesses using traditional systems. The majority of aquaculture ponds use earthen ponds and only a small part use tarpaulin pond system, especially for cultivating *Clarias gariepinus* (Figure 1).

Figure 1. Types and conditions of small-scale aquaculture ponds used in Bantul Regency, Yogyakarta, Indonesia.

Type and quantity of samples. There are three types of samples, namely water, sediment and fish samples. The location of small-scale aquculture is determined based on the distribution of data on small-scale aquculture groups sourced from the Monograph of the Maritime and Fisheries Service of Yogyakarta Special Region Province (KKP 2021). Based on data from the DIY Provincial Maritime and Fisheries Service, there are 4 sub-district areas that utilize the flow of the Opak River for fish aquaculture, namely location 2, location 3, location 4 and location 5 (affected locations) and as a comparison location 1 was chosen which was not affected by liquid waste disposal activities leather tanning industry (upstream river area). The type of fish aquaculture pond chosen was *Clarias gariepinus* and *Oreochromis niloticus* pond, determined based on the highest amount of fish production and consumption levels in both Bantul district and Special Region of Yogyakarta Province. From five research locations, 2 types of fish were selected, namely tilapia and catfish. Water, sediment and fish samples at each research location were taken from four ponds with 3 replications, so that there was a total of 360 samples.

Sample collection and preparation. Water samples were taken at half of the total depth of the ponds. The tool used to take water samples is a plastic scoop with a stem (SNI 2008). The water samples taken were preserved by adding concentrated HNO_3 (3 mL HNO_3/L water sample) then cooled to 4°C (APHA 2005). Sediment samples were taken as much as ±100 grams using a shovel instead of a grab sampler (USEPA 2001). Sediment samples were placed in plastic and stored at 4°C. Fish samples were taken randomly, the samples obtained were then rinsed with clean water, put in a cooler and taken to the laboratory on the same day to be frozen at -20°C until the preparation process was carried out (Rajeshkumar & Li 2018).

Hexavalent chromium analysis. Water samples that have been treated with preservatives are put into a cooler at a temperature of 4°C before extraction. Extraction is carried out in the Chemical Laboratory Universitas Kristen Duta Wacana Yogyakarta. A 100 ml water sample was used then added with 10 mL of concentrated HNO_3. The sample is then heated until it reaches a volume of 50 mL. The addition of concentrated HNO_3 and heating of the sample was repeated. The extract was then filtered using filter paper soaked with 1% HNO_3, then stored in a sample bottle. For solid samples (sediment and fish), the samples obtained were extracted using the acid method (USEPA 2001), by measuring wet weight with an analytical balance, then the samples were heated to reduce the water content in an oven at a temperature of 60°C until the samples were dry. After that, the dry weight was weighed again and the sample was ground with a mortar, then the sample was stored in a closed container. A sample of 3 grams was taken and then added with 18 mL of HCl and 6 ml of concentrated HNO_3. The sample was then heated until it reached a volume of ±10 mL. The addition of concentrated HCl and HNO_3 solutions was repeated and the sample was heated. The extract was then filtered using filter paper soaked in 1% HNO_3. Measurement of extraneous chromium levels was carried out using AAS (atomic absorption spectrophotometry) based on SNI 06-6989.17-2004 (SNI 2004). Analysis process was conducted with AAS PinAAcle 900T Perkin Elmer.

Bioconcentration and bioaccumulation factors. Bioconcentration (BCF) and bioaccumulation (BAF) factors of hexavalent chromium in fish were calculated by referring to the equation of Türkmen et al (2011), as follows:

$$BCF\ Cr = \frac{content\ of\ Cr\ in\ fish\ meat}{content\ of\ Cr\ in\ water}$$

BCF values higher than 1000 mg L^{-1}, between 100 and 1000 mg L^{-1}, and lower than 100 mg L^{-1} in an aquatic organism indicate high, moderate, and low accumulative properties, respectively (Amriani et al 2011).

$$BAF\ Cr = \frac{Cr\ (fish\ sample)}{Cr\ (sediment)}$$

Where: Cr (fish sample) is the Cr concentration measured in the fish (mg kg^{-1} dry weight); Cr (sediment) is the Cr concentration measured in the sediment (mg kg^{-1} dry weight). BAF was calculated using the mean concentration value of each element present in the fish sample and sediment. BAF higher than 1 indicates that Cr experiences bioaccumulation or biomagnification (Ibhadon et al 2014).

