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Impact of Mud Volcano Lava to the Aquatic Life Using the Fish Biological Study Case in Lusi Mud Volcano Indonesia

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ABSTRACT

Investigation of the effect of flowing mud volcano lava to the aquatic life in the river using study case of LUSI mud in Indonesia had been conducted by *in-situ* biomonitoring including measurement of biological response of caged fish and river body quality. Fishes were caged in the downstream sections of the mud effluent and control cage was placed at the upstream section. Similar hierarchy of metal found in the effluent, water and sediment i.e., Al>Fe>Pb>Mn>Cu>Zn>Cr>Cd. Concentrations of Total Suspended Solid (TSS), total aluminum, total iron and lead at the downstream effluent site which showed maximum values of ([TSS = 4177] [Al = 23.70] [Fe = 6.97] [Pb = 1.27] mg L^{-1}) were significantly higher than that of the upstream control ([TSS = 381] [Al = 4.30] [Fe = 1.38] [Pb = 0.38] mg L^{-1}). Excluding lead in the downstream sites (153 mg kg^{-1}), all sediment levels were found to be below the possible effect level. Metal levels in fish bodies from the downstream sites showed higher level than control, with maximum level observed in aluminum species, 377.9 mg kg^{-1}. Fishes which were exposed in the downstream sampling sites showed low survival rate value of 10-0% with survival period of = 21 days, gill alteration in a level of irreparable lesion with Histopathologic Alteration Index (HAI) value of 121-233 while the fishes in the control cage had a survival rate of 93-66% for 28 days and normal state of gills with HAI value of 0.0-0.3. Mainly by increasing colloidal aluminum LUSI mud volcano lava into the river results in adverse effect on the downstream water quality and fish life.

Key words: LUSI, mud volcano, *in situ* biomonitoring, gill histological alteration, fish biology

INTRODUCTION

LUSI mud volcano is a term referring to the hot mud that is being emitted from a mud volcano at an average rate 90,000 m^3/day in Sidoarjo, East Java, Indonesia since 29 May 2006. The cause of the eruption may be linked to gas exploration activities (UNDAC, 2006; Istadi *et al*., 2009). Since 2007, the mud flow was channeled into the Porong River to prevent problems from occurring at the village nearby. As a consequence, the degradation of physico-chemical characters as predicted would lead to biological system disturbance in Porong River. An urgent evaluation of the Porong aquatic ecosystem was necessary due to the role of Porong River as the most important hydrological system in the Sidoarjo regency. The total stream chemical potential energy contributes about 29 million US dollars per year to the regency (Karr *et al*., 2008).

Mud has been flowing directly into Porong River for almost six years and there has never been any investigation done on the effect of the mud on organism *in situ*. A biomonitoring approach could reflect on the impact of fauna habitating in the river ecosystem. *In situ* biomonitoring has great potential for linking biomarker data with community and ecosystem level responses (Barbee *et al*., 2008). Due to the possibility to bioaccumulation, the heavy metal compounds should be mandatory in the monitoring. While the acute effect could be analyzed by survival rate, the chronic effects of contaminants are associated with sub lethal concentrations of the pollutants which lead to histological alterations.

Histopathological biomarkers are a reflection of the overall health of the entire population in an ecosystem. Histopathological techniques have the advantage of allowing investigators to examine specific target organs and cells as they are affected by exposure to environmental chemicals. Additionally, histopathology provides a mean to detect both acute and chronic adverse effects of exposure in the tissues and organs of individual organisms (USEPA, 1987; Schlenk *et al*., 2008). Fish gills are the first target of water-borne pollutants due to their direct contact with the surrounding water (Patel and Bahadur, 2010). A previous laboratory study of the Sidoarjo mud effect on milkfish (*Chanos chanos*) indicated that liver histology alterations had taken place (Hidayati, 2010).

The current research could provide an early warning of the risk of the uncontrolled LUSI mud release so that steps could be taken in order to minimize the impact on the aquatic populations, as well as subsequent economic and environmental problems. The aim of the study was to investigate the biological response of caged Mozambique tilapia (*Oreochromis mossambicus*) with reference to survival, gill histopathology and metal content in their Whole Body Composites (WBC) due to the flow of mud volcano effect in the river body.

MATERIALS AND METHODS

Map in Fig. 1 illustrates that the LUSI mud volcano is located in a semi urban district of Sidoarjo, East Java, Indonesia. The construction of a large pond with embankments in the LUSI mud eruption area as well as the channeling of the excess watery mud to the adjacent river are recent efforts to minimize the adverse impact of the mud flow on human infrastructures. In this study, the discharge of watery mud is assumed to be effluent that had been directly spilling into the Porong River (±0.5 km from LUSI mud area, ±12 km from the mouth river) since 2007. Porong River itself had modified to be a flood control canal and for agricultural water supply (Ramu, 2004).

Water and sediment samples were collected from January 2011 to February 2012 and they were done during the five surveys conducted and represented both dry and wet seasons. Three sampling sites were located downstream of the discharge area, where caged fishes were exposed to the mud effluent (Fig. 1). The sampling sites namely P1 (7°32'41.8"S; 112°42'31.67"E) which represented the area of initial discharge of the effluent, P2 (7°32' 42.91"S; 112°43' 49.53"E) as an intermediate site and P3 that represented a site far from the effluent (4 km) with a coordination of 7°32' 36.57"S; 112°44' 35.06"E. A control area located at an upstream location, about 6 km above the discharge area did not receive any LUSI mud.

The experimental fish, namely the Mozambique tilapia, *Oreochromis mossambicus* was selected because it was considered relatively tolerant to a wide range of levels of water temperature, Dissolved Oxygen (DO), salinity, pH, light intensity and photoperiod. Tilapia can persist in a highly polluted habitat and have potential for biological monitoring of environmental pollution (Balcazar *et al*., 2004; Ueng *et al*., 1996). Hence, it was expected to survive in the polluted water

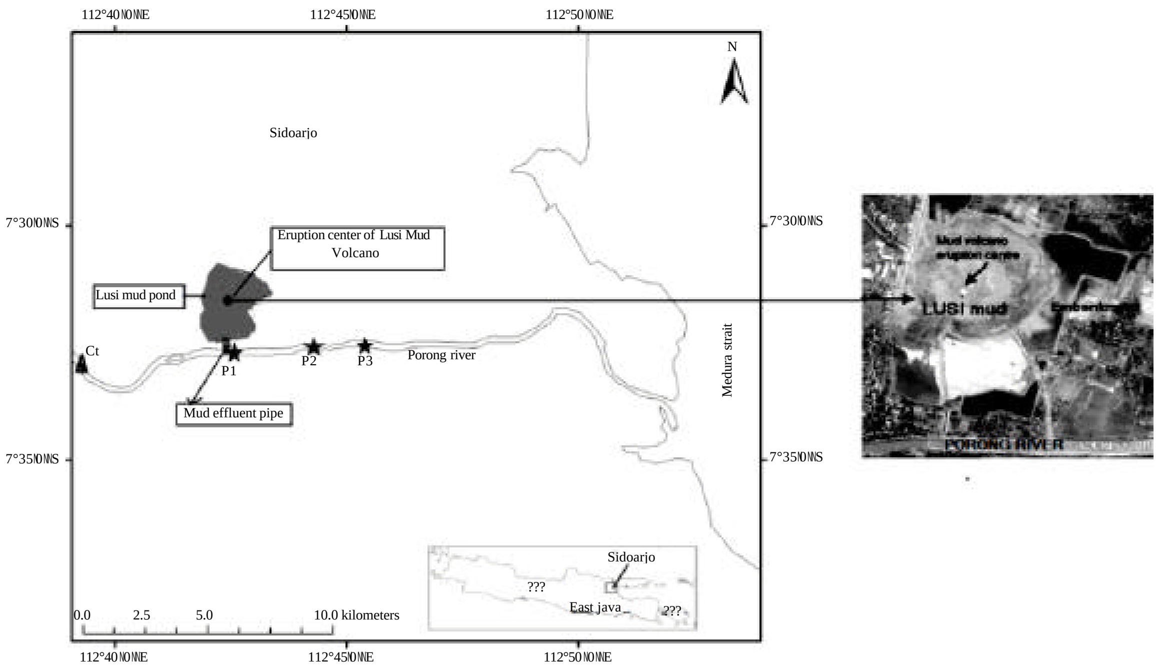

Fig. 1: Map of sampling sites. Triangle: Control sampling site Ct; Stars: Sampling sites P1, P2 and P3 (satellite image of LUSI mud volcano was modified from (CRISP, 2010)

for the particular treatment time of exposure as was needed in the study. The juvenile fish with body standard length of 5±0.4 cm and weight of 4.5±0.9 g were used in the study and they were obtained from commercial aquaculture ponds.

Rectangular-shaped cages were made from materials that did not contain any metal elements i.e., the nets, plastic strings and frame stabilizers are made from plastic pipes (Length×width×height = 40×30×30 cm). A preliminary study revealed that the fish cages were properly and safely immersed during the dry season. A duplicate fish cage with 40 fishes each was immersed at each sampling site and anchored to the river bed with 10 m plastic string coiled around concrete blocks. Daily fish mortality was observed and recorded as Survival Rate (SR) and prior to the analysis of trace metal and histology. The fish samples were stored in a 4°C cool box. An extra fish cage was provided at each sampling site as fish stock that will provide the needed properly sized fish samples for the trace metal analysis. The tissue sample of each individual fish was obtained by grinded the entire body, including the head, skin, gill, muscle and bones. The trace metal analysis was conducted using the whole body composite where the body tissue samples from several fishes are combined, thoroughly homogenized and treated as a single sample (USEPA, 2000; USEPA, 2009b).

Water samples were collected prior to collection of sediment samples at the fish cage sampling sites. Measurements of pH, Dissolved Oxygen (DO) were carried out on site using the water quality meter TROLL® 9500 while Total Suspended Solids (TSS) were analyzed in the laboratory using gravimetric method according to US EPA Method 160.2 (USEPA, 1971).

Water samples for metal analysis were filtered using 0.45 μm membrane filter and then preserved in HNO_3, with the pH adjusted to 2. The sediment samples were taken using the AMS

5 lb. sand/silt dredge and placed into polyethylene containers. Samples of sediment and fish from treatment cages were stored immediately at 4°C prior to metal analysis. Furthermore, acid digestion of water samples, bulk of sediment and WBC of caged fishes were prepared prior to metal analysis which include Al, Cu, Pb, Mn, Cr, Fe, Zn and Cd using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) following the EPA method 200.7 (USEPA, 1994). Moreover, sediment composition was also classified referring to particle size according to Wentworth Grade Scale. Particle size is determined by passing a sample of sediment through a series of sieves from 1.25 mm to 63 μm. The cumulative percentage of materials retained on the sieves is calculated (UNEP/WHO, 1996).

Referring to the survived fish samples, about three to six replications of gill samples that were fixed using 10% buffered formalin were dehydrated using a progressive series of ethanol dilutions and then embedded in paraffin (xylene was used for intermediate impregnation). This was followed by sectioning (2-3 μm thick) using a rotary microtome, followed by staining with Haematoxylin and Eosin (Lillie, 1965). The histological images were obtained using a Microscope (Olympus BX 41) that was connected to an Evolution LC Camera.

