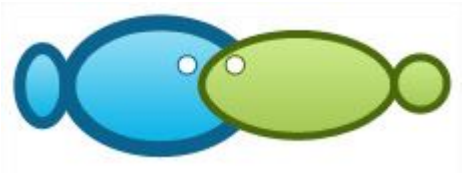

The occurrence of fish diseases in mercury (Hg)-using artisanal gold mining area of the Talawaan watershed, North Sulawesi Province, Indonesia

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Abstract. Fish are a high quality protein and nutrient source, but mercury (Hg) contamination in aquatic ecosystems can cause health risks to fish and human. Hg and its potential environmental problems have been a great concern since the appearance of a small-scale gold mining community in the Talawaan watershed, North Sulawesi, Indonesia. Research on the occurrence of fish diseases in the area of Hg-using artisanal gold mining (AGM) in the Talawaan watershed (TW), North Sulawesi Province, Indonesia, was conducted and aimed to evaluate the diseases by parasite and bacterial contamination on wild and cultured fishes. Nine sampling sites in total were selected in this research, where 8 sites were selected in the TW (7 those were in the AGM, and 1 outside of AGM as control site), and another one was in out of the TW also as a control site. Wild and cultured fishes were collected within the TW, also cultured fish from the control site outside the TW. Water quality, which consists of organic, inorganic, Hg, and bacterial parameters, was analysed. The Hg accumulation in fish in riverine area was observed by collecting water samples and fish in the sampling sites. Fish diseases determined by the presence of parasites and bacteria in the samples were also observed. In this case, isolation via Thiosulfate Citrate Bile Salts Sucrose (TCBS) and Trypic Soya Agar (TSA) for 24 hours and purification processes were performed. The results showed that several species of parasites and bacteria were found in wild fish species and varied according to fish species and sampling sites. In the cultured fish, parasites and bacteria were found almost in all of sampling sites both within and outside the TW. Water quality and Hg contamination in fish were in good condition during both dry and wet seasons. Overall, fish disease due to parasites and bacteria occurred in the AGM areas and the control area in the TW, as well as the control area outside TW.

Key Words: fish diseases, parasites, bacteria, mercury, Talawaan watershed.

Introduction. Fish are a high quality protein and nutrient source. However, fish might also be infected by diseases that may affect human beings who depend on fish as their main source of protein and nutrient. Infectious diseases are a major problem in fish culture. There are some fish diseases and infections that can be transmitted from the fish and the water in which they are bred to humans. The incidence of the transmission of diseases is dependent upon several factors; the type of organism, the susceptibility of the host, and environmental factors such as water quality (Durborow 1999). The presence of fish diseases is closely related to the condition of the water and the entire aquatic environment (Arwin et al 2016). Parasites, bacteria, viruses, and fungi are the causes of the fish diseases (Irianto 2005). Hg contamination is ubiquitous in aquatic ecosystems and can pose health risks to fish and their consumers (Lepak et al 2016). Such diseases can cause a systemic illness leading to high mortality of fish. However, defficiency or malnutrition is also another cause.

In the North Sulawesi Province, in Indonesia, particularly in the North Minahasa Regency, fish farming is one of the income sources of the community (Manumpil et al 2015). This regency is located within the Talawaan watershed (TW), which has a wide

area for the fish farming industry since it provides water in amount of about 150 m^3 sec^{-1} (MUDA 2013). However, in the same area within the watershed is located an artisanal gold mining (AGM) infrastructure using mercury (Hg) for an amalgam method to obtain the gold (Limbong et al 2003; Lingkubi 2004; Lasut & Yasuda 2010; BKIPMKHP 2017a). Due to this AGM activity, an environmental concern arose that the fish farming industry from this area could be affected. Interestingly, information about diseases related to bacteria and parasites, and the present of Hg particularly in organism such as wild and cultured fish in the TW is limited.

The present study aims to evaluate the occurrence of fish diseases in the TW in relation to the AGM activities, particularly the diseases consisting of parasite and bacterial contamination in wild and cultured fishes. This study is related to two prior studies published only in Indonesian language (Manumpil et al 2015; BKIPMKHP 2017a).

Material and Method

Study area and sampling sites. TW is located in the northern part of the Minahasa Peninsula, North Sulawesi Province, Indonesia (Figure 1). The watershed covers an area of 34,000 ha, geographically located on 01°04'30" - 01°06'55" N and 124°08'35" - 125°00'28" E. The Talawaan river flows to the northwest through the watershed from the upper land area to the sea, in which it branches into three rivers (Talawaan, Kima, and Bailang) (BPLH Sulut 2004). At the upper land area of the TW is located an area of AGM where Hg is used in the amalgam method to obtain the gold using trommels (Martens 2000; Limbong et al 2003; Lasut et al 2010; Lasut & Yasuda 2010).