Analysis of socio-economic factors and chromium exposure. The socio-economic conditions of society play an important role in exposure and public health; therefore, indicators are needed that show socio-economic differences in exposure and health risks. The selection of participants and socioeconomic factors was carried out adopting Tyrrell et al (2013), namely participants selected as respondents aged 17–74 years with other socioeconomic indicators such as place of residence, gender, age and body weight were also measured to determine disparities in exposure and health risks. Socio-economic variables are measured using instruments in the form of questionnaires.

Health risk assessment

Non-carcinogenic risk. Health risk analysis of consumption of fish products from small-scale aquaculture by the community is determined based on the hazard quotient (HQ) value. HQ is the ratio of chronic daily intake (CDI) divided by the oral reference dose (RfD) of the heavy metal chromium. RfD is an estimate of daily oral exposure to a toxic substance that is unlikely to pose a lifetime risk of significant harmful effects (Varol et al 2017; Mwakalapa et al 2019). Based on USEPA (2018) the RfD value for the heavy metal chromium is set at 0.003. Estimated daily intake (EDI), average daily intake (ADI), maximum edible amount (MEA) and hazard quotient (HQ) values are determined using the formula (USEPA 2008; Hajduga et al 2019; Pinzón-Bedoya et al 2020) as following:

$$EDI = C * DFC/BW \qquad (1)$$

To estimate the average daily intake (ADI) value, the equation is used:

$$ADI = EDI * ED * EF / AT * 10^{-3} \qquad (2)$$

Next, the MEA and HQ values are calculated using the equation:

$$HQ = ADI/RfD \qquad (3)$$
$$MEA = RfD * BW/C \qquad (4)$$

Where C is the metal concentration µg/g in wet weight, DFC is fish consumption in mg/day, BW is the body weight of adults in Indonesia of 58.8 kg (BPS 2023), ED is the duration of exposure, life expectancy for Indonesians is 73.93 years (BPS 2023), EF is the frequency of exposure (365 days/year), AT is the average exposure time for non-carcinogens (365 days/year×ED) (USDOE 2011), and RfD is the reference dose for Cr of 0.003 USEPA (2018). If the HQ value is less than or equal to 1, then it is assumed that exposure to the heavy metal chromium does not pose a non-carcinogenic human health risk throughout a lifetime of exposure (USEPA 2011, 2012). On the other hand, if there is more than one, exposure to chromium can pose a non-carcinogenic risk to humans.

Carcinogenic risk. Carcinogenic risk is the cumulative probability of a person developing cancer during lifetime exposure to carcinogenic chromium pollutants. The carcinogenic health risk associated with fish consumption is determined based on the target cancer risk (TR) value and calculated using the formula as (5) as follows:

$$TR = EDI \times CSF \times 10^{-3} \qquad (5)$$

Where CSF is the oral cancer slope factor (CSF) value, for the heavy metal chromium determined at 0.5 mg/kg-day (USEPA 2000). Meanwhile, the EDI value is an estimate of the daily intake of heavy metals from fish consumption depending on the concentration of heavy metals in the fish and the amount of fish consumed. EDI is calculated using the following equation:

$$EDI = \frac{C \ x \ DFC}{BW}$$

Where C is the average concentration of heavy metals in fish (mg/kg in wet weight); daily fish consumption (DFC) is the consumption level (fish consumption rate; mg/person/day) obtained from direct bio survey results and BW is the average body weight of Indonesian adults, namely 58.8 kg (BPS, 2023).