The gill histological alteration data were analyzed using a semi-quantitative method, namely the Histopathologic Alteration Index (HAI), adopted from Flores-Lopes and Thomaz (2011) with modifications. The pattern of alterations and severity of stages were observed to determine the value of HAI as follows: Stage (1) epithelial hypertrophy, hyperplasia and lifting of gill epithelium, disorganization, fusion and shortening of secondary gill lamellae, stage (2) hemorrhaging and rupture of lamellar epithelium, hypertrophy and hyperplasia of chloride cells, stage (3) telangiectasis, cell degeneration and necrosis. The HAI value was calculated for each observed stage using the formula:

$$HAI = (1X\ S1)+(10X\ S2)+(100X\ S3)$$

where, S represents the sum of the number of alterations at each particular stage. Then the HAI value was interpreted as having organ function status which was determined according to Poleksic and Mitrovic-Tutundzic (1994) and Flores-Lopes and Thomaz (2011). HAI values from 0 to 10 indicate normal gills; 11-20: slight damage; 21-50: moderate changes; 50-100: severe lesions and values above 100 indicate irreparable lesions (Poleksic and Mitrovic-Tutundzic, 1994; Flores-Lopes and Thomaz, 2011).

RESULTS

Reports of previous geochemical studies of bulk mud from LUSI pond by United Nation Disaster Assessment and Coordination (UNDAC, 2006) and United States Geological Survey (USGS, 2008) as well as results of current analysis of effluent composition which is shown in Fig. 2, indicated that aluminum and iron were found to be the major metals both in water and mud portion. Based on USGS (2008) survey, the mean level of metal composition (unit mg kg^{-1}) in the mud pond showed the following hierarchical order i.e., [Al = 83550]>[Fe = 49000]>[Mn = 924]>[Zn = 95.3]>[Cr = 90.6]>[Cu = 24.7]>[Pb = 18.7]>[Cd = 0.09] was greater than that measured in mud of the effluent, excluding lead.

Figure 2, the hierarchical of metal concentrations (mg L^{-1}) in the water effluent demonstrated the following order Al (8.1)>Pb (6.95)>Fe (2.76)>Cu (0.64)>Mn (0.35)>Zn (0.09)>Cd (0.02)>Cr (not detected) for the wet season 2011 while in dry season 2011 observed that Al (7.8).>Pb

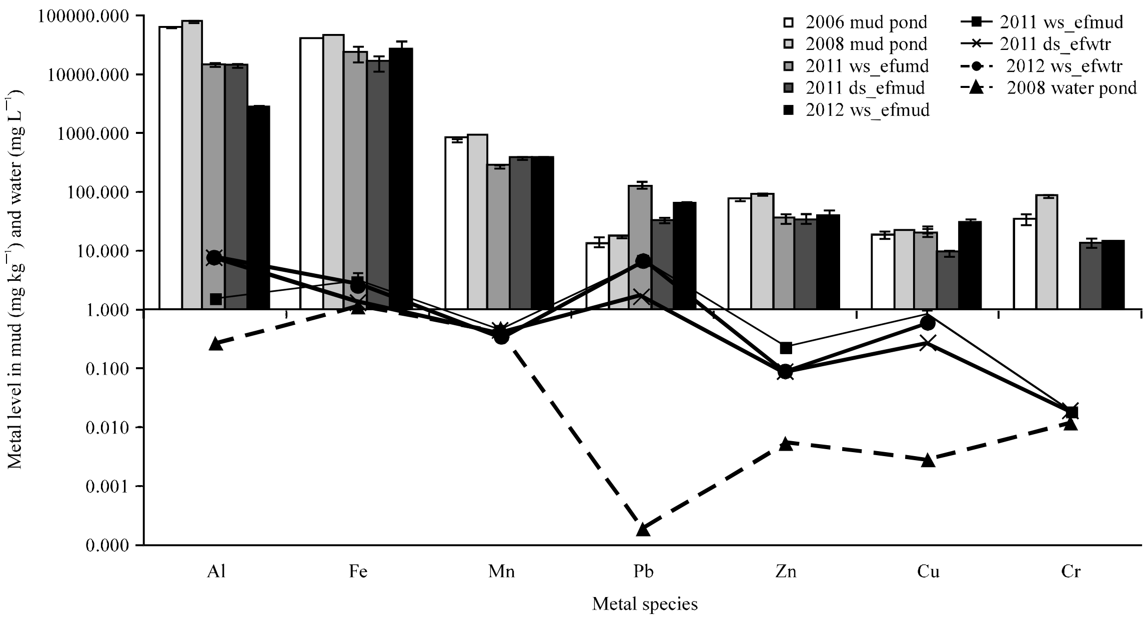

Fig. 2: Comparison of chemical composition of LUSI mud in the collecting pond and as effluent. The metal composition of mud and water from LUSI mud pond was adopted from UNDAC (2006) and USGS (2008). The seasonal chemical composition of mud constituents in the effluent is coded as ws_efmud and ds_efmud and while the separated water is termed ws_efwtr and ds_efwtr. The label of 2006, 2008, 2011 and 2012 indicate the year of the surveys. ds: Dry season; ws: Wet season

Table 1: Water physico-chemical quality of sampling sites. Unit of TSS and DO = mg L^{-1}.

Sampling time Season/ month_year	Control			Site P1			Site P2			Site P3		
	TSS	DO	pH	TSS	DO	pH	TSS	DO	pH	TSS	DO	pH
Wet/Jan_11	122±25	5.0±0.60	7.0±0.1	943±175	3.4±0.10	8.2±0.03	422±140	3.3±0.1	8.4±0.0	493±165	4.3±0.2	8.2±0.1
Dry/June_11	132±63	4.7±0.00	7.5±0.5	4177±1233	3.5±0.01	7.0±0.03	1147±328	4.8±0.0	6.0±0.0	927±82	5.2±0.2	7.0±0.0
Dry/July_11	381±58	4.4±0.00	7.5±0.5	1163±169	3.9±0.03	7.0±0.03	459±105	4.0±0.2	6.0±0.0	709±189	3.0±0.0	7.0±0.0
Wet/Jan_12	122±48	7.0±0.30	7.0±0.0	672±116	6.8±0.10	7.3±0.26	1241±60	7.1±0.0	7.1±0.1	1013±179	7.0±0.1	7.0±0.1
Wet/Feb_12	40±12	6.8±0.05	6.9±0.2	676±81	6.7±0.10	6.8±0.10	582±95	6.1±0.6	7.3±0.1	752±5	6.1±0.6	7.0±0.0

(1.73)>Fe (1.34)>Mn (0.48)>Cu (0.29)>Zn (0.09)>Cr (0.02)>Cd (not detected). Furthermore, in wet season 2012 showed trend that Pb (6.95)>Fe (3.43)>Al (1.56)>Cu (0.91)>Mn (0.48)>Zn (0.24)>Cr (0.02)>Cd (not detected).

Table 1, showed that the water samples exhibited average temperatures of 28-30°C and Dissolved Oxygen (DO) in the level of 3.3-7.1 mg mL^{-1}. Almost all observed pH levels in all the sampling sites were in the safe level according to National Recommended Water Quality Criteria (USEPA, 2009a) that stipulated a range of 6.5-9.0. Meanwhile, the pH level in dry season at site P2 was observed to be acidic (pH = 6.0) which is out of the range. Water salinity at the control and downstream sites i.e P1 and P2 were in values of (0.0‰) while the site at P3 (that is closer to the river mouth) was in a range level of 0.0-1.0‰. The highest TSS value was 4176.8 mg L^{-1} found during the dry season at site P1 which is nearest to the LUSI mud effluent and the lowest was found in the control site during the wet season in the level of 40 mg L^{-1}.

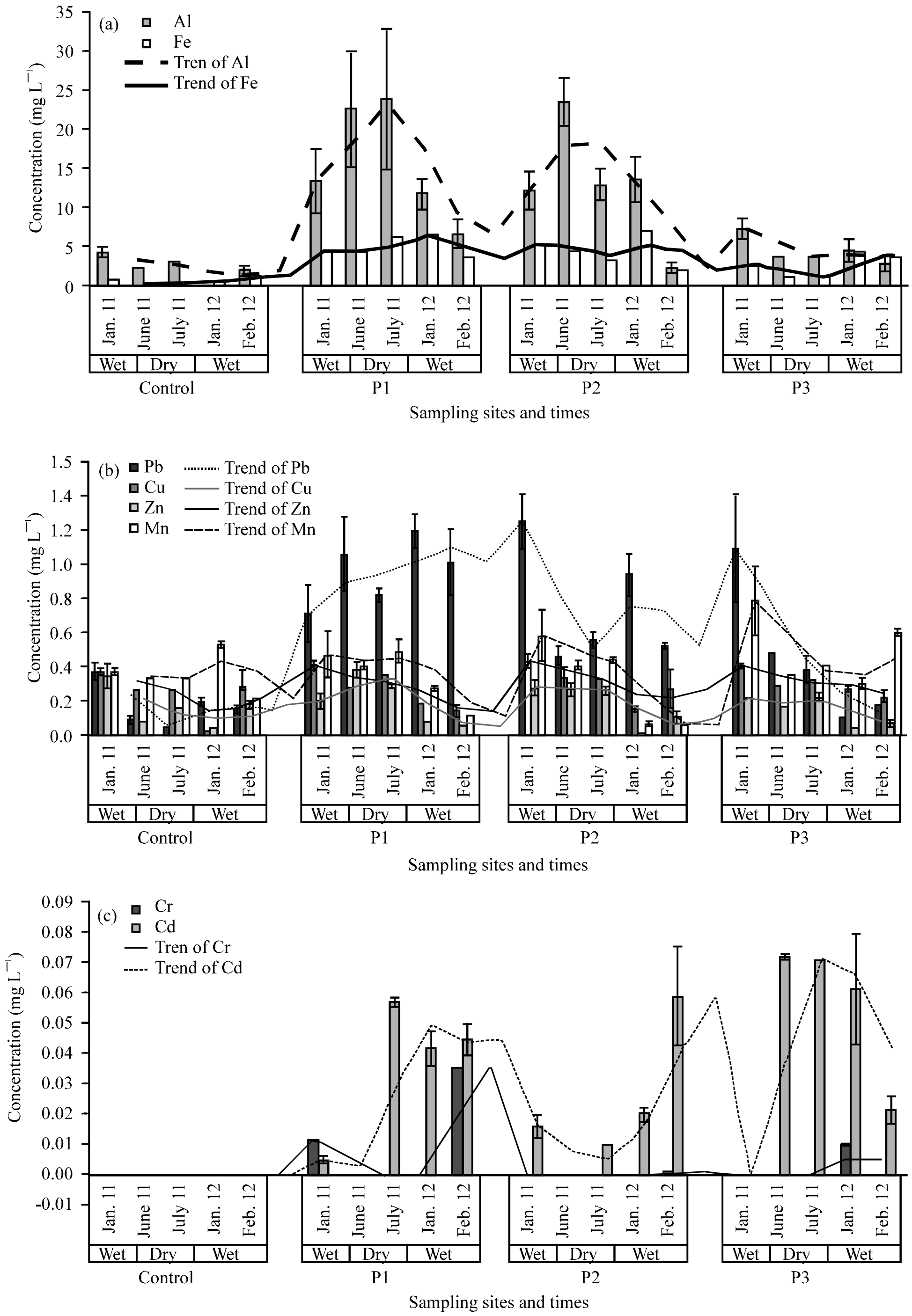

Fig. 3(a-c): Trend of metal concentration in water major metal, (a) Aluminum and iron, (b) Lead copper zinc and Manganese and (c) Chromium and Cadmium

Figure 3 exhibits the spatial and seasonal metal concentration in water of Porong River. In general, the trend of metal composition that was found in the water of Porong downstream sites (P1;P2 and P3) was similar to the composition found in the effluent with the hierarchical order i.e., Al >Fe>Pb>Mn>Zn or Cu>Cr>Cd.