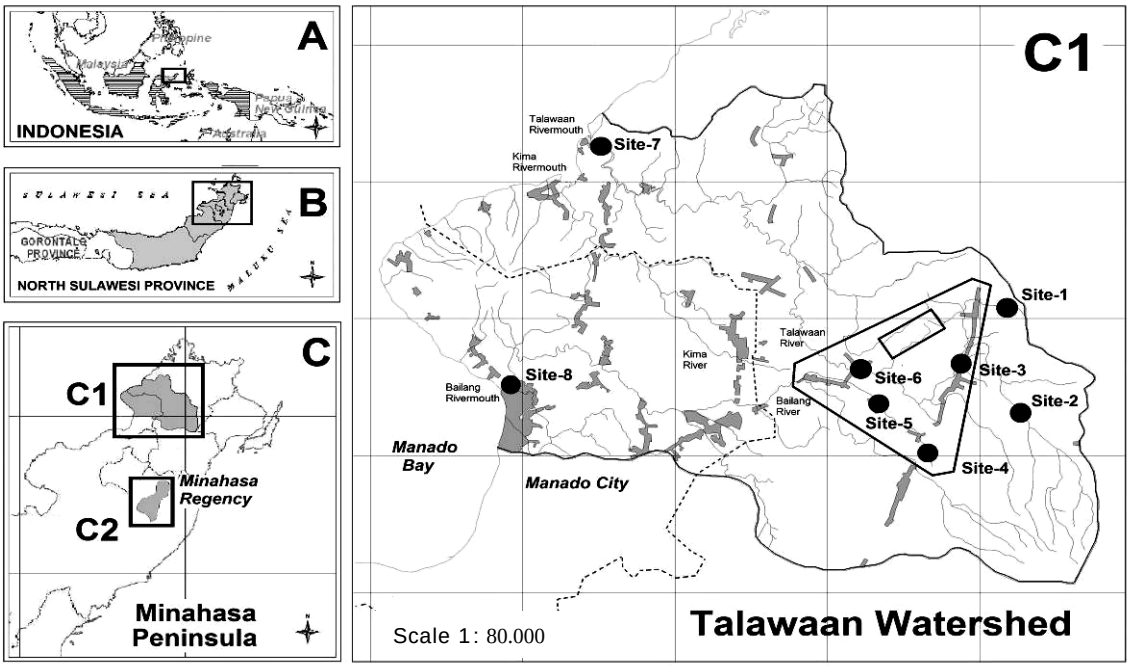

Figure 1. Study area and sampling site: A. Indonesia; B. North Sulawesi Province; C. Minahasa Peninsula; C1. Talawaan watershed; C2. Tondano watershed.

The AGM activities cover an area of the TW, which is indicated by a rectangle-shaped line (Figure 1, C1). However, before 2004, it covered a wide area, which is indicated by a triangle-shaped line on the map. Nine sampling sites were selected, 8 sites (site 1 to site 8) within the TW (Figure 1, C1) and a site (site 9) was in the neighboring watershed of Tondano (Figure 1, C2). Two sites were set up as controls, such as site 2 (as the control within the TW) and site 9 (as the control from outside the TW watershed).

The description and geographical coordinates of the sites are shown in Table 1. The sampling sites are interconnected to each other through rivers; based on this, 2 parts of river are flowing through the TW to coastal area (Figure 1, C1). Firstly, the upper

areas of sites 1 and 3 are interconnected with the lower areas of sites 6 and 7 through the river of Talawaan. Besides, the water from site 2 is flowing through site 3. Secondly, the upper areas of sites 4 and 5 are interconnected with the lower area of site 8 through the river of Bailang. The upper areas of sites 1, 2, 3, 4, 5 and 6 are freshwater and the lower areas of sites 7 and 8 consist of brackish water at the river mouth. The sites 3, 4, and 5 are in the AGM area, and sites 2 and 9 were considered as the control areas respectively within and outside of the TW.

Table 1

Sampling sites and sampling activities

Sampling sites	*Geographical coordinates*	*Elevation (m)*	*Description of locations*	*Sampling activities*
Site 1	1°33'18.88"N 124°59'58.64"E	243.2	Wasian village and river	Water quality
Site 2*	1°30'41.13"N 124°0'53.16"E	327.4	Tatelu village (BBAT-Tatelu)	Fish diseases (cultured fish)
Site 3	1°31'39.11"N 124°59'21.41"E	212.8	Warukapas village	Fish diseases (cultured fish); Deposit-Hg sediment
Site 4	1°30'30.47"N 124°58'52.09"E	217.3	Dimembe village	Fish diseases (cultured fish); Deposit-Hg sediment
Site 5	1°30'53.56"N 124°58'8.78"E	161.5	Tetey village	Fish diseases (wild fish); Deposit-Hg sediment
Site 6	1°31'49.91"N 124°57'37.92"E	125.0	Talawaan village and river	Fish diseases (wild fish); Fish diseases (cultured fish); Water quality; Deposit-Hg sediment
Site 7	1°36'47.31"N 124°52'29.60"E	10.1	Talawaan Bajo village and rivermouth	Fish diseases (wild fish); Water quality; Deposit-Hg sediment
Site 8	1°31'32.90"N 124°50'41.98"E	15.8	Bailang village and rivermouth	Fish diseases (wild fish); Water quality; Deposit-Hg sediment
Site 9**	1°22'64.54"N 124°89'33.82"E	683.1	Minahasa Regency	Fish diseases (cultured fish)

*adapted from BKIPMKHP (2017a); **adapted from Manumpil et al (2015).