Data analysis. Data from calculations of hexavalent chromium concentrations in water, sediment, fish, consumption patterns, daily intake and health risks were analyzed qualitatively and descriptively using tables. The data was also analyzed quantitatively using the ANOVA test by first carrying out normality and homogeneity tests. The normality test is carried out to determine that the data is distributed normally, by looking at the *p*-value of the normality test results. If the $p>0.05$ then the data is normally distributed. Meanwhile, the homogeneity test was carried out using Levine's test of equality of error variance statistics using the SPSS 21.0 for Windows. The homogeneity test results have a value of $p>0.05$, which indicates that the data sample was obtained from a homogeneous population. Data analysis was also carried out using the T-test to determine differences in levels of hexavalent chromium contamination between *Clarias gariepinus* and *Oreochromis niloticus* aquaculture ponds.

Results. All small-scale aquaculture ponds at locations affected by the disposal of liquid waste from the leather industry have been contaminated with hexavalent chromium. Hexavalent chromium has been found in all media, including water, sediment and fish.

Hexavalent chromium concentration in water and sediment. Hexavalent chromium concentrations in water and sediment samples vary based on location and type of aquaculture system. The hexavalent chromium concentration in water samples was found to be in the range of 0.0200-0.2200 mg L^{-1} in *Clarias gariepinus* ponds with an average of 0.0875 mg L^{-1}, slightly higher than the chromium concentration in *Oreochromis niloticus* ponds with a range of 0.0100-0.2900 mg L^{-1} and the average was 0.0582 mg L^{-1}. Meanwhile, hexavalent chromium was not found in aquaculture pond water samples in areas not affected by liquid waste disposal (Table 1). Based on location, hexavalent chromium concentrations were found to be higher in locations close to wastewater discharge points and tended to fluctuate downstream. The same thing also happened to the hexavalent chromium concentration in the sediment. The average hexavalent chromium concentration in *C. gariepinus* pond sediments was 0.4327 mg kg^{-1}, higher than the average hexavalent chromium concentration in *O. niloticus* ponds, namely 0.1718 mg kg^{-1}.

Table 1

Concentration of hexavalent chromium in water and sediment (mg L^{-1} kg^{-1})

Sampling station	*Clarias gariepinus*			*Oreochromis niloticus*		
	Range	*Mean*	*SD*	*Range*	*Mean*	*SD*
Pond water						
Location 1	0.0000-0.0000	0.0000	0.00000	0.0000-0.0000	0.0000	0.00000
Location 2	0.0200-0.2100	0.0983	0.05306	0.0100-0.0300	0.0217	0.00937
Location 3	0.1000-0.2200	0.1433	0.04141	0.0100-0.0300	0.0158	0.00793
Location 4	0.1200-0.1600	0.1417	0.01337	0.2000-0.2900	0.2467	0.03200
Location 5	0.0200-0.0900	0.0542	0.01881	0.0000-0.0200	0.0067	0.00651
Sediment						
Location 1	0.0000-0.0300	0.0158	0.00793	0.0000-0.0400	0.0150	0.01382
Location 2	0.0500-1.4000	0.7000	0.42717	0.0400-0.2000	0.1250	0.05854
Location 3	0.5000-0.9000	0.7025	0.13039	0.1000-0.3000	0.1833	0.07177
Location 4	0.1900-0.5700	0.3400	0.13638	0.2500-0.5100	0.3708	0.08501
Location 5	0.2100-0.7800	0.4050	0.14451	0.0800-0.4000	0.1650	0.09986

Based on the results of ANOVA analysis and T-test, it shows that there are significant differences in chromium concentrations in water and sediment samples between research locations (*p*: 0.001) and between types of fish ponds (*p*: 0.001).

Hexavalent chromium accumulation in fish. Hexavalent chromium contamination in aquaculture ponds causes fish to be exposed to chromium during the cultivation period. All fish samples, both *C. gariepinus* and *O. niloticus* in the four affected areas, found higher hexavalent chromium accumulation compared to the control area (Location 1). The accumulation level of hexavalent chromium in fish in the affected area ranges from 0.2000-2.4000 mg kg^{-1} (*C. gariepinus*) and 0.2000-3.0000 mg kg^{-1} (*O. niloticus*). This value is much higher than the level of hexavalent chromium accumulation in fish in the control area, both in *C. gariepinus* with a range of 0.0100-0.0300 mg kg^{-1} and *O. niloticus* with a range of 0.0100-0.0200 mg kg^{-1} (Table 2).