The trend of aluminum indicated the seasonal fluctuation, primarily at locations that are near to the effluent i.e., P1 and P2. Maximum concentration of aluminum was found in the dry season i.e., 23.70 mg L^{-1} at P1 and 23.41 mg L^{-1} at P2 which was significantly greater ($p<0.05$) than in the wet season which exhibited range levels of 12.09-13.30 mg L^{-1} in year 2011 and 6.61-13.53 mg L^{-1} in year 2012. Moreover, aluminum concentrations at sites P1 and P2 in the dry season demonstrated either spatial or temporal differences with that observed at site P3 which represented the downstream section far from the effluent (3.68-3.70 mg L^{-1}). They also showed differences with the control site in upstream where aluminum concentration ranged from 0.37-3.40 mg L^{-1}. Meanwhile, there were no significant differences between aluminum concentration in the control and P3 sites.

Unlike with aluminum behavior, the maximum iron concentration ([P1 = 6.97 mg L^{-1}]; [P2 = 6.60 mg L^{-1}]) was observed in the wet season. There were statistically temporal differences of iron concentration but not for seasonal pattern. Spatially, iron concentration at the control site (0.29-1.38 mg L^{-1}) was significantly lower than iron concentrations in the downstream sites of P1 (3.56-6.60 mg L^{-1}) and P2 (1.97-6.97 mg L^{-1}). Moreover, the differences in iron concentrations between control and P3 sites were only observed in the wet season of 2012 (4.38 mg L^{-1}).

By comparing with the water quality criteria that is presented in Table 2, the average aluminum (as total aluminum) and iron (as total iron) concentrations from all the surveys done were at sites P1(15.55 mg L^{-1}; 4.99 mg L^{-1}) and P2 ([12.83 mg L^{-1} and 4.40 mg L^{-1}]) which were much higher than the standard for total aluminum that is stipulated in the Guidelines for Interpreting Water Quality Data, Province of British Columbia (Anonymous, 1998) where the recommended level for total aluminum is 5 mg L^{-1} while that for total iron is 1.7 mg L^{-1} (Randall *et al.*, 1999). The average concentration of aluminum in site P3 (4.39 mg L^{-1}) was below the standard but the iron concentration was in a level above the limit (2.57 mg L^{-1}). Meanwhile, the mean concentrations of aluminum and iron in the control site (2.43 mg L^{-1}; 0.68 mg of Fe L^{-1}) were in the safe limit.

Similar to the pattern of the water effluent, the range of lead concentration in the water of the downstream site of Porong River was found to be in a higher level than manganese concentration (Fig. 2, 3 and Table 2). The highest lead concentration (P1 = 1.21 mg L^{-1}; P2 = 1.27 mg L^{-1}) was demonstrated in the wet season. Significant seasonal differences were observed in P2 and P3

Table 2: Comparison of the observed metal concentration with water quality standard (WQC)

		Range concentration of observed metal between Jan 2011-Feb 2012				USEPA WQC for freshwater aquatic life		Indonesian government	WQC for wild
Metal	MDL	P1	P2	P3	Control	Acute	Chronic	Reg. for class III	life and livestock
Al (total)	0.0250	6.61-23.70	2.31-23.41	2.86-7.24	0.37-4.30	NA	NA	NA	5*
Fe (total)	0.0100	3.56-6.60	1.97-6.97	1.09-4.38	0.29-1.38	NA	NA	NA	1.7**
Mn	0.0010	0.11-0.50	0.06-0.59	0.31-0.80	0.21-0.54	0.50	0.10	0.10	NA
Zn	0.0050	0.06-0.39	0.01-0.28	0.04-0.24	0.03-0.35	0.12	0.12	0.05	NA
Cu	0.0050	0.14-0.42	0.16-0.44	0.23-0.41	0.028-0.38	NA	NA	0.02	0.3*
Pb	0.0050	0.72-1.21	0.47-1.27	0.10-1.11	0.051-0.38	0.065	0.0025	0.03	NA
Cr III	0.0050	ND-0.035	ND-0.001	ND-0.011	ND	0.57	0.07	NA	NA
Cd	0.0050	ND-0.057	ND-0.059	ND-0.072	ND	0.002	0.00025	0.01	0.08*

ND: Not detected, NA: No available data

with the trend that the wet season is higher than the dry season. Like the spatial trends of aluminum and iron, the lead concentration at the control site (0.051-0.38 mg L^{-1}) is significantly lower than that found at sites along downstream P1 and P2. The lead concentrations in all sampling sites were above the standard reference, 0.07 mg L^{-1} (USEPA, 2009a).

The peak of zinc concentration observed in the dry season at site P1 was detected to range from 0.29-0.39 mg L^{-1} and for site P2 (0.27 mg L^{-1}) which exceeded the standard reference (0.12 mg L^{-1}). These concentrations were significantly greater than that measured in the control site (0.08-17 mg L^{-1}). Whereas during the wet season, it was observed that the similarity of zinc concentration between control and the downstream sites were due to the declining zinc concentration.

The downstream sites exhibited a range value of manganese concentration ([P1 = 0.21-0.50; P2 = 0.07-0.59; P3 = 0.31-0.80] mg L^{-1}]) that is generally not different with the control (0.21-0.54 mg L^{-1}). Mean of manganese concentration of samples from sites P2 and P3 in the wet season were detected to be above the stipulated standard according to USEPA (2009a) i.e., 0.5 mg L^{-1}. The copper species concentration in water seems to have similar behavior with manganese which did not exhibit spatial and seasonal differences. The copper concentrations were in the range of P1 (0.14-0. 42 mg L^{-1}); P2 (0.16-0.44 mg L^{-1}); P3 (0.23-0.41 mg L^{-1}) and control (0.03-0.38 mg L^{-1}) with the highest detected in site P2 during the wet season of 2011. According to the Indonesian government regulations (Anonymous, 2001), copper concentration was in a level above the standard quality which stipulated a level of only 0.02 mg L^{-1}.

Chromium was only detected at the downstream sites in the wet season i.e., ([P1 = 0.035; P2 = 0.001; P3 = 0.011] mg L^{-1}); with those at P1 and P3 found to be below the safe level for Cr III (USEPA, 2009a). Similar to chromium, cadmium was only detected in the downstream sites i.e., P1 (0.005-0.057 mg L^{-1}), P2 (0.016-0.059 mg L^{-1}), P3 = (Not detected-0.072 mg L^{-1}) while it was not detected in the control site. In general, cadmium concentrations that were detected at the downstream sites exceeded the WQC according to USEPA (2009a) that stipulated a level of only 0.002 mg L^{-1}. However, they were in a safe level to wildlife and livestock water supply (0.08 mg L^{-1}).

Results of sediment composition analysis (Fig. 4) exhibited that the site nearest to the effluent pipe i.e., P1, contained the highest mud portion (77.1%) while levels at the other sites were below 12%. The rest of the sediment composition at site P1 consisted of 22.9% very fine sand.

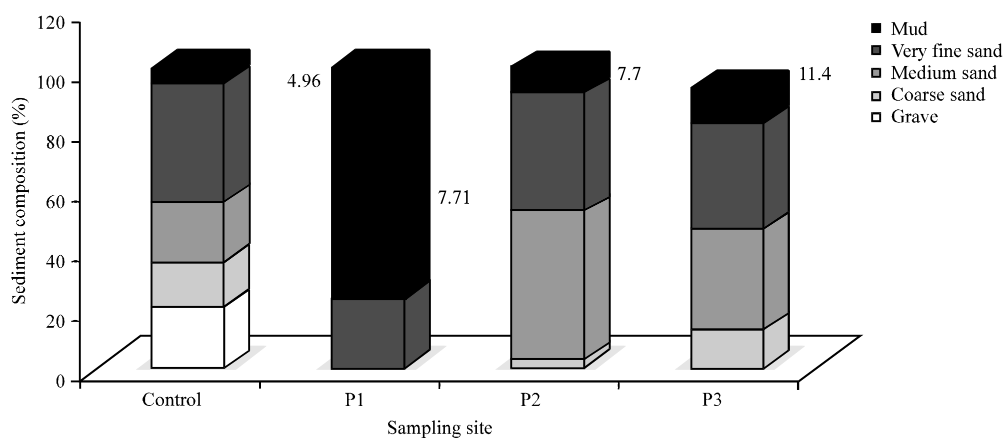

Fig. 4: Sediment composition in Porong River. A number in each bar represents the mud portion

Table 3: Summary of metal levels in sediments at the sampling sites of Porong River

Site and time		Al ($g\ kg^{-1}$)		Fe ($g\ kg^{-1}$)		Mn ($g\ kg^{-1}$)		Pb ($mg\ kg^{-1}$)		Cu ($mg\ kg^{-1}$)		Zn ($mg\ kg^{-1}$)		Cr ($mg\ kg^{-1}$)		Cd ($mg\ kg^{-1}$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control																	
Wet	s1	28.93	1.87	18.12	1.66	0.70	0.20	88.16	3.99	23.05	3.78	37.82	1.56	ND	ND	0.077	0.003
Dry	s2	26.49	1.50	19.34	5.39	0.47	0.13	85.00	13.23	30.60	1.22	33.43	0.21	ND	ND	ND	ND
Dry	s3	25.92	3.02	18.80	1.69	0.54	0.06	90.00	10.00	29.59	0.72	31.33	1.15	ND	ND	ND	ND
Wet	s4	14.94	0.19	17.51	0.85	0.55	0.02	47.57	13.22	40.72	3.11	25.05	2.78	0.13	0.03	ND	ND
Wet	s5	13.51	0.65	15.17	0.36	0.68	0.03	28.52	39.09	41.56	3.50	22.88	0.78	ND	ND	ND	ND
P1																	
Wet	s1	36.89	2.75	17.64	2.45	0.74	0.06	101.0	10.63	27.09	1.79	37.77	2.25	7.67	0.74	0.203	0.049
Dry	s2	10.20	0.08	17.72	0.32	0.50	0.01	119.9	3.70	13.32	2.36	52.11	0.25	0.45	0.05	ND	ND
Dry	s3	17.46	0.01	21.08	2.33	0.57	0.00	153.4	2.05	32.51	3.01	61.55	0.65	ND	ND	ND	ND
Wet	s4	10.02	1.41	18.94	3.30	0.61	0.02	91.34	18.90	93.60	17.2	37.59	2.01	2.33	0.31	ND	ND
Wet	s5	12.81	1.39	18.78	2.86	0.46	0.09	88.60	2.47	58.32	3.07	46.57	0.98	10.90	1.46	ND	ND
P2																	
Wet	s1	26.82	6.69	24.19	1.89	0.60	0.05	100.7	30.07	19.08	4.03	49.01	0.52	16.34	2.65	0.310	0.080
Dry	s2	14.40	2.73	18.94	2.68	0.48	0.03	107.3	5.75	13.59	2.34	44.38	0.25	10.94	0.05	ND	ND
Dry	s3	13.14	0.03	17.05	0.79	0.47	0.01	108.3	5.51	23.88	3.31	43.22	1.07	ND	ND	ND	ND
Wet	s4	11.29	2.58	17.32	1.57	1.07	0.15	56.27	4.48	50.68	11.99	38.38	2.74	36.09	3.69	ND	ND
Wet	s5	10.77	0.15	18.56	0.47	0.45	0.01	91.45	17.50	70.27	12.99	42.37	3.73	6.61	0.64	ND	ND
P3																	
Wet	s1	32.07	0.57	22.72	4.37	0.62	0.02	56.29	9.93	19.57	0.44	49.49	0.47	19.17	4.23	0.413	0.075
Dry	s2	23.24	3.07	22.56	4.61	0.58	0.06	125.2	7.89	21.92	0.15	39.29	1.96	ND	ND	ND	ND
Dry	s3	34.63	0.15	22.35	0.98	0.63	0.01	132.4	1.52	31.91	0.59	38.79	4.32	ND	ND	ND	ND
Wet	s4	11.36	1.15	20.01	2.56	0.44	0.03	44.92	12.96	37.72	3.05	33.08	6.83	28.26	8.46	ND	ND
Wet	s5	14.83	0.39	17.43	1.32	0.50	0.01	37.78	2.83	73.12	15.7	33.92	2.49	0.02	ND	ND	ND
PELs*		NA		NA		NA		112		108		271		160		4.2	
Soil**		69.68		45.10		0.77		10.9		37.0		70		11.00		0.08	
Clay**		NA		47.00		0.85		25.00		42.00		100		NA		6.1	