Sample collection and preparation. In order to observe diseases and Hg accumulation in fish, fish samplings were performed in September 2016. Wild and cultured fishes were sampled haphazardly. The wild fishes were caught by using a hand-line along the rivers at sites 5, 6, 7, and 8, and the cultured fishes were caught by using a net in fish ponds at sites 3, 4, and 5. Sample collection and preparation of cultured fish from control areas (sites 2 and 9) were conducted by BKIPMKHP (2017a) and Manumpil et al (2015), respectively.

For disease observation on wild fish, species and number of samples depended on the fish catch obtained; and regarding cultured fish, two species were selected, such as Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) with 16 (range of total length: 10.8-11.4 cm; range of body weight: 294.4-322 g) and 2 (range of total length: 10.5-13.0 cm; range of body weight: 325-455 g) samplings, respectively, at each site. For Hg accumulation in wild fish, three species were selected, such as crescent perch (*Terapon jarbua*), stripped snakehead (*Channa striata*), and barramundi (*Lates calcarifer*) at sites 7 and 8; and regarding cultured fish, *C. carpio* and *O. niloticus* were selected at sites 3, 4, and 5.

All samples were kept in plastic bags individually and placed in a cool box with coolant gel during transportation from the field to the laboratory. This was to ensure that the samples could not contaminate each another and were kept in fresh condition for further analysis.

Fish diseases determination. Two types of fish diseases were determined, i.e. parasite and bacterial. The determination was conducted in the laboratory of Balai Karantina Ikan, Pengendalian Mutu, dan Keamanan Hasil Perikanan (BKIPMKHP) Kelas II Manado. The parasite and bacteria were determined using the method mentioned in Manumpil et al (2015) and BKIPMKHP (2017a). The presence of ectoparasites was checked by scraping the whole fish (Noga 2010) under the microscope with 100 times of magnification. Lamellae of gills were also checked for the parasites. The identification of the parasites was conducted using a guidance of Kabata (1985). The determination of bacteria was conducted in two steps, namely isolation and purification. The isolation was performed via Thiosulfate Citrate Bile Salts Sucrose (TCBS) and Trypic Soya Agar (TSA) for 24 hours. The purification was conducted in order to determine bacterial characteristics. The identification of bacteria was conducted using the guidance of Austin & Austin (1993). In addition, the data from the control areas (site 2 and site 9) were adapted from BKIPMKHP (2017a) and Manumpil et al (2015), respectively.

Mercury (Hg) measurement. The accumulation of Hg in fish was also measured. The measurement was performed at the laboratory of Balai Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan Kelas II, Manado, Indonesia. The Hg was measured using AAS with protocol of the APHA-AWWA-WPCF (1990).

Water quality measurement. The measurement of water quality was only conducted in July 2015 for the dry season and March 2016 for the wet season. Four indicators were used, i.e., organic, inorganic, metal, and bacterial. The bacterial indicator was determined using the total coliform (TC) parameter; the organic and inorganic indicators were determined using the parameters of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia ($N\text{-}NHNO_3$), dissolved oxygen (DO), nitrate ($N\text{-}NO_3$), nitrite ($N\text{-}NO_2$), and total phosphate ($P\text{-}PO_4$); while metal and other indicators were determined using mercury (Hg), temperature, and pH. The measurement of the BOD_5 and COD was conducted using the method presented by Rice et al (2012) with some modifications for measuring the DO that used the portable La Motte DO-4000. Respectively, the $N\text{-}NO_3$, $N\text{-}NO_2$), and PO_4^{-3} were measured using cadmium-reduction and ascorbic acid methods (Rice et al 2012); and an Atomic Absorption Spectrophotometers (AAS) method was used for the metal parameter with the protocol of the APHA-AWWA-WPCF (1990). The TC was measured using the multiple-tube technique (APHA-AWWA-WPCF 1990) with the highest measurement of > 24,200 MPN (most probable number per 100 mL). The temperature and pH were measured *in situ*. All methods followed the Indonesian national standard (BSN 2012). The water samples (0.5 liter) were collected from sites 1, 6, 7, and 8 in July 2015 and March 2016. Site 1 and 6 represented upstream samples, and sites 7 and 8 as downstream samples. All measurements and laboratory analyses were performed at the laboratory of Water Laboratory Nusantara (WLN), Manado, Indonesia (the accredited laboratory in Indonesia).