Table 2

Accumulation of Cr^{+6} contaminants in fish (mg kg^{-1})

Sampling station	*Clarias gariepinus*			*Oreochromis niloticus*		
	Range	*Mean*	*SD*	*Range*	*Mean*	*SD*
Location 1	0.0100-0.0300	0.0225	0.00754	0.0100-0.0200	0.0158	0.00515
Location 2	0.2000-2.1000	1.3508	0.67737	1.0000-2.0000	1.5833	0.51493
Location 3	1.3400-2.4000	1.8983	0.32882	1.0000-3.0000	1.5833	0.66856
Location 4	1.0000-1.4600	1.2333	0.15605	1.3300-1.9200	1.5792	0.21505
Location 5	0.8000-1.4000	1.0642	0.16882	0.2000-1.0000	0.6583	0.31467

Table 2 shows that the accumulation of hexavalent chromium is found to be higher in areas close to the waste disposal point and tends to fluctuate downstream. The results of the ANOVA analysis proved that there was a significant difference in hexavalent chromium accumulation between research locations (*p*: 0.001) with an alpha of 0.05. The average accumulation value of hexavalent chromium in *C. gariepinus* (1.11382 mg kg^{-1}) was found in higher concentrations than in *O. niloticus* (1.0839 mg kg^{-1}). This result was confirmed by the T-test which showed a significant difference in hexavalent chromium accumulation in *C. gariepinus* and *O. niloticus* (*p*: 0.001 at alpha 0.005). However, the average value of hexavalent chromium accumulation tends to fluctuate based on location and type of fish.

Bioaccumulation factor (BCF). Table 3 shows a comparison of BCF and BAF chromium values in *C. gariepinus* and *O. niloticus*. The BCF value of chromium at locations affected by waste flows ranges from 8.695 to 22.815 in *C. gariepinus* and ranges from 6.447 to 118.055 in *O. niloticus*. Meanwhile, the bioconcentration mechanism was not found in the control area (Location 1), because chromium was not found in the water samples. Figure 3 explains the difference in mean BCF and BAF values between *C. gariepinus* and *O. niloticus*. The BCF value of *O. niloticus* was higher (49.150) compared to *C. gariepinus* (13.513). BCF values were found to be higher in areas close to the location of liquid waste disposal from the leather tanning industry (Locations 2 and 3). The same thing was also found in the BAF value, that the BAF value for *O. niloticus* (7.214) was much higher than the BAF value for *C. gariepinus* (2.642). BAF values were found to be higher in areas close to the location of liquid waste disposal from the leather tanning industry.

Table 3

BCF and BAF values of chromium pollutants in sediment and fishes

Sampling station	*BCF Values*			*BAF Values*		
	Clarias gariepinus	*Oreochromis niloticus*	*Sig. ANOVA*	*Clarias gariepinus*	*Oreochromis niloticus*	*Sig. ANOVA*
Location 1	0.000	0.000	0.003	0.925	0.895	0.023
Location 2	22.815	88.89		2.590	16.527	
Location 3	13.910	118.055		2.722	9.583	
Location 4	8.695	6.447		4.104	4.380	
Location 5	22.145	32.357		2.870	4.685	
Mean	13.513	49.150		2.642	7.214	
Sig. T-Test	0.003			0.003		

Table 3 shows the comparison of BCF and BAF values between *C. gariepinus* and *O. niloticus.* Based on ANOVA and T-test analysis, it shows that there are significant differences in BCF and BAF values between research locations for both *C. gariepinus* and *O. niloticus* (*p*: 0.003 and 0.023 with alpha 0.05). The BAF value of *O. niloticus* was significantly higher than that of *C. gariepinus* (*p*: 0.003 with alpha 0.05).