Code of sampling times, s1: January 2011; s2: June 2011; s3: July 2011; s4: January 2012; s5: February = 2012*) PELs (possible effect level) according Canadian Sediment Quality Guidelines (CSQG, 2002);**) major elements that are found in reference to soil and clay standards (UNDAC, 2006; Suprapto *et al*., 2007). ND: Not detected, NA: No available data, sd: Standard deviation. Bold font means higher than the standard reference

The metal levels in Porong River sediments that are listed in Table 3 exhibit a general trend in the following order i.e. Al>Fe>Mn>Pb>Cu>Zn>Cr>Cd. Statistically, there were no spatial and seasonal differences of iron level between the sediment samples. Overall, the major metal species i.e., aluminum, iron and manganese in all sediment samples did not exceed the level that was found in reference to soil and clay (UNDAC, 2006).

Moreover, the most observed metals in the sediments were in levels that are lower than the Possible Effect Level (PELs) according to Canadian Sediment Quality Guidelines for aquatic life (CSQG, 2002), excluding the lead level in downstream sites during the dry season i.e., P1 (119-153 $mg\ kg^{-1}$) and P3 (125-132 $mg\ kg^{-1}$) which were found to exceed the PELs of lead (112 $mg\ kg^{-1}$).

Table 4 gives that the hierarchical aluminum in WBC of caged fishes was in the following order i.e., P2 (377.92 $mg\ kg^{-1}$)>P1 (69.6 $mg\ kg^{-1}$)>P3 (63.52 $mg\ kg^{-1}$)>control (36.70 mg Al kg^{-1}). The

Table 4: Metal level in fish whole body composite (WBC)

Sampling Sites	Metal level at 7 days of the whole body composites of caged fish (mg kg^{-1} dry weight body)							
	Al	Fe	Mn	Pb	Cu	Zn	Cr	Cd
Control	36.70±0.57	26.42±2.72	15.18±0.8	ND	9.70±0.47	13.98±6.4	<MDL	<MDL
P1	69.55±26.01	78.46±21.0	44.23±8.2	2.09±0.76	27.75±0.3	30.80±8.4	<MDL	<MDL
P2	377.92±30.1	96.12±6.61	54.03±0.3	6.24±0.46	30.13±0.6	28.19±2.2	<MDL	<MDL
P3	63.52±19.22	29.93±0.44	36.85±0.6	1.42±0.01	30.17±0.1	11.93±1.9	5.30±0.10	0.62±0.01
MTL in feed of fish	NA	NA	NA	10	100	250	NA	10
MTL in feed of rodent	200	500	2000	10	500	500	100	10

MTL: Maximum tolerable level in animal feed according the NRC, 2005; MDL: Method detection limit ND: Not detected; NA: No available data

aluminum level in WBC of caged fishes at site P2 exceeded the Maximum Tolerable Level (MTL) in animal feed (rodent and fish) according to the National Research Council (NRC, 2005) that stipulated a level of 200 mg kg^{-1} and was also significantly higher ($p<0.05$) than sites P1, P3 and control. For comparison, a whole body channel catfish sample collected from the Guadalupe River in Texas and the Gila River in Arizona contained 56.1 mg Al kg^{-1} wet weight and 67 mg kg^{-1} wet weight respectively (Lee and Schultz, 1994; Baker and King, 1994). Similar to aluminum, the trend of iron and lead in caged fish WBC was in the following sequences i.e. P2 (96.12 mg kg^{-1})>P1 (78.46 mg kg^{-1})>P3 (29.93 mg kg^{-1})>control (26.4 mg kg^{-1}) while the trend of lead was P2 (6.24 mg kg^{-1})>P1 (2.09 mg kg^{-1})>P3 (1.42 mg kg^{-1})>control (not detected). The iron and lead levels in WBC of caged fishes in all sampling sites were below the Maximum Tolerable Level (MTL) in animal feed according to the (NRC, 2005) that stipulated a level of 500 and 10 mg kg^{-1}, respectively. In all the sampling sites, manganese level in WBC of caged fishes were detected in a range of 15.18 mg kg^{-1}-54.03 mg Mn kg^{-1} which is within the safe limit (>2000 mg kg^{-1}) according MLT in animal feed (NRC, 2005).

The level of copper in WBC of caged fishes from all the sampling sites was in a range of 9.70-30.17 mg kg^{-1} which was below the MLT in animal feed for copper that stipulated a level of 100 mg kg^{-1} for fish and 500 mg kg^{-1} for rodent. Zinc level in caged fish WBC from the control (upstream) site (13.98 mg kg^{-1}) did not show statistically any difference with zinc level that are observed in the upstream sites (range of average 11.93-30.80 mg kg^{-1}). As observed with other essential metals, zinc level were found to be below the MLT in fish feed (250 mg kg^{-1}). Meanwhile, chromium and cadmium that are detected in WBC of caged fishes at site P3 (5.30, 0.62 mg kg^{-1}) were also found to be below the MTL for animal feed (100, 10 mg kg^{-1}) (NRC, 2005).

The normal gills were observed in caged fishes from the control site. The secondary lamellae are covered by a thin epithelial (Fig. 5) which allows for short diffusion distances and promotes efficient exchange of oxygen and soluble metabolic wastes. Chloride cells that provide the primary means for maintaining internal ionic homeostasis were located between the secondary lamellae on gill filaments called primary lamellae (Yonkos *et al.*, 2000).

Based on semiquantitive analysis obtained, caged fish gills which are exposed for 7-28 days in the control site exhibit low value of Histological Alteration Index (HAI) of between 0.0-0.3 (Table 5) which could be considered that the gills were in normal functioning of the organ (Flores-Lopes and Thomaz, 2011). Contrary to the control, the gills of caged fishes that are exposed in the downstream sites exhibited symptoms of severe gill alterations such as hyperplasia chloride cells and focal necrotic cells (Fig. 6 and 7) as well as showed higher value of HAI (>100) as listed in Table 5.

Table 5: Histological alteration index of caged fish gills

Location	Exposure period (days)	Means of HAI	Histological alteration status
CP	7	0.0	Normal
CP	14	0.0	Normal
CP	21	0.0	Normal
CP	28	0.3	Normal
P1	7	119.5	irreparable lesion
P1	14	200.3	irreparable lesion
P1	21	233.0	irreparable lesion
P1	28	NS	NS
P2	7	121.0	irreparable lesion
P2	14	NS	NS
P2	21	NS	NS
P2	28	NS	NS
P3	7	11.7	slight damage
P3	14	112	irreparable lesion
P3	21	167.0	irreparable lesion
P3	28	NS	NS

NS: No sample anymore

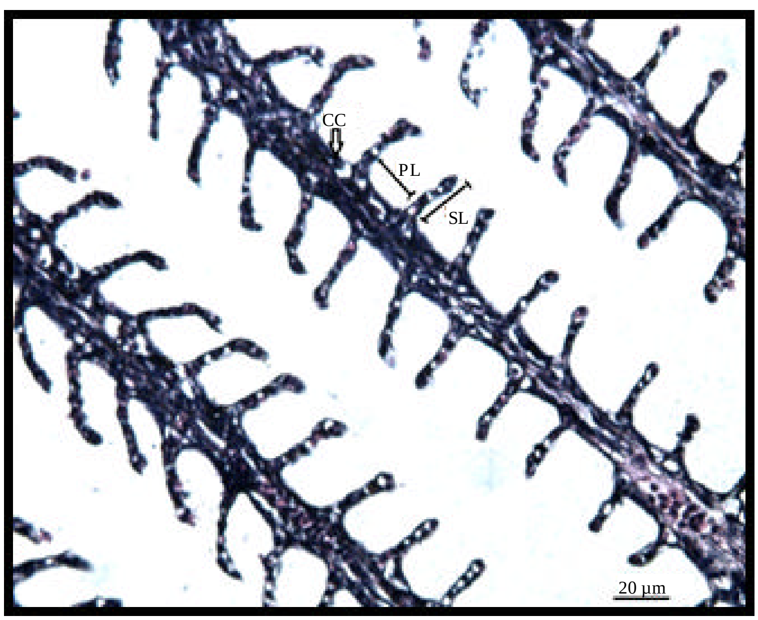

Fig. 5: Histopathology of the caged fish gills from the control site. Gill exhibits normal of PL (primary lamellae); SL (secondary lamellae). The chloride cells (CC) were located in the primary lamellae