Results and Discussion. Table 2 shows the parasite and bacterial occurrences in the wild fishes of TW at four sampling sites (5, 6, 7, and 8). Six species of parasites (*Trichodina* sp., *Dactylogyrus* sp., *Chilodonella* sp., *Diplectanum* sp., *Epistylis* sp., and *Gnathia* sp.) and 3 species of bacteria (*Plesiomonas shigelloides*, *Aeromonas* sp., and *Plesiomonas* sp.) were observed in the fishes that live in those areas. Different parasites and bacteria were identified by fish species and sampling sites.

The parasite *Trichodina* sp. was observed only in the freshwater fish Mozambique tilapia (*Oreochromis mossambicus*) from sites 5 and 6, three spot cichlid (*Cichlasoma trimaculatum*), marble goby (*Oxyeleotris marmorata*), and bonylip barb (*Osteochilus vittatus*) from site 5. *Dactylogyrus* sp. was observed only in *O. mossambicus* at site 6, and *O. hasselti* from sites 5 and 6. *Epistylis* sp. was observed only in *O. mossambicus* and *C. trimaculatum* from site 5. Moreover *Chilodonella* sp. and *Diplectanum* sp. were observed only in the brakishwater from site 7 in silver barb (*Barbonymus gonionotus*), and *Gnathia* sp. in stripped snakehead (*Channa striata*) from site 8.

The bacterium *P. shigelloides* was observed in both fresh and brackish water. In fresh water it was observed in site 5 (in *O. marmorata* and *O. vittatus*) and site 6 (in *O. mossambicus*), and in brackish water in the site 7 (in the bigeye trevally, *Caranx sexfasciatus*). The *Aeromonas* sp. was observed only in freshwater in site 5 (in *O. mossambicus* and *O. vittatus*) and in site 6 (in *O. mossambicus* and *C. trimaculatum*). The bacterium *Plesiomonas* sp. was only found in site 7 at gill of *B. gonionotus*.

Table 2

Parasites and bacteria observed in wild fish at the Talawaan watershed

Sampling sites	*Fish species*	*Presence of parasites*					*Presence of bacteria*			
		Tr	*Da*	*Cl*	*Di*	*Ep*	*Gn*	*Ps*	*Ae*	*Pl*
Site 5	*O. mossambicus*	+	-	-	-	+	-	-	+	-
	C. trimaculatum	+	-	-	-	+	-	-	-	-
	O. marmorata	+	-	-	-	-	-	+	-	-
	C. striata	-	-	-	-	-	-	-	-	-
	O. vittatus	+	+	-	-	-	-	+	+	-
Site 6	*O. mossambicus*	+	+	-	-	-	-	+	+	-
	O. vittatus	-	+	-	-	-	-	-	-	-
	C. trimaculatum	-	-	-	-	-	-	-	+	-
	O. marmorata	-	-	-	-	-	-	-	-	-
	Monopterus albus	-	-	-	-	-	-	-	-	-
Site 7	*C. sexfasciatus*	-	-	-	-	-	-	+	-	-
	Leiocassis micropogon	-	-	-	-	-	-	-	-	-
	B. gonionotus	-	-	+	+	-	-	-	-	+
	Terapon jarbua	-	-	-	-	-	-	-	-	-
Site 8	*C. striata*	-	-	-	-	-	+	-	-	-
	Lates calcarifer	-	-	-	-	-	-	-	-	-
	Glossamia wichmanni	-	-	-	-	-	-	-	-	-
	C. sexfasciatus	-	-	-	-	-	-	-	-	-
	Rasboroides vaterifloris	-	-	-	-	-	-	-	-	-

Tr: *Trichodina* sp.; Da: *Dactylogyrus* sp.; Cl: *Chilodonella* sp.; Di: *Diplectanum* sp.; Ep: *Epistylis* sp.; Gn: *Gnathia* sp.; Ps: *P. shigelloides*; Ae: *Aeromonas* sp.; Pl: *Plesiomonas* sp.; "+" = detected; "-" = not detected.

Table 3 shows parasite and bacterial occurrences in the cultured fish from 5 sampling sites (2, 3, 4, 5, and 9). Two species of parasites (*Trichodina* sp. and *Dactylogyrus* sp.) and 10 species of bacteria (*Moraxella* sp., *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Listeria* sp., *Cryptosporidium hominis*, *Pasteurella haemolytica*, *Bacillus* sp., *Pseudomonas anguilliseptica*, *Aeromonas salmonicida*, and *Edwardsiella ictaluri*) were observed in the *C. carpio* and *O. niloticus*. At the control area of site 2 in TW, no parasites and bacteria were observed in the fish samples, while in the site 9, only the parasites *Trichodina* sp. and *Dactylogyrus* sp. were observed. Almost all species of parasites and bacteria were observed in the fishponds in sites 3, 4, and 5.

