Sains Malaysiana 46(3)(2017): 373–380
http://dx.doi.org/10.17576/jsm-2017-4603-03

Evaluation of the Effect of Sidoarjo Mud on Aquatic Life Using Chromatophores and the Microstructure of Fish Scales

(Penilaian Kesan Lumpur Sidoarjo Terhadap Hidupan Akuatik Menggunakan Kromatofor dan Mikrostruktur Sisik Ikan)

DEWI HIDAYATI, NORELA SULAIMAN*, B.S. ISMAIL, M. SHUHAIMI-OTHMAN & M.E. DE BELLARD

ABSTRACT

The Sidoarjo mud is the first visible phenomenon of a mud volcano that occurs in a human settlement and which is subsequently channelled into a river. Clay, aluminium and iron were reported to be the dominant contaminants that could possibly come into contact with and accumulate on the surface of local fish and initiate alteration in scale microstructure. The aim of this study was to evaluate the extent of water body contamination in the Sidoarjo mud by evaluating the chromatophore density and microstructure deformation of fish scales that act as biomarkers. Scale samples were obtained from caged Mozambique tilapia (Oreochromis mossambicus) *fish that were placed downstream and upstream of the Sidoarjo mud spillway pipes. With respect to melanophore density, it was found that the scales of fish exposed in the downstream section were significantly lower in chromatophores (<50 chr/mm²) than the control scales in fish from the upstream station (>100 chr/mm²). This study suggested that the density of chromatophores was closely related to the concentration of total suspended solids (*r *= 0.69), which was possibly enhanced by iron (*r *= 0.56). Using scanning electron microscopy analysis, some deformation, i.e. irregularity of spherule shape and increasing