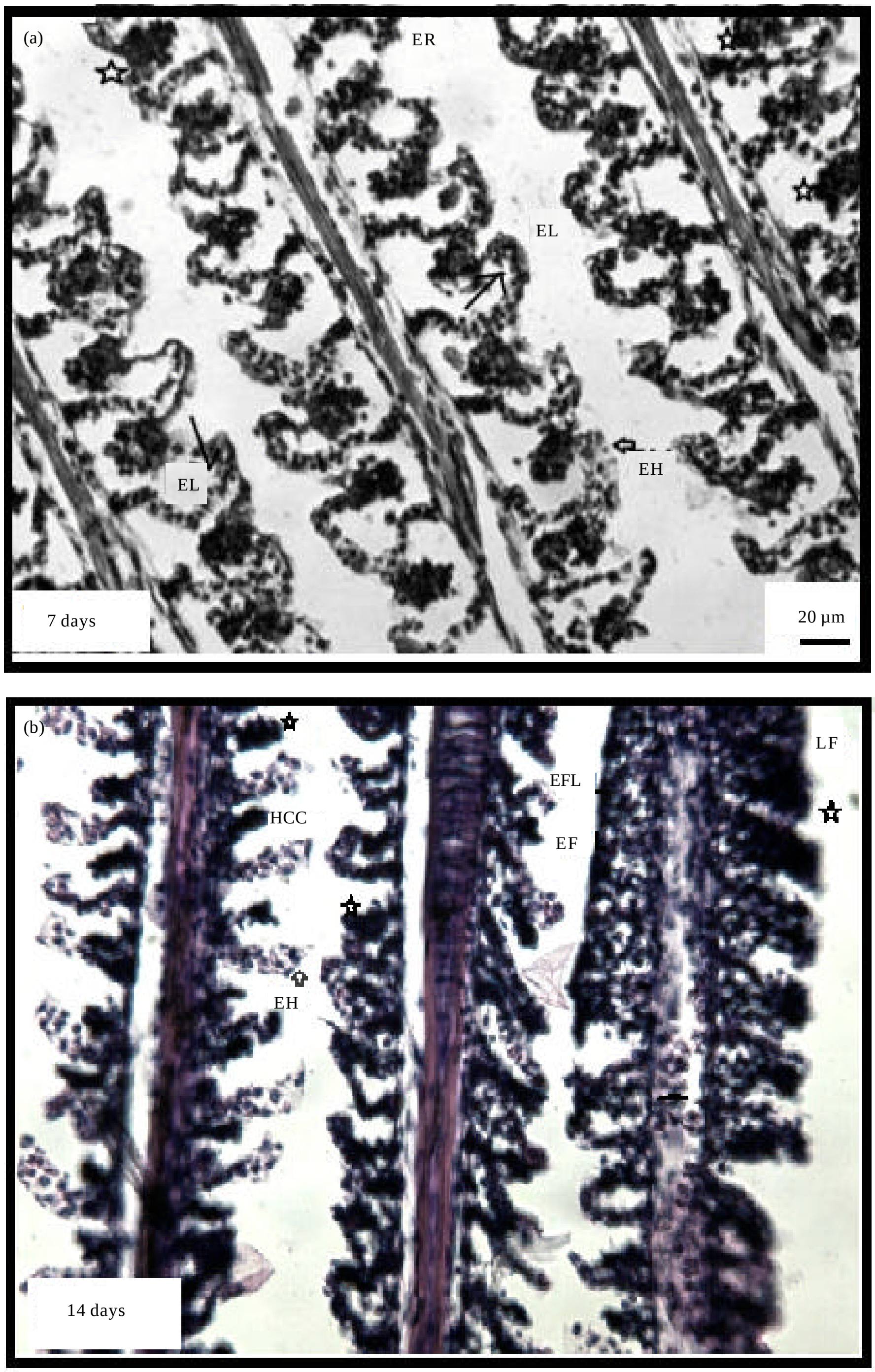

Fig. 6: Histopathology of the caged fish gills from site P1 at 7 and 14 days exposure period indicatedalteration of epithelial hypertrophy (EH); epithelial lifting (EL); epithelial rupture (ER) and focal necrotic cells (*). 14 days exposure period showed LF (lamellae fusion); extensive fusion lamellae (EFL), extention of the hyperplasia chloride cells (HCC) and increased area of focal necrotic cells (*)

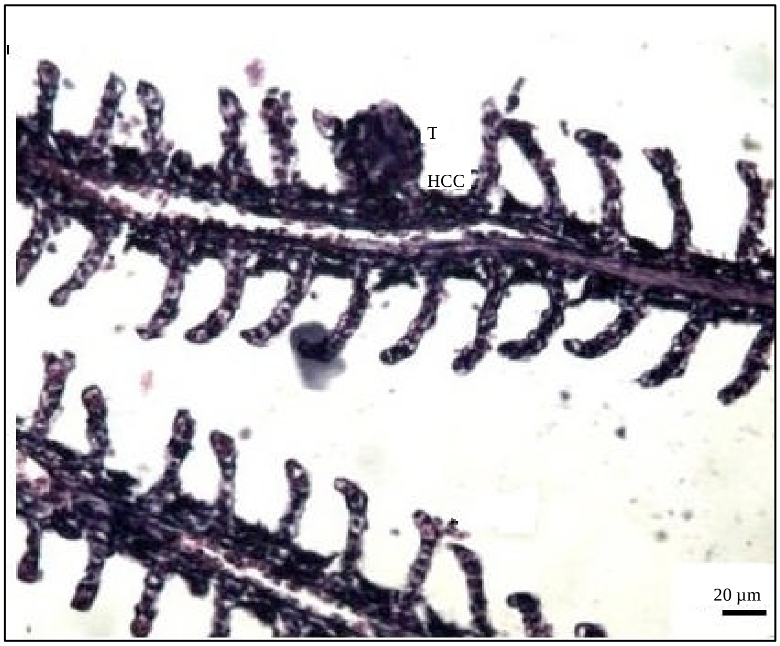

Fig. 7: Histopathology of the caged fish gills from site P3 at 14 days exposure period. Gill shows alteration symptoms: T (Telangiectasis), HCC (Hyperplasia of the chloride cells)

DISCUSSION

It is important to describe the chemical composition of mud in the LUSI pond as the origin of the mud effluent, however, the chemical composition in the effluent pipe is highlighted as a point source of pollution in Porong River. Refer to Fig. 2 exhibited that the aluminum, iron and lead were found as the major metal in LUSI mud pond and effluent. The high level of aluminum and iron was possibly related the abundance of aluminum (16%) and iron (9%) in the earth crust (Taylor and McLennan, 1985). Meanwhile, the lead source is probably from earth crust, windblown dust (Taylor and McLennan, 1985; ATSDR, 2007) and might also come from traffic near the LUSI location as well as human activities in the LUSI pond (digging and pumping of mud) that allowed concentrations of these substances in this spectrum to occur (UNDAC, 2006) The mean level of metal composition in the mud pond was greater than that measured in mud of the effluent, excluding lead. Lead that observed at mud pond was 19 mg kg^{-1} while in effluent was found in range of 35-139 mg L^{-1}. The high of lead level is possibly related with the digging and pumping of mud activity prior to be channeled in effluent pipe.

Moreover, the metal species that were found in water of the effluent exhibited higher concentrations than that found in the pond, for instance aluminum concentration level in the effluent ranged from 1.6-8.1 mg L^{-1} while the water samples from the pond was 0.28 mg L^{-1}. These findings might indicate that the excess water from the pond that was channeled to the effluent pipe had become concentrated due to the accumulation process.

In general, the physicochemical quality in all sampling sites included temperature, DO, pH and salinity was good for fish activities. Tilapia can still maintain swimming activities at an O_2 level of 1-2 mg L^{-1} while most warm water fishes can tolerate and fare well in the temperature range of nearly 30-35°C (Kutty, 1968; Kutty and Saunders, 1973; Kutty, 1987; Uchida *et al.*, 2000).

Since the mud effluent was released without any special process, the Total Suspended Solid (TSS) is the most suspected pollutant that could have occurred in the receiving water body. Results of TSS concentration in all downstream sites (P1, P2 and P3) that observed in range of 672-4177 mg L^{-1} were exceeded the standard reference of water supply for Class III that is regulated by the Indonesian government (Anonymous, 2001) and Water Quality Criteria for European Freshwater Fish in APEM (2007) that stipulated a level of 400 mg L^{-1}. More strictly, USEPA (2006) stipulates suspended solids criteria for aquatic life in vary from 30 mg L^{-1} up to 263 mg L^{-1}. However, Alabaster and Lloyd (1980) reported that the probable suspended solid concentration would need to exceed 100,000 mg L^{-1} to result in mortality over a short time frame while no deaths have been recorded in concentrations of between 200 and 1,000 mg L^{-1}.

The high concentration of TSS at site P1 was possibly contributed by input of clay from effluent. United States Geological Surveys or USGS (2008) reported that clay was found as dominant material in Sidoarjo mud (73.6%). The differences of seasonal pattern of TSS was observed among the sampling sites, where the peak of TSS concentration at site that near the effluent discharge, P1, was found during the dry season and declined at wet season. Contrary, concentration of TSS in the early part of the wet season at sites P2 and P3 was higher than that of the dry season. It probably occurred due to the flushing of the diluted mud from site P1 to sites P2 and P3 during the wet season.

USGS (2008) reported that the metal composition in the water of LUSI pond might be represented substantially by colloidal contributions, as the samples were filtered only to 0.45 μm prior to analysis (Kharaka and Hanor, 2007; Kimball *et al.*, 1995; USGS, 2008). Colloids are very fine solid particles (0.001-10 μm in diameter) which are suspended in solution (USEPA, 1999). The colloidal iron concentrations in surface water were found to be in the range size of 0.025-0.45 μm (Kuma *et al.*, 1998). Due to the similar water sampling methods that were conducted by USGS, the aluminum and iron concentrations in the current study was possibly dominated by the colloidal stage. In nature, dissolved aluminum concentrations in water with near-neutral pH values usually ranged from 0.001 to 0.05 mg L^{-1} (WHO, 1998). However, aluminum concentrations that are measured in all samples were found to be in the range level of 0.37-23.70 mg L^{-1} which means that they had exceeded the natural value. As explained previously, it possibly occurred due to the samples consisting of either dissolved or in colloidal form. For reference, Church *et al.* (1999) reported that the concentration of dissolved aluminum and colloidal aluminum at pH of 6.35 was 0.09 and 3.6 mg L^{-1} respectively while colloidal iron exceeded 1 mg L^{-1}.

Refer to the concentration of aluminum, iron and cadmium revealed that the water quality at all downstream sites were not good for aquatic life and even for wild life and livestock. Meanwhile, the maximum concentration of manganese, zinc, copper and lead in all sampling sites were not safe for aquatic life (USEPA, 2009a; Anonymous, 1998; Randall *et al.*, 1999). The similarity of hierarchical order of metal composition between water of Porong downstream sites and that was found in the water effluent reflected the possibility contribution of effluent to the Porong downstream quality. The Pearson correlation indicated that the metal composition in LUSI effluent has strong contribution to several metal concentrations at the downstream sites which included aluminum ($r = 0.64$), copper ($r = 0.55$), zinc ($r = 0.82$) and cadmium ($r = 0.72$). Meanwhile, other observed downstream metal concentrations such as iron, manganese and lead were possibly contributed by any source of discharge streams which included the LUSI effluent.

The high mud portion at sampling site that nearest the effluent pipe, i.e., P1 (Fig. 4), possibly affected by the mud effluent. Therefore, contribution the mud effluent to the chemical composition of downstream sediment is might be occurred. As previously described, lead in mud and water of effluent was found higher than that found in mud pond. Moreover, lead was the only metal found in the sediment that exceeded the permissible level. According to the Pearson correlation, lead in downstream sediments might have been contributed by the water of the effluent (r = 0.82) and enhanced by the runoff from upstream (r = 0.52). In addition, ATSDR, 2007 reported that a significant fraction of lead carried by river water is from surface particulate matters from runoff. In this study, lead concentration in water effluent ranged from 1.73-6.95 mg L^{-1} while at the upstream it was detected in a range of 0.051-0.38 mg L^{-1} (Table 3). The source of lead at the upstream section was probably from the industrial and anthropogenic activities in the surrounding area (Hejabi *et al.*, 2010; Ajibola and Ladipo, 2011; Conceicao *et al.*, 2013). The upstream of Porong River is part of the longest river in East Java namely the Brantas River that receives the intermixing of urban and industrial pollution (Ramu, 2004).

Although the sites of P1 and P2 were located near the initial stage of the effluent, however, in the dry season the aluminum level in sediments at sites P1 and P2 were lower than that of P3 and the control site. It possibly occurred because sites P1 and P2 were near the effluent which was located in the mixing zone between the effluent and surface water, hence, aluminum probably dominated in the colloidal form. As a comparison, nearly half of the colloidal aluminum in the Animas River was formed in the. mixing zone (Schemel *et al.*, 1999) whereas, in the site that was far from the mixing zone, the colloid form would be aggregated and tend to precipitated then accumulated in the bed sediment.