Health risk assessment. Biosurvey results show that there are differences in fish consumption patterns among communities in each research area. The average level of fish consumption per day ranges between 21.43 – 45.71 mg day. The level of fish consumption by the community (DFC) and the level of hexavalent chromium concentration in fish (C) determine the value of daily chromium intake (EDI), the maximum amount consumed (MEA) and the level of health risk (HQ and TR). Table 4 describes the values for daily chromium intake (EDI), maximum amount consumed (MEA) and health risks (HQ and TR). In areas with high concentrations of hexavalent chromium in fish, high levels of fish consumption also contribute to high daily hexavalent chromium intake values and health risks. The highest non-carcinogenic health risk (HQ) values were found at Location 4 (1.041) > Location 3 (0.900) > Location 2 (0.513) > Location 5 (0.207) > Location 1 (0.014). The non-carcinogenic health risk value in the control area (Location 1) was proven to be the least different from the area affected by the tannery industry's wastewater discharge.

Table 4

Summary of non-carcinogenic and carcinogenic health risk analysis

Location	*C*	*DFC*	*EDI*	*ADI*	*MEA*	*HQ*	*TR*
Location 1	0.02	42.85	0.015	0.0000409	0.026	0.014	7.5×10^{-6}
Location 2	1.47	25	0.625	0.0015402	0.120	0.513	3.1×10^{-4}
Location 3	1.41	45.71	1.096	0.0027009	0.125	0.900	5.4×10^{-4}
Location 4	1.74	42.85	1.268	0.0031247	0.101	1.041	6.3×10^{-4}
Location 5	0.86	21.43	0.313	0.0007713	0.205	0.257	1.4×10^{-4}

Table 4 shows that high fish consumption in communities in locations 3 and 4 is in the dangerous category and has the potential to cause health problems because the HQ value is close to and greater than 1. Meanwhile, in other areas, fish consumption is not dangerous. The cancer risk (TR) value at the control location (Location 1) is smaller than 1.0×10^{-6}, so it can be ignored. Meanwhile, in waste-affected locations, the TR value exceeds 1.0×10^{-4}, so consumption of fish contaminated with hexavalent chromium has the potential to cause cancer.

Discussion

Level of hexavalent chromium contamination in aquaculture pond. The results of the research show that the use of Opak River water contaminated with hexavalent chromium from the liquid waste disposal activities of the leather tanning industry is a factor causing contamination of small-scale aquaculture ponds. All water, sediment and fish samples from aquaculture ponds at affected locations (locations 2-5) were contaminated with hexavalent chromium at higher concentrations compared to control locations (Location 1). Even at control sites, hexavalent chromium was not detected in any water samples. This proves that liquid waste disposal activities from the leather tanning industry are a factor causing hexavalent chromium contamination in small-scale aquaculture ponds developed by the community. According to Xu et al (2023), among various sources of chromium pollution, leather tanning is the main factor causing chromium pollution in the environment.

This research also found that the concentration of hexavalent chromium in sediment samples was higher than in water samples. Chromium binds more easily with organic materials and is quickly deposited into sediments (Chimela et al 2016; Ehiemere et al 2022). Heavy metals such as chromium will interact more easily with sediment, causing sequestration (Brady et al 2015). The process of entering heavy metals into sediment increases the concentration of pollutants in the sediment, thereby reducing their concentration in the water (Nurkhasanah 2015). Furthermore, Udosen et al (2016) stated that sediment acts as a natural absorber of heavy metals in aquatic ecosystems, thereby reducing the bioavailable fraction in water. Considering these characteristics, sediments also function as metal reservoirs compared to receiving water bodies (Bai et al 2018). Due to river water quality monitoring, simply measuring the concentration of heavy metals in water media is neither accurate nor sufficient to identify metal inputs in the water system (Ismail et al 2016). However, measurements of heavy metal concentrations in sediments must be carried out to more accurately describe the level of water pollution (Abdel-Khalek et al 2016).

The average concentration of hexavalent chromium in water samples has exceeded the quality standards required for aquaculture. The maximum chromium concentration required in Government Regulation Number 82 (Government Regulations of the Republic of Indonesia 2001) is 0.05 mg/L. The same concentration is also set by the United Nations Environment Programme and the World Health Organization for the criteria for protecting aquatic ecosystems with a maximum allowable concentration (MAC) Cr value of 0.05 mg L^{-1} (UNEP 2008). The average chromium concentration in *Clarias gariepinus* pond water samples was 0.0875 mg L^{1}, slightly higher than the average in *Oreochromis niloticus* pond of 0.0582 mg L^{-1}. The results of this research indicate that the liquid waste disposal activities of the leather tanning industry have caused contamination of aquaculture ponds by hexavalent chromium. Therefore, business organizations can focus on improving their waste disposal practices and implementing sustainability approaches to improve waste properties and reduce hazardous waste. Implementing these practices can help improve water quality by reducing the entry of pollutants into waters while improving fish quality and health (Manalo & Hemavathy 2023).