The quantification of the presence of parasites and bacteria in the cultured fish from sites 3, 4, and 5 within the TW is presented in Figures 2 and 3, respectively. In the sample of *O. niloticus*, the parasite *Trichodina* sp. was the most present (25% at sites 3 and 4), while at the site 4 it was *Dactylogyrus* sp. (50%). These were followed by the bacteria *A. hydrophila* at site 4 (56%) and site 3 (44%), and *P. shigelloides* (31%) at site 5. These also occurred in the sample of *C. carpio*. All samples (100%) at site 4 were observed for parasite (*Trichodina* sp.) and bacteria (*A. hydrophila*); however, in some samples from all sites, no parasites nor bacteria were observed.

Table 3

Parasites and bacteria observed in cultured fish at the Talawaan watershed

Sampling sites	*Species*	*Presence of parasites*		*Presence of bacteria*									
		Tr	*Da*	*Mo*	*Ah*	*Ps*	*Li*	*Ch*	*Ph*	*Ba*	*Pa*	*As*	*Ei*
Site 2*	*O. niloticus*	-	-	-	-	-	-	-	-	-	-	-	-
	C. carpio	-	-	-	-	-	-	-	-	-	-	-	-
Site 3	*O. niloticus*	+	+	+	+	-	-	-	-	-	-	-	-
	C. carpio	+	-	-	+	-	-	-	-	-	-	-	-
Site 4	*O. niloticus*	+	+	+	+	+	+	-	-	-	-	-	-
	C. carpio	+	-	-	+	-	-	-	-	-	-	-	-
Site 5	*O. niloticus*	+	+	-	-	+	-	+	+	+	-	-	-
	C. carpio	-	+	-	-	+	-	-	-	-	-	-	-
Site 9**	*O. niloticus*	+	+	-	-	-	-	-	-	-	-	-	-

Tr: *Trichodina* sp.; Da: *Dactylogyrus* sp.; Mo: *Moraxella* sp.; Ah: *A. hydrophila*; Ps: *P. shigelloides*; Li: *Listeria* sp.; Ch: *C. hominis*; Ph: *P. haemolytica*; Ba: *Bacillus* sp.; Pa: *P. anguilliseptica*; As: *A. salmonicida*; Ei: *E. ictaluri*; *BKIPMKHP (2017a); **Manumpil et al (2015); "+" = detected; "-" = not detected.

The source of water flowing through site 3 is different from the one going through sites 4 and 5, it comes from sites 1 and 2 (outer area of former AGM in TW); this is interconnected with the river of Talawaan in which site 7 is the end of the rivermouth. Sites 4 and 5 are interconnected with the river of Bailang in which site 8 is the end of the rivermouth.

In North Sulawesi, KHV was generally found in *C. carpio* (BKIPMKHP 2017a). In 2016, a survey conducted by BKIPMKHP in the area of Warukapas (site 3) and Dimembe (site 4) found negative contamination of *Pesudomonas anguilliseptica, Aeromonas salmonicida*, *Edwarsiella ictaluri*, to the fish *Mochokus niloticus* and *C. carpio*; and KHV to *C. carpio* and *M. niloticus* (BKIPMKHP 2017a). Another survey also conducted in 2017 found a negative contamination of *A. salmonicida*, *P. anguilliseptica,* and *E. ictaluri* to *C. carpio* and *M. niloticus*; and a negative contamination of KHV and *Herpes ictaluri* as well (BKIPMKHP 2017b).

The environmental condition of the TW was observed by measuring the water quality status using several parameters (organic and inorganic) during two different seasons (dry and wet) at 4 sampling sites (sites 1, 6, 7, and 8). The results of the measurement are shown in Table 4 and Figure 4. Seemingly, all the organic parameters were comparable within the sampling sites and the seasons. It means the water flowing through the watershed from site 1 to site 7, passing through the site 6 has a good status as well as the water going to site 8. This situation was also indicated by the inorganic parameters (Figure 4). Except for site 6 during the dry season, the inorganic parameters were comparable within the sites and seasons. For other water quality parameters, Hg was not detected in all sites' and seasons' samplings; total coliform was > 24,200 MPN for all sampling sites and seasons; and the pH was in range of 6.99-7.93 and 6.89-8.29 during the dry and wet seasons, respectively. However, at the rivermouth of Bailang river (site 8), the quality of the water was bad since wastewater from houses was not treated prior to discharge (Lasut et al 2008).