The most of the trace metals in Whole Body Composite (WBC) of caged fishes that are exposed at the downstream sites showed higher levels than that found in the upstream site (control). It possibly occurred due to the metal concentration in water of downstream sites generally higher than that found in upstream which increasing opportunity to be taken up and accumulated in fish (Jezierska and Witeska, 2006). The hierarchical of metal level in WBC of caged fishes which showed following order i.e., Al>Fe>Mn>Cu>Zn>Pb>Cr>Cd could be reflected in the species metal bioavailability in the fish body. Similar with water metal concentration, aluminum and iron were observed as the major metals in caged WBC.

As the mixing zone of the effluent that was dominated by aluminum and iron, sites P1 and P2 could potentially be dominated by colloidal aluminum and colloidal iron (Witters *et al.*, 1996) (Table 2 and Fig. 3). Effect of these major metals to the experiment caged fishes is adjusted by ambient water quality i.e. pH and TSS (LaZerte, 1984; Burrows and Hem, 1977; Pagenkopf, 1983; Moiseenko *et al.*, 1995; Jezierska and Witeska, 2006; Wiggins-Lewis *et al.*, 2002). Although there were no significant differences of total aluminum concentration in the water of sites P1 and P2, conversely, significant differences in metal levels of caged fish WBC (P1 = 69.55 mg Al kg^{-1}; P2 = 377. 92 mg Al kg^{-1}) were found which indicated the differences of bioavailability.

The highest level of aluminum in the fish body at site P2 must be related to the rich aluminum concentration and together with lower pH and lower TSS (Table 2 and Fig. 3: Al = 23.41 mg L^{-1}; pH = 6.00; TSS = 459-1147 mg L^{-1}) encouraged the aluminum to dissolve and therefore, increase the bioavailability of aluminum. Contrary, the high aluminum level at site P1 (23.70 mg L^{-1}) was possibly maintained in the colloidal form due to the neutral pH and highest TSS concentration (pH = 7.0; TSS = 1163-4177 mg L^{-1}). pH level and TSS concentration at site P1 encouraged the formation of stable metal complexes such as with colloidal size of silicate to form aluminosilicate which have extremely low dissolution kinetic and were not expected to be absorbed by aquatic organisms (Wiggins-Lewis *et al.*, 2002), hence, the aluminum in caged fish WBC at site P1 was lower than that in site P2.

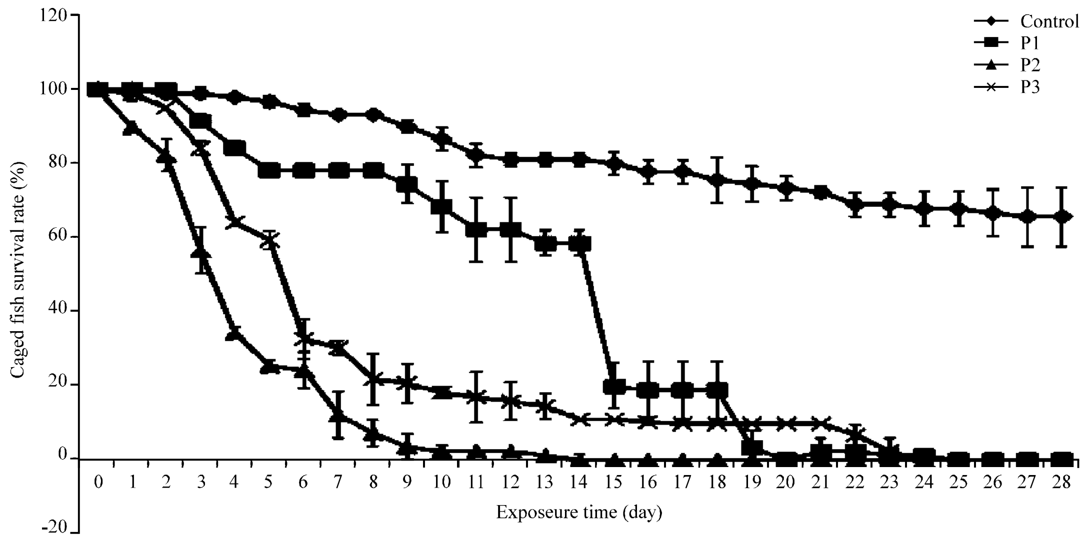

Fig. 8: Survival rate (SR) of caged fishes. The caged fishes from upstream or control (Ct) survived until 28 days and showed higher SR than those of the downstream (P1, P2, P3)

The excess aluminum in caged fishes at site P2 (Table 4) has a high risk of lethal disturbance (Jezierska and Witeska, 2006), therefore, possibly contributed to the caged fish survival rate (SR) value in this sampling site (Fig. 5). Meanwhile, aluminum concentrations in other sampling sites and other trace metal species in WBC of caged fishes were in a safe level for animal feed. However, in certain safe limits, several metals such as Fe, Mn, Cu and Zn play an important role as a necessary nutrient and are essential for normal growth and metabolism of all living organisms, hence, relatively non-toxic to aquatic biota (Wiener and Giesy, 1979; Cole, 1983; Schroeder *et al.*, 1966; Carbonell and Tarazona, 1994).

As displayed in Fig. 8, the caged fish at the upstream (or control) survived for 28 days with a weekly Survival Rate (SR) of between 93-66%. Meanwhile, fish from the downstream sites exhibited shorter survival period = 21 days with SR value of 10-0%. The lowest SR was found in site P2 which exhibited SR of 24±6.3% for 7 days. In general, the hierarchical trend of SR, from highest to lowest SR was in the following order i.e., control>P1>P3>P2.

By adopting the reference of the *in situ* acute (96 h) and 7 days full chronic toxicity test that was developed by Meletti and Rocha (2002) and Duke and Mount (1991) as well as the graph of SR as displayed in Fig. 5, showed SR value at site P2 after 4 days was at a level of 32% (or fifty percent of fish population died <96 h) which means that the effect of LUSI effluent receiving water at site P2 was acute. The effect of LUSI effluent receiving water at site P3 was categorized as sub chronic as the fifty percent mortality of fish population occurred after 6 days. The longer period (14 days) of fifty percent mortality caged fish population was observed at site P1 which means the effect of LUSI effluent receiving water at P1 was chronic.

Using the analogy of the whole effluent toxicity (USEPA, 2004), the toxic effect of LUSI mud effluent to the caged fishes might have occurred as the aggregate toxic effect from all pollutants contained in the effluent with possibly different concentrations due to the dilution factor as the differences of distance to the point of discharge. Moreover, aluminum was highlighted as the main factor of the LUSI effluent toxic effect because it was found to be the major metal and showed statistical difference compared to the control in all observed medium parameters (effluent, water, fish body). Since gills are continuously exposed to ambient water, they absorb oxygen and water-soluble chemicals (Chezhian *et al*., 2010; Patel and Bahadur, 2010) and the caged fish gills were suspected as the affected organ that is responsible for fish survival. The positive correlation showed between histological alteration index (HAI) and concentration of aluminum (r = 0.65) and TSS (r = 0.55) indicated that HAI might be correlated to the complex formation that is created by suspended solids with aluminum.

The effect of LUSI effluent to the gills might have occurred due to the physical damage and chemical alteration. LUSI mud particle size of less than 10 μm could possibly be responsible for the complexity of aluminosilicate in the downstream sites which then could possibly have clogged up onto the gill surfaces, adhering the space between the gill filaments, thus, the physical damage and fatal effect to fish mortality (USGS, 2008; Muraoka *et al*., 2011; Chapman *et al.,* 1987; APEM, 2007), whereas, the thin layers of epithelial tissues are vulnerable to alterations by changes in the surrounding physico-chemical conditions (Patel and Bahadur, 2010; Schlenk *et al*., 2008).

Fish respond to toxic exposure in fairly specific ways but often the same response is elicited by a variety of chemicals or chemical mixtures (Braunbeck, 1994). At the initial stage, the clogged fine mud particles, primarily colloid aluminum, could potentially induced the abrasion of the delicate epithelial lining, hence triggering the adaptive respond of the gills by adjusting its structures and resulting in histological alteration such as epithelial and chloride cell hyperplasia, epithelial lifting and disorganization of the gill lamellas as presented in Fig. 6 (Phippen *et al*., 2008; Kumar *et al*., 2012). With longer periods of exposure, chronic symptoms, more massive alteration namely lamellar fusion were observed (Fig. 6) which then led to death due to loss of large respiratory areas (Karlsson-Norrgren *et al*., 2006; Roberts, 1989) whereas, hypoxia induced blood vessel expansion called telangiectasis resulting in the most severe irreversible alteration, necrosis (Fig. 6 and 7) (Karlsson-Norrgren *et al*., 2006; Shimizu *et al*., 1996).

Furthermore, the level of pH and TSS could alter the different behavior and effect of aluminum to the physiological and structure of the gills. Structurally, the gill HAI value of caged fishes at sites P2 was 121, indicating that the gills were in irreparable lesions (Table 5). At site P2, a pH level of 6 can potentially encourage the aluminum to dissolve, thereby, increase the monomeric aluminum (Al^{3+}) (Gensemer, 1991). It was found that monomeric aluminum at pH 4.8-6.5 caused fish mortality as failure of ionic regulation (Leivestad *et al*., 1987). Therefore, the fine LUSI particles together with both the aluminum colloidal species in the dissolved stage were suspected to be responsible for the physico-chemical alteration which was reflected by alterations such as epithelial and chloride cell hyperplasia. More severely, the chloride cell hyperplasia with high levels of aluminum present in the gills may also interfere with the ionic influx across the epithelia and chloride cells (Roberts, 1989).

Acute aluminum toxicity occurred by binding to functional groups of apical and intracelullar gill epithelial, hence, disrupting its barrier properties, iono and osmoregulatory dysfunction which resulted in accelerated cell necrosis, sloughing and death of the fish (Exley *et al*., 1991). Heavy metal ions can readily bind to the gills and disrupt the respiratory and iono-regulatory function (Playle, 1998). Accordingly, the acute toxic effect value at site P2 (Fig. 8) might have occurred due to the interference of excess aluminum in the gill cells which induced ionic balance disturbance and enhanced by the failure of respiration that is related to gill histological alteration.

As previously described, sites P1 and P2 could potentially be dominated by colloidal aluminum (Witters *et al*., 1996) (Fig. 3), however, the values of TSS and pH in site P1 (Table 1) had the advantage of reducing aluminum toxicity and therefore the mortality of caged fishes at site P1 was lower than that in P2. While the high TSS concentration provides aluminum to complexation with organic ligand and silicate, the neutral pH allowed the extensive polymerization of the large molecules in the water, thereby, diminishing the aluminum toxicity to the gills. The higher aluminium polymers are less toxic than monomers (Rosseland and Staurnes, 1994; Wiggins-Lewis *et al.*, 2002). Moreover, aluminum at higher pH values as showed in site P1 appeared to have no effect on sodium fluxes (Dalziel *et al.*, 1986). The chronic effect of water quality at site P1 to the caged fishes was possibly related to gills physically damaged by LUSI particulates and colloidal aluminum with similar mechanism as previously discussed. Colloidal aluminum that is freshly formed in the water column can be particularly toxic to fish (Witters *et al.*, 1996). The chronic effect was marked by histological alteration namely lamellae fusion as presented in Fig. 7 (Karlsson-Norrgren *et al.*, 2006). During the 21 days exposure the HAI value in site P1 was a high 233 which indicated that the gills are in the level of irreparable lesion.