Hexavalent chromium accumulation in fish. The entry of hexavalent chromium into aquaculture ponds causes the hexavalent chromium to be distributed in the water and sediment media, then it will be absorbed and accumulates in the fish's body. Absorption and accumulation of hexavalent chromium in fish bodies can occur through water, sediment and food (Ahmad & Sarah 2014). Sriuttha et al (2017), state that aquatic animals, such as fish, can accumulate Cr from water, sediment and food. Contamination of aquaculture ponds by chromium pollutants can cause the migration of heavy metals into the fish's body through bioconcentration and bioaccumulation (Baki et al 2018; Korkmaz et al 2019; Arisekar et al 2020). The high level of heavy metal accumulation in fish indicates the high level of pollutant concentration in the aquatic environment (Zhao et al 2012). Accumulation levels of heavy metals in commercial fish species have received great attention and increasing interest in their use as bioindicators to assess the integrity of aquatic environmental systems (Jiang et al 2005; Chi et al 2007; USEPA 2011).

Accumulation of hexavalent chromium in fish was found to be significantly different (p: 0.001 with alpha 0.05) between control locations and locations affected by tannery industry wastewater discharge. Hexavalent chromium concentrations also tend to differ between locations in the affected area, these differences are caused by differences in fish type, age and aquaculture systems developed. Hexavalent chromium accumulation in *O. niloticus* fish is slightly higher than *C. gariepinus* fish, this indicates that hexavalent chromium accumulation in fish is not only influenced by the type of fish and its biological characteristics such as food habit but is influenced by many interacting factors such as entry routes, characteristics, metabolism, and the environmental conditions in which the species lives (Ozmen et al 2008; Zhao et al 2012). Rajeshkumar and Li (2018) stated that heavy metal accumulation is also influenced by uncontrolled environmental variables, such as water currents, temperature, pH, electroconductivity, etc. Based on researchers' observations, the low accumulation of hexavalent chromium in *C. gariepinus* is caused because the water tends to stagnate so that chromium will quickly be disposed of in the sediment and the fish are still relatively young, 2-4 months old. Meanwhile, in

O. niloticus ponds the water generally flows continuously, the majority are earthen ponds so there is a lot of sediment, and the age of the fish samples is close to consumption age, namely 4-6 months.

Hexavalent chromium concentrations in *C. gariepinus* and *O. niloticus* fish averaged 1.1382 mg kg^{-1} and 1.0839 mg kg^{-1}, all of which were still below the maximum concentration limit set by the Director General of the Food and Drug Supervisory Agency, namely 2.5 mg/kg^{-1} (Dirjen POM 1989). The results of this research are much higher than the results of previous research on chromium accumulation in fish conducted by Rahman et al (2012) in Bangladesh with accumulation levels ranging from 0.09–0.4 mg kg^{-1}; Leung et al (2014) in China found accumulation levels in fish ranging from 0.18–0.85 mg kg^{-1}. The high accumulation of chromium in fish was caused by research carried out in aquaculture ponds that used water contaminated by liquid waste from the tanning industry. The discovery of chromium contaminants in food, especially *C. gariepinus* and *O. niloticus*, is an initial indication that the threat to human health through fish consumption is real. The liquid waste disposal activity of the leather tanning industry is a source of river water pollution and contamination of aquaculture ponds along the downstream Opak River. The release of industrial waste containing heavy metals poses a serious threat to the environment and human health (Nakkeeran et al 2018).