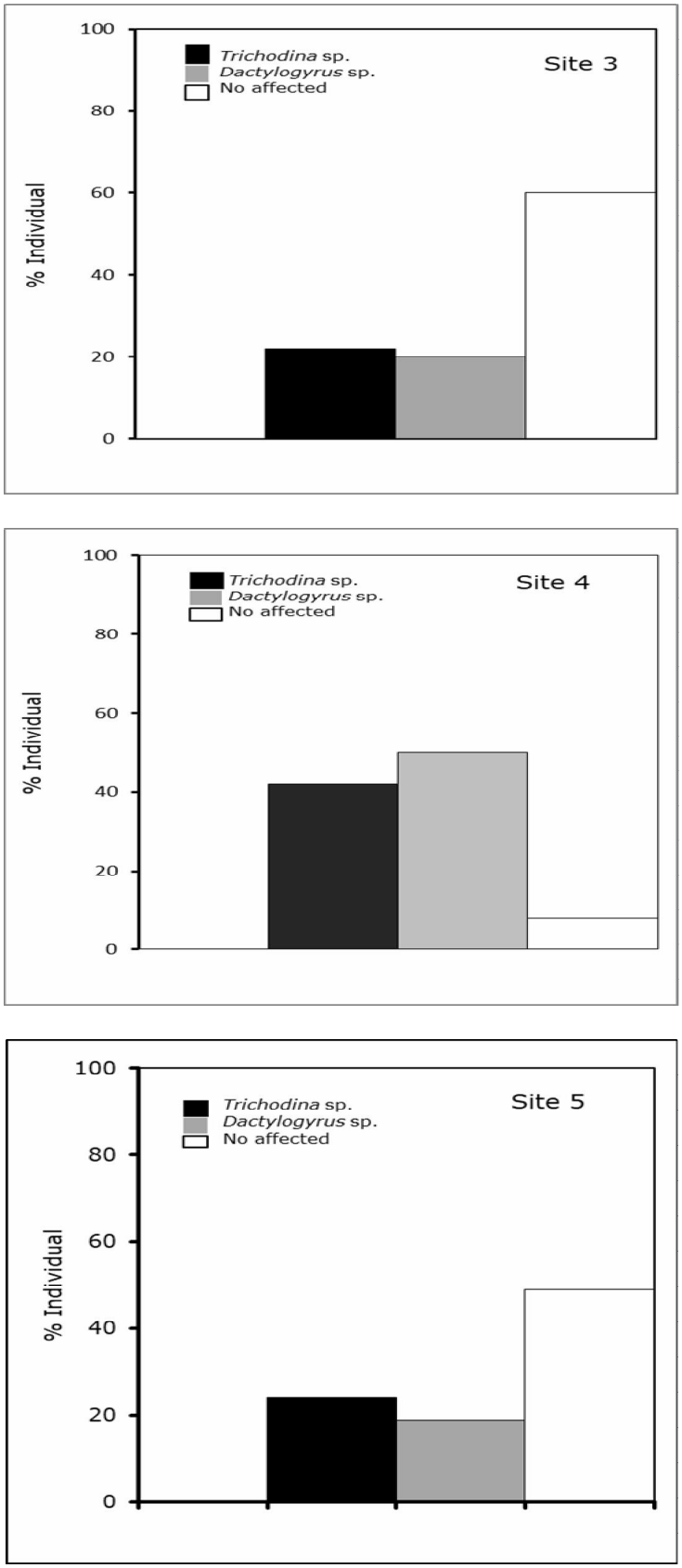

Figure 2. The presence of parasites in cultured fish samples at the sampling sites in the Talawaan watershed.