Due to the aggregate toxic effect from all pollutants, contribution of other observed metals was also considerable. Several metal concentrations in the downstream sites that were much higher than the standard references including iron, lead, copper and cadmium (Table 2) potentially could have toxic effect on the fishes. Moreover, the acute toxic of aggregate pollutants could have increased in site P2 because at pH 6 ion activities of Fe and Cu increased across all levels of Al addition (Gensemer, 1991). The precipitation of complex iron to the gills would lead to mortality (Phippen *et al.*, 2008) while copper potentially disturbed the gill permeability to water and ions (Lauren and McDonald, 1986). However, as a toxic and non essential metals (Liden *et al.*, 2001), cadmium and lead potentially danger because they could bioaccumulate and biopersist in tissues over time (Etesin and Benson, 2007). The lead in the ion form, primarily at lower pH and TSS could enter into the body tissues of fish through the gills by binding itself to the mucus layer (MacDonald *et al.*, 2002). Furthermore, mucus may form a respiratory exchange obstruction *per se* and may act as substrate for rapid growth of bacteria (Roberts, 1989). By enhancing the aluminum toxicity, concentrations of iron, lead and copper also contributed to the lethal effect of caged fish in the downstream sites.

Gill HAI at site P3 after 21 days of exposure was in level 167 indicating that gill function status was in the irreparable alteration. The gills of caged fishes that are exposed at site P3 showed severe histological alteration, telangiectasia and hyperplasia of chloride cells (Fig. 7) which indicated the physical and chemical trauma (Roberts, 1989). Cadmium found in caged fish bodies at site P3 indicated cadmium bioavailability, whereas in the dissolved form, cadmium might have been taken up by the gills which posed high toxicity even at very low level (Kumar and Singh, 2010). Cadmium in level of 0.002 mg L^{-1} caused acute effect to the aquatic life (USEPA, 2009a). While maximum cadmium at site P3 in the current study was in the level of 0.072 mg L^{-1}. The prime target of cadmium on the gills is chloride cells that provide the primary means for maintaining internal ionic homeostasis. This cell is also known as MRC (mitochondria-rich cell) and is associated with enzymes of Na-K ATPase which indirectly energizes NaCl secretion by this cell (Yonkos *et al.*, 2000; Evans *et al.*, 2005; Garcia-Santos *et al.*, 2006). Site P3 that represented the location furthest from the effluent is also nearest to the mouth river, with fluctuation salinity of 0-1‰. During the transition from fresh water to sea water, gills adapt to the increased salinity by responding to a rapid signal that stimulates chloride secretion (Zadunaisky, 1996). Therefore, due to the chloride cell dysfunction and the failure of NaCl secretion which possibly could have occur, thus, the fatal effect of the caged fish mortality in site P3. Accordingly, the low SR value of caged fishes at site P3 (30%) was possibly caused by respiratory and osmoregulatory alteration that are contributed by chemical effects of pollutant aggregates, mainly cadmium and physical damaged by LUSI particles.

In conclusion, the flow of LUSI mud volcano lava into the river results in adverse effect in the downstream quality including the abundance of complex metal-suspended solids primarily colloidal aluminum accompanied with other discharge streams which potentially could have lethal effect to the fishes due to the gill structural alteration and the excess aluminum level in the fish body. Declining suspended solids accompanied by persistent abundance of colloidal aluminum along the downstream sites present a higher risk to the aquatic ecosystem.

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REFERENCES

APEM, 2007. Review of UKTAG (United Kingdom Technical Advisory Group) proposed standard for suspended solids: A final scientific report Advances in Production Engineering and Management, WWF-UK., UK.

ATSDR, 2007. Toxicological profile for lead. U.S. Department of Health and Human Sevices, Atlanta, Georgia, U.S. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id = 96andtid = 22.

Ajibola, V.O. and M.K. Ladipo, 2011. Sediment quality of effluent discharge channels from six industrial sites in Lagos, Nigeria. Int. J. Environ. Res., 5: 901-908.

Alabaster, J.S. and R. Lloyd, 1980. Water Quality Criteria for Freshwater Fish. Published by Arrangement with the Food and Agriculture Organization of the United Nations by Butterworths, United Kingdom, ISBN-13: 9780408106733, pp: 2-14.

Anonymous, 1998. Guidelines for interpreting water quality data. British Columbia Ministry of Environment, Lands and Parks, British Columbia, Canada.

Anonymous, 2001. Peraturan pemerintah Republik Indonesia nomor 82 tahun 2001 tentang pengelolaan kualitas air dan pengendalian pencemaran [The Republic of Indonesia number 82 of 2001 about water quality management and air pollution control]. President of the Republic of Indonesia, Jakarta, Indonesia.

Baker, D.L. and K.A. King, 1994. Environmental Contaminant Investigation of Water Quality, Sediment and Biota of the Upper Gila River Basin Arizona. U.S. Fish and Wildlife Service, Phoenix, Arizona.

Balcazar, J.L., A. Aguirre, G. Gomez and W. Paredes, 2004. Culture of hybrid red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*) in marine cages: Effects of stocking density on survival and growth. Proceeding of the 6th International Symposium on Tilapia in Aquaculture, September 12-13, 2004, Manila, Philippine, pp: 479-483.

Barbee, G.C., J. Barich, B. Duncan, J.W. Bickhan and C.W. Matson *et al*., 2008. *In situ* biomonitoring of PAH-contaminated sediments using juvenile coho salmon (*Oncorhynchus kisutch*). Ecotoxicol. Environ. Saf., 71: 454-464.

Braunbeck, T., 1994. Detection of Environmentally Relevant Concentrations of Toxic Organic Compounds Using Histological and Cytological Parameters: Substance-Specificity in the Reaction of Rainbow Trout Liver. In: Sublethal and Chronic Effects of Pollutants on Freshwater Fish, Maller, R. and R. Lloyd (Eds.). Blackwell Science Publishers, Boston, pp: 15-21.

Burrows, W.D. and J.D. Hem, 1977. Aquatic aluminium: Chemistry, toxicology and environmental prevalence. CRC Crit. Rev. Environ. Control, 7: 167-216.

CRISP, 2010. Centre for remote imaging, sensing and processing. CRISP, National University of Singapore. http://www.crisp.nus.edu.sg/coverages/EJmudflow/index20101117.html.

CSQG, 2002. Canadian Sediment Quality Guidelines (CSQG) for the protection of aquatic life. Canadian Council of Ministers of the Environment, Canada.

Carbonell, G. and J.V. Tarazona, 1994. Toxicokinetics of copper in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicol., 29: 213-221.

Chapman, P.M, J.D. Popham, J. Griffin, D. Leslie and J. Michaelson, 1987. Differentiation of physical from chemical toxicity in solid waste fish bioassays. Water Air Soil Pollut., 33: 295-308.

Chezhian, A., N. Kabilan, T.S. Kumar, D. Senthamilselvan and K. Sivakumari, 2010. Impact of common mixed effluent of sipcot industrial estate on histopathological and biochemical changes in estuarine fish lates calcarifer. Curr. Res. J. Biol. Sci., 2: 201-209.

Church, S.E., B.A. Kimball, D.L. Fey, D.A. Ferdere, T.J. Yager and R.B. Vaughn, 1999. Source, transport and partitioning of metals between water, colloids and bed sediments of the Animas River, Colorado. U.S. Geological Survey Open-File Report 97-0151.

Cole, G.A., 1983. Textbook of Limnology. 3rd Edn., CV Mosby Co., St. Louis, Toronto and London, ISBN-10: 0801610044, Pages: 401.

Conceicao, F.T., G.R.B. Navarro and A.M. Silva, 2013. Anthropogenic influences on Cd, Cr,Cu, Ni, Pb and Zn concentrations in soils and sediments in a watershed with sugar cane crops at Sao Paulo State, Brazil. Int. J. Environ. Res., 7: 551-560.

Dalziel, T.R.K., R. Morris and D.J.A. Brown, 1986. The effects of low pH, low calcium concentrations and elevated aluminium concentrations on sodium fluxes in brown trout, *Salmo trutta* L. Water Air Soil Pollut., 30: 569-577.

Duke, T.W. and D.I. Mount, 1991. Toxic Effects on Individuals, Populations and Aquatic Ecosystems and Indicators of Exposures to Chemicals. In: Methods for Assessing Exposure of Human and Non-Human Biota, Tardiff, R.G. and B. Goldstein (Eds.). John Wiley and Sons, New York, pp: 393-404.

Etesin, M.U. and N.U. Benson, 2007. Cadmium, copper, lead and zinc tissue levels in Bonga Shad(*Ethmalosa fimbriata*) and Tilapia (*Tilapia guineensis*)Caught from Imo River, Nigeria. Am. J. Food Technol., 2: 48-54.

Evans, D.H., P.M. Piermarini and K.P. Choe, 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of nitrogenous waste. Physiol. Rev., 85: 97-177.

Exley, C., J.S. Chappel and J.D. Birchal, 1991. A mechanism for acute aluminium toxicity in fish. J. Theor. Biol., 151: 417-428.

Flores-Lopes, F. and A.T. Thomaz, 2011. Histopathologic alterations observed in fish gills as a tool in environmental monitoring. Braz. J. Biol., 71: 179-188.

Garcia-Santos, S., A. Fontainhas-Fernandes and J.M. Wilson, 2006. Cadmium tolerance in the Nile tilapia (*Oreochromis niloticus*) following acute exposure: Assessment of some ionoregulatory parameters. Environ. Toxicol., 21: 33-46.

Gensemer, R.W., 1991. The effects of pH and aluminum on the growth of the acidophilic diatom *Asterionella ralfsii* var. Americana. Limnol. Oceanogr., 36: 123-131.

Hejabi, A.T., H.T. Basavarajappa and A.M.Q. Saeed, 2010. Heavy metal pollution in Kabini River sediments. Int. J. Environ. Res., 4: 629-636.

Hidayati, D., 2010. An evaluative study on Sidoarjo mud flow after phytoremediation treatment in milk fish (*Chanos chanos*) liver. IPTEK J. Technol. Sci., 21: 28-31.

Istadi, B.P., G.H. Pramono, P. Sumintadireja and S. Alam, 2009. Modeling study of growth and potential geohazard for LUSI mud volcano: East Java, Indonesia. J. Mar. Pet. Geol., 26: 1724-1739.

Jezierska, B. and M. Witeska, 2006. The Metal Uptake and Accumulation in Fish Living in Polluted Waters. In: Soil and Water Pollution Monitoring, Protection and Remediation, Twardowska, I., H.E. Allen, M.M. Haggblom and S. Stefaniak (Eds.). Vol. 69, Springer, Netherlands, pp: 107-114.

Karlsson-Norrgren, L., I. Bjorklund, O. Ljungberg and P. Runn, 2006. Acid water and aluminium exposure: Experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish Dis., 9: 11-25.

Karr, M.J., I.P.A. Wiguna and A. Widodo, 2008. Final report evaluation of mud flow disaster alterrnatives in Sidoarjo regency, East Java, Indonesia. Collaborative Project by UNEP and AUSAID (Australian Embassy, Jakarta Indonesia), pp: 1-104.

Kharaka, Y.K. and J.S. Hanor, 2007. Deep Fluids in the Continents: I. Sedimentary Basins. In: Surface and Ground Water, Weathering and Soils: Treatise on Geochemistry, Drever, J.I. (Ed.). 2nd Edn., Vol. 5, Elsevier, New York, USA., pp: 1-48.

Kimball, B.A., E. Callender and E.V. Axtmann, 1995. Effects of colloids on metal transport in a river receiving acid mine drainage, Upper Arkansas River, Colorado, USA. Applied Geochem., 10: 285-306.