Even though the level of contamination is still within safe limits, considering the community's fish consumption patterns which tend to be high and continue to increase, serious attention is needed in monitoring and preventing chromium pollution in aquaculture ponds. Fish consumption is not the only chromium intake, but intake can also come from water, vegetables, rice, etc., which are also contaminated with chromium. The diversity of food sources in humans can cause the accumulation of heavy metals in the body which can ultimately reach detrimental levels, causing serious health risks, such as cancer (Badamasi et al 2019; Yu et al 2020) and neuro degenerative diseases (Cicero et al 2017; Chen & Chau 2019; Yu et al 2020). In particular, hexavalent chromium is the most toxic form (Tumolo et al 2020; Chen et al 2022), and enters the body through various ways (consumption, inhalation and skin contact) which will cause pathological changes in human organs and systems and even increase the incidence rate and mortality of many cancers (Deng et al 2019; Sharma et al 2022). Prolonged human exposure can cause gastrointestinal upset, respiratory problems, kidney and liver damage, and altered genetic material, among other conditions (Shanker et al 2005).

Values of bioconcentration and bioconcentration factors. At locations affected by the disposal of liquid waste from the tannery industry (locations 2-5), bioconcentration and bioaccumulation mechanisms of hexavalent chromium occurred in *C. gariepinus* and *O. niloticus* with varying values. Meanwhile, in unaffected locations (control, Location 1), bioconcentration did not occur, but bioaccumulation still occurred, although at very low values. The BCF value in *C. gariepinus* ranges from 6.447-118.055 and in *O. niloticus* ranges from 8.695-22.815, this indicates that chromium contamination in aquaculture ponds causes a bioconcentration mechanism in fish. Yasmeen et al (2016) and Mensoor and Said (2018) stated that water contaminated with heavy metals can increase the heavy metal content in fish muscles. This means that the Opak River water which is contaminated with chromium and used as a water source for aquaculture is a source of chromium pollution in fish.

The BCF value of chromium in fish varies relatively based on the type and location of the aquaculture ponds. Variations in BCF values can be caused by differences in Cr concentrations in water and organism characteristics such as body weight, age and metabolism (Haryanti & Martuti 2020). Awaliyah et al (2021) reported that the age and weight of fish affect heavy metal concentrations. Arkianti et al (2019), said that the BCF value is influenced by the type of heavy metal, organism, length of exposure, and water environmental conditions. The BCF for these two types of fish tends to be higher in the aquaculture ponds at Location 2 which is close to the waste disposal point for the tannery industry. This shows that the potential for exposure to Cr through water media at this location is higher than at other locations.

Meanwhile, the BAF value in this study experienced fluctuations, not influenced by proximity to the pollutant source. However, it is more influenced by the large concentration of Cr in the sediment and intrinsic factors in fish. Eneji et al (2011), stated that the rate of heavy metal bioaccumulation in fish depends on the metal concentration in the sediment, eating habits, and the ability of the fish to digest contaminants. *C. gariepinus* has a higher BAF value than *O. niloticus*, this is because *C. gariepinus* is a benthic and omnivorous species. According to Madu et al (2017), *C. gariepinus* tends to bioaccumulate contaminants from sediment due to its habitat and food preferences. The results of this study also confirm the high BAF value, that chromium is found in higher concentrations in sediment than in water. The high concentration of Cr in sediment is a determining factor in the BAF value. However, the BAF value for *O. niloticus* and *C. gariepinus* in all study locations has a value of less than 1. However, according to Kouakou et al (2016), the BAF value has shown the potential for bioaccumulation and biomagnification of chromium in the aquatic food chain.

Fish consumption and health risk assessment. Risk assessment is based on the accumulated concentration of hexavalent chromium in fish consumed, the average consumption rate of fish, and the body weight of the adult population. The daily fish consumption level at locations 3 and 4 ranges from 42.85 – 45.71 mg/day with an Indonesian average adult body weight of 58.8 kg, getting an estimated daily intake (EDI) value ranging from 1.096-1.268 mg/kg bw/day. This value is the highest figure in locations affected by waste disposal. The high EDI values in these two locations are caused by the high accumulation of chromium in fish and high levels of fish consumption. However, the EDI value is still below the threshold limit set by Drug and Food Control of the Republic of Indonesia (Dirjen POM 1989) for chromium in food, namely 2 mg/kg. However, considering the accumulative nature of chromium and its chronic toxic nature, long-term consumption still has the potential to cause health problems. This is confirmed by the results of the non-carcinogenic health risk analysis, with HQ values close to or even more than 1 (HQ ranges from 0.900-1.041) which illustrates that fish consumption in these two areas has moderate to high health risks.