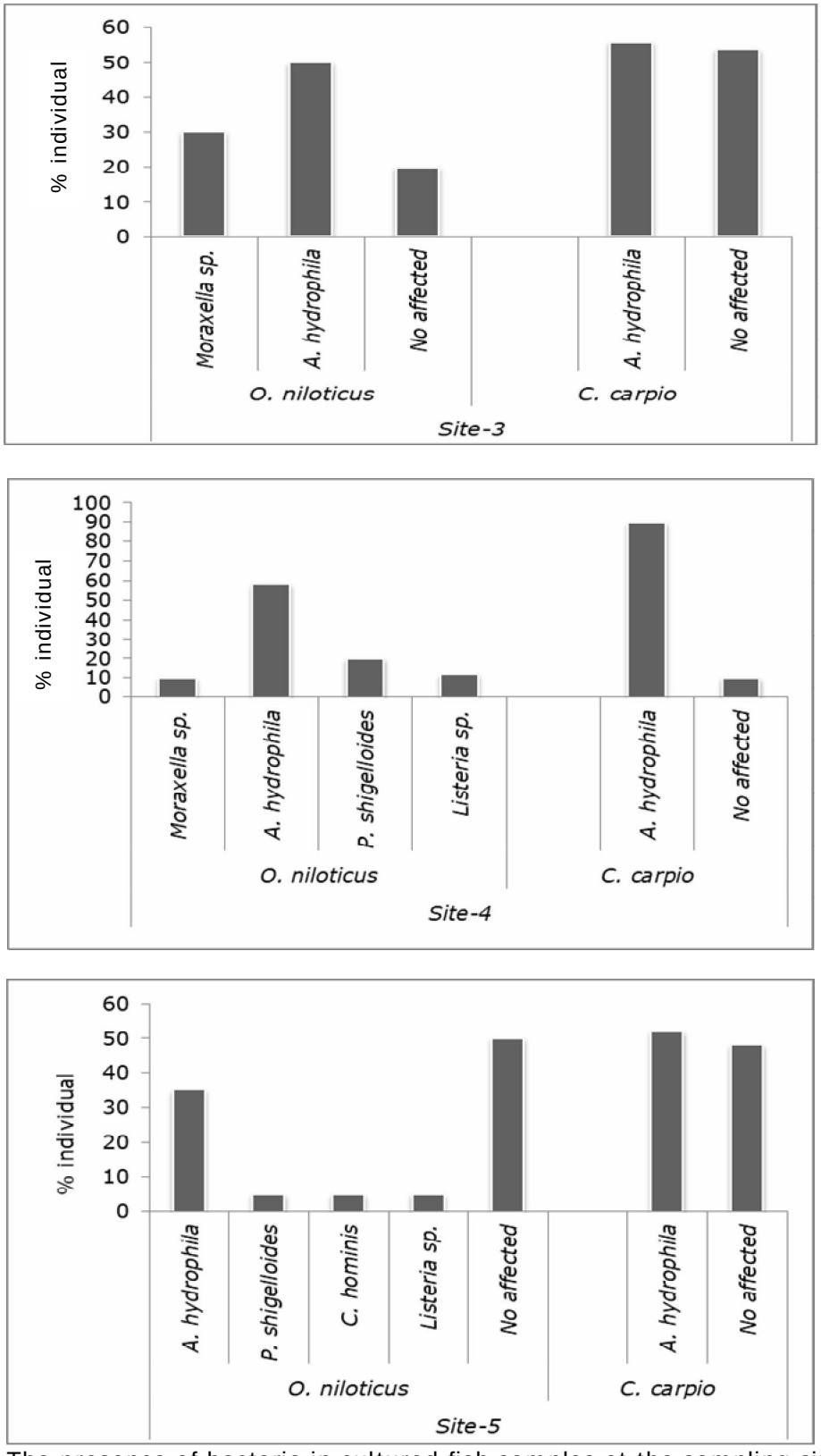

Figure 3. The presence of bacteria in cultured fish samples at the sampling sites in the Talawaan watershed.

Before 2004, the AGM covered an area of ±822 ha in the upper land of the TW in 8 villages (Wasian, Tatelu, Tatelu Rondor, Tatelu Warukapas, Talawaan, Kolongan, Tetey, and Mapanget) of the Dimembe District of the North Minahasa Regency (BPLH Sulut 2004; Lingkubi 2004). The number of miners was about 2500 to 3000. The metallic forms of Hg and cyanide (CN) are used to obtained gold. One factory consisted of 13 mill balls (trommel), a cylindrical-shape equipment to use Hg. The number of trommel and CN ponds in the area was about 1994 and 58 units, respectively (Lingkubi 2004). Nowadays, both techniques are used in combination in order to recover the gold effectively in the area indicated by rectangle line (Figure 1).