Kuma, K., A. Katsumoto, J. Nishioka and K. Matsunaga, 1998. Size-fractionated iron concentrations and fe(III) hydroxide solubilities in various coastal waters. Estuarine Coastal Shelf Sci., 47: 275-283.

Kumar, P. and A. Singh, 2010. Cadmium toxicity in fish: An overview. GERF Bull. Biosci., 1: 41-47.

Kumar, V., A.K. Abbas and J.C. Aster, 2012. Robbins Basic Pathology. 9th Edn., Saunders/Elsevier, Canada, pp: 2-5.

Kutty, M.N., 1968. Influence of ambient oxygen on the swimming performance of goldfish and rainbow trout. Can. J. Zool., 46: 647-653.

Kutty, M.N. and R.L. Saunders, 1973. Swimming performance of young atlantic salmon (*Salmo salar*) as affected by reduced ambient oxygen concentration. J. Fish. Res. Board Can., 30: 223-227.

Kutty, M.N., 1987. Site selection for aquaculture: Chemical features of water. Working Paper ARAC/87/WP/12(9), African Regional Aquaculture Centre, United Nation Development Programme, Food and Agriculture Organization (FAO) of the United Nations. http://www.fao.org/docrep/field/003/ac183e/AC183E00.htm

LaZerte, B.D., 1984. Forms of aqueous aluminum in acidified catchments of central Ontario: A methodological analysis. Can. J. Fish Aquat. Sci., 41: 766-776.

Lauren, D.J. and D.G. McDonald, 1986. Influence of water hardness, pH and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Can. J. Fish Aquat. Sci., 43: 1488-1496.

Lee, M.C. and T.W. Schultz, 1994. Contaminants investigation of the Guadalupe and San Antonio rivers of Texas. U.S. Fish and Wildlife Service. Corpus Christi, Texas. http://www.fws.gov/southwest/es/Documents/R2ES/GuadalupeSanAntonioRivers.pdf

Leivestad, H., E. Jensen, H. Kjartansson and L. Xingfu, 1987. Aqueous speciation of aluminium and toxic effects on Atlantic salmon. Proceedings of the International Symposium on Ecophysiology of Acid Stress in Aquatic Organisms, January 13-16, 1987, Antwerpen, Belgiam, pp: 387-398.

Liden, C., M. Bruze and T. Menne, 2001. Metals. In: Textbook of Contact Dermatitis, Rycrof, R.J.G, T. Menne, P.J. Frosch and J.P. Lepoittavin (Eds.). 3rd Edn., Springer, Germany, pp: 933-937.

Lillie, R.D., 1965. Histopathologic Technic and Practical Histochemistry. 3rd Edn., McGraw-Hill, New York.

Macdonald, A., L. Silk, M. Schwartz and R.C. Playle, 2002. A lead-gill binding model to predict acute lead toxicity to rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 133: 227-242.

Meletti, P.C. and O. Rocha, 2002. Development of a chamber for *in situ* toxicity tests with small fishes. Braz. J. Biol., 62: 187-190.

Moiseenko, T.I., L.P. Kudryavtseva, I.V. Rodyushkin, V.A. Dauvalter, A.A. Lukin and N.A. Kashulin, 1995. Airborne contamination by heavy metals and aluminum in the freshwater ecosystems of the Kola Subarctic region (Russia). Sci. Total Environ., 160-161: 715-727.

Muraoka, K., K. Amano and J. Miwa, 2011. Effects of suspended solids concentration and particle size on survival and gill structure in fish. Proceedings of the 34th World Congress of the International Association for Hydro-Environment Engineering and Research, June 26-July 1, 2011, Brisbane Convention and Exhibition Centre, Brisbane, Australia, pp: 2893-2900.

NRC, 2005. Mineral Tolerance of Animals. 2nd Edn., National Academies Press, Washington, DC., USA., ISBN: 10: 0309096545, Pages: 510.

Pagenkopf, G.K., 1983. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, ph and water hardness. Environ. Sci. Technol., 17: 342-347.

Patel, J.M. and A. Bahadur, 2010. Histopathological alterations in catla catla induced by chronic exposure of copper ions. J. Cell Tissue Res., 10: 2365-2370.

Phippen, B., C. Horvath, R. Nordin and N. Nagpal, 2008. Ambient water quality guidelines for iron. Water Stewardship Division. Ministry of Environment, British Columbia. http://www.env.gov.bc.ca/wat/wq/BCguidelines/iron/iron_tech.pdf.

Playle, R.C., 1998. Modelling metal interactions at fish gills. Sci. Total Environ., 219: 147-163.

Poleksic, V. and V. Mitrovic-Tutundzic, 1994. Fish Gills as a Monitor of Sublethal and Chronic Effects of Pollution. In: Sublethal and Chronic Effects of Pollutants on Freshwater Fish, Muller, R. and R. Lloyd (Eds.). Fishing News Books, Oxford, UK., pp: 339-352.

Ramu, K.V., 2004. Brantas river basin case study Indonesia. http://siteresources.worldbank.org/INTSAREGTOPWATRES/Resources/Indonesia_BrantasBasinFINAL.pdf

Randall, S., D. Harper and B. Brierley, 1999. Ecological and ecophysiological impacts of ferric dosing in reservoirs. Hydrobiologia, 395-396: 355-364.

Roberts, R.J., 1989. Fish Pathology. 2nd Edn., Bailliere Tindall, London, pp: 67-78.

Rosseland, B.O. and M. Staurnes, 1994. Physiological Mechanisms for Toxic Effects and Resistance to Acidic Water: An Ecophysiological and Ecotoxicological Approach. In: Acidification of Freshwater Ecosystems: Implications for the Future, Steinberg, C.E.W. and R.F. Wright (Eds.). Wiley, New York, pp: 228-246.

Schemel, L.E., B.A. Kimball and K.E. Bencala, 1999. Colloid formation and the transport of aluminum and iron in the animas river near Silverton, Colorado. Proceedings of Toxic Substances Hydrology Program Technical Meetings, Volume 1, March 8-12, 1999, Charleston, South Carolina, pp: 59.

Schlenk, D., R. Handy, S. Steinert, M.H. Depledge and W. Benson, 2008. Biomarker. In: The Toxicology of Fishes Boca Raton, Di Giulio, R.T. and D.E. Hinton (Eds.). Press Taylor and Francis Group, USA., pp: 709-712.

Schroeder, H.A., D.D. Balassa and I.H. Tipton, 1966. Essential trace metals in man: Manganese. A study in homeostasis. J. Chronic Dis., 19: 545-571.

Shimizu, S., Y. Eguchi, W. Kamiike, Y. Itoh and J. Hasegawa *et al*., 1996. Induction of apoptosis as well as necrosis by hypoxia and predominant prevention of apoptosis by Bcl-2 and Bcl-XL. Cancer Res., 56: 2161-2166.

Suprapto, S.J., R. Gunradi and Y.R. Ramli, 2007. Geokimia sebaran unsur logam pada endapan lumpur Sidoarjo (Geochemical distribution of metal in Sidoarjo mud). Buletin Pusat Sumber Daya Geol., 2: 1-9.

Taylor, S.R. and S.M. McLennan, 1985. The Continental Crust: Its Composition and Evolution. Blackwell, Oxford, ISBN-13: 978-0632011483, pp: 312.

UNDAC, 2006. Environmental assessment hot mud flow East Java Indonesia. Final Technical Report, United Nation Disaster Assessment and Coordination, pp: 1-56.

UNEP/WHO, 1996. Water Quality Monitoring: A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. In: Sediment Measurements, Bartram, J. and R. Ballance (Eds.). UNESCO, WHO and UNEP, London, UK., pp: 1-15.

USEPA, 1971. Residue, non-filterable (gravimetric, dried at 103-105°C). Method 160.2, United States Environmental Protection Agency, pp: 1-3. http://www.umass.edu/tei/mwwp/acrobat/epa160_2nonfiltres.pdf

USEPA, 1987. Recommended protocols for fish pathology studies in Puget sound. U.S. Environmental Protection Region 10, Puget Sound Water Quality Authority, pp: 1-91. http://www.psparchives.com/publications/our_work/science/protocals_guidelines/fishpath.pdf

USEPA, 1994. Methods 200.7 Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. United States Environmental Protection Agency, pp: 1-58. http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_200_7.pdf

USEPA, 1999. Guidance manual for compliance with the interim enhanced surface water treatment rule: Turbidity provisions. EPA 815-R-99-010. http://water.epa.gov/lawsregs/rulesregs/sdwa/mdbp/upload/2001_01_12_mdbp_turbidity_cover_tu.pdf

USEPA, 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Vol. 1, 3rd Edn., Risk Assessment and Fish Consumption Limits, Washington, DC., pp: 7.14-7.18.

USEPA, 2004. National Whole Effluent Toxicity (WET) implementation guidance under the NPDES program. EPA 832-B-04-003. http://water.epa.gov/scitech/methods/cwa/wet/upload/2004_12_28_pubs_wet_draft_guidance.pdf

USEPA, 2006. Framework for Developing Suspended and Bedded Sediments (SABS) Water Quality Criteria. OW and ORD, Washington, DC., pp: 9-10.

USEPA, 2009a. National recommended water quality criteria. United States Environmental Protection Agency, Office of Water Office of Science and Technology. http://www.epa.gov/ost/criteria/wqctable/

USEPA, 2009b. The national study of chemical residue in lake fish tissue. EPA 823 R.09.006, Office of Water and USEPA, Office of Science and Technology, pp: 15-17.

USGS, 2008. Preliminary analytical results for a mud sample collected from the LUSI mud volcano, Sidoarjo, East Java, Indonesia. Open-File Report 2008-1019, U.S. Department of the Interior and U.S. Geological Survey, Reston, Virginia.

Uchida, K., T. Kaneko, H. Miyazaki, S. Hasegawa and T. Hirano, 2000. Excellent salinity tolerance of mozambique tilapia (*Oreochromis mossambicus*): Elevated Chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci., 17: 149-160.

Ueng, Y.F., C. Liu, C.F. Lai, L.M. Meng, Y.Y. Hung and T.H. Ueng, 1996. Effects of cadmium and environmental pollution on metallothionein and cytochrome P450 in tilapia. Bull. Environ. Contam. Toxicol., 57: 125-131.

WHO, 1998. Guidelines for Drinking-water Quality. 2nd Edn., World Health Organization, Geneva.

Wiener, J.G. and J.P. Giesy, 1979. Concentrations of Cd, Cu, Mn, Pb and Zn in fishes in a highly organic softwater pond. J. Fish. Res. Board Can., 36: 270-279.

Wiggins-Lewis, M., J.V. Nabholz and R. Jones, 2002. TSCA New Chemicals Program (NCP) chemical categories. U.S. Environmental Protection Agency. http://www.epa.gov/oppt/newchems/pubs/cat02.pdf.

Witters, H.E., S. VanPuymbroeck, A.J.H.X. Stouthart and S.E.W. Bonga, 1996. Physicochemical changes of aluminium in mixing zones: Mortality and physiological disturbances in brown trout (*Salmo trutta* L.). Environ. Toxicol. Chem., 15: 986-996.

Yonkos, L.T., D.J. Fisher, R. Reimschuessel and A.S. Kane, 2000. Atlas of fathead minnow normal histology. University of Maryland Aquatic Pathobiology Center. http://aquaticpath.umd.edu/fhm/

Zadunaisky, J.A., 1996. Chloride cells and osmoregulation. Kidney Int., 49: 1563-1567.