This study also reports that the cancer risk (TR) value due to lifelong consumption of fish has the potential to cause cancer with a TR value of more than the limit set by the USEPA (2018), namely between 1.0×10^{-4} (1 in 10,000) to 1.0×10^{-6} (1 in 1,000,000). In the affected locations the TR value is between 1.4×10^{-4} to 6.3×10^{-4}, so the health risk in the form of the potential for cancer is included in the unacceptable category. Meanwhile at the control location (Location 1) the TR value was less than 1.0×10^{-6}, so small that it could be ignored (Varol et al 2017). Based on the HQ values (locations 3 and 4) and TR in all affected locations, consumption of hexavalent chromium-contaminated fish can cause non-carcinogenic and carcinogenic risks to local communities. Considering this, in the future we must focus on efforts to prevent and control chromium pollution in river ecosystems and aquaculture ponds and to reduce potential health risks faced by local communities. This effort can be done through: 1) increasing the effectiveness of waste treatment, 2) monitoring contamination in food products regularly, and 3) conducting further studies related to health risk management to determine the amount and frequency of safe fish consumption. This step can help ensure food safety and minimize potential health risks through the consumption of small-scale aquaculture carried out by communities along the Opak River.

Conclusions. The use of river water contaminated with hexavalent chromium for aquaculture causes contamination in all aquaculture ponds. Hexavalent chromium contamination in aquaculture ponds causes hexavalent chromium accumulation in fish with a range of 0.2000-2.4000 mg kg^{-1} in *Clarias gariepinus* and 0.2000-3.0000 mg kg^{-1} in *Oreochromis niloticus*. The level of hexavalent chromium accumulation in fish is influenced by the location, type and age of fish and aquaculture system such as type of pond and water circulation system. BCF and BAF values differed significantly both based on location and fish type. Consumption of fish contaminated with hexavalent chromium has the potential to cause non-carcinogenic health risks (HQ ranges from 0.900-1.041) and the potential for cancer with a TR value of 1.4×10^{-4} to 6.3×10^{-4}. The highest sequential HQ values were found at Location 4 (1.041) > Location 3 (0.900) > Location 2 (0.513) > Location 5 (0.207) > Location 1 (0.014).

Acknowledgements. The authors are grateful to the Faculty of Biotechnology, Universitas Kristen Duta Wacana, Environmental Laboratory of the Environmental and Forestry Service DIY Province for providing funding and facilities. The authors are also grateful to respondents, the promoter team for their collaboration in research and providing many inputs to improve this article. We would like to thank the Bantul District Fisheries and Marine Department and field extension workers for their support, as well as the fish farmers who helped us with sample collection.

Conflict of interest. The authors declare that there is no conflict of interest.

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Received: 22 February 2024. Accepted: 17 April 2024. Published online: 30 October 2024.
Authors:
Djoko Rahardjo, Development Studies Doctoral Program, Faculty of Interdisciplinary Satya Wacana Christian University, Salatiga, Indonesia, e-mail: djoko@staff.ukdw.ac.id
Gatot Sasongko, Interdisciplinary Faculty, Satya Wacana Christian University, Jl. Diponegoro No. 52-60, Salatiga, Indonesia, e-mail: gatot.sasongko@uksw.edu
Suwarno Hadisusanto, Faculty of Biology, Gadjah Mada University, Jl. Teknika Selatan, Sekip Utara Bulaksumur Yogyakarta, Indonesia, e-mail: suwarnohs@ugm.ac.id

How to cite this article:
Rahardjo D., Sasongko G., Hadisusanto S., 2024 Hexavalent chromium contamination and implications for human health risks related to fish consumption from small-scale aquaculture ponds in Bantul Regency, Yogyakarta, Indonesia. AACL Bioflux 17(5):2276-2293.