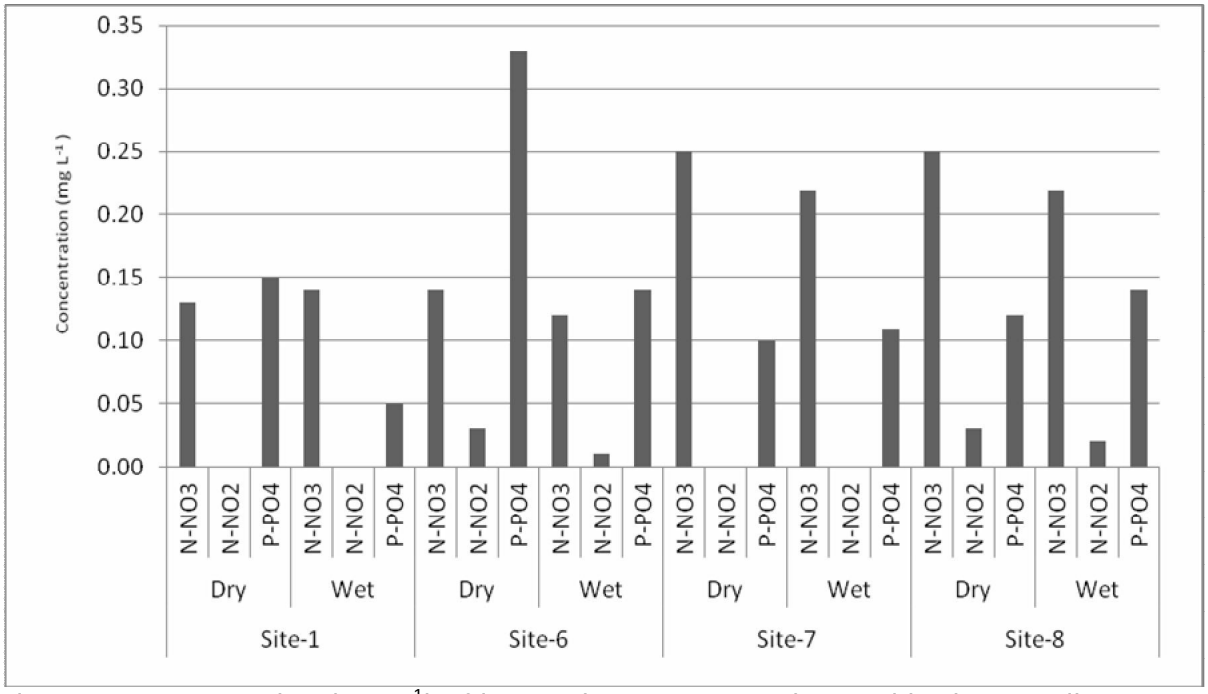

Figure 4. Concentration (mg L^{-1}) of inorganic parameters observed in the sampling areas.

Table 4

Concentration of organic matters during the sampling period at several selected sites of the Talawaan watershed

Parameters (mg L^{-1})	*Dry season*				*Wet season*			
	Site 1	*Site 6*	*Site 7*	*Site 8*	*Site 1*	*Site 6*	*Site 7*	*Site 8*
BOD_5	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
COD	< 10	< 10	< 10	< 10	< 10	12	42	51
$N\text{-}NH_3$	< 0.02	0.03	< 0.02	0.89	0.04	0.05	< 0.02	0.58
DO	7	7	8	7	7.54	7.12	7.54	7.24

BOD_5 = biological oxygen demand; COD = chemical oxygen demand; $N\text{-}NH_3$ = ammonia; DO = dissolved oxygen.

By overlapping the study area of Lasut & Yasuda (2010) and the present study, one can see that the concentrations of total Hg inside the area in site 3, site 4, site 5, and site 6 were 955 ppb, 115 ppb, 171 ppb, and 2357 ppb, respectively. Outside the area in site 7 and site 8 the total Hg concentrations were 97 ppb and 361 ppb, respectively. The concentration of Hg inside the mining area was far higher than outside.

The presence of bacteria and parasites is caused by the polluted water environment. The results reported that pollution in coastal and marine areas has caused the deaths of many species, threatening human health and potentially undermining the function of ecosystems permanently (Austin & Austin 1993). The Hg is found in river water, in this research was not in high concentrations due to the water flow, but the effect of this heavy metal when accumulated is more toxic and thus dangerous than other

metals such as lead (Pb) and cadmium (Cd) (McLusky & Elliot 2004). Hg is dangerous because it is easily absorbed in the body of the fish and damages the cells (Tschakert 2010). As shown in Table 5 mercury found in cultured and wild fish in TW with varies concentration. Tiger bass fish (*Terapon jarbua*) is the highest concentration that found in downstream of Talawaan river. Perhaps the most significant impact of a mining project even in small scale, is its effects on water quality and availability of water resources within the watershed (Gibb & O'Leary 2014). All things that are not good happen in the upstream, will also affect downstream or coastal areas.

Table 5

Mercury accumulation in fish in the Talawaan watershed

Sites	*Species*	*Source*	*Average body length (cm)*	*Average body weight (gr)*	*Average Hg concentration (mg kg^{-1})*
Site 3	*C. carpio*	Cultured fish	10.5	325	0.009
Site 4	*C. carpio*	Cultured fish	11.0	325	0.028
Site 5	*C. carpio*	Cultured fish	11.8	330	0.005
Site 7	*T. jarbua*	Wild fish	10.0	320	0.175
Site 8	*C. striata*	Wild fish	10.7	322	0.029
	L. calcarifer	Wild fish	12.3	345	0.026

Conclusions. We found that the occurrence of fish diseases due to parasites and bacteria occurred in the AGM area and the control area in the TW, as well as in the control area outside the TW; however, they varied according to fish species and sites. Most of parasites and bacteria occurred in freshwater sites. The parasites *Trichodina* sp. and *Dactylogyrus* sp., and the bacteria *P. shigelloides* and *Aeromonas* sp. were the common species in wild fish in freshwater condition. While the parasites *Trichodina* sp. and *Dactylogyrus* sp., and the bacteria *Moraxella* sp., *A. hydrophila*, and *P. shigelloides* were the most common in cultured fish bred in freshwater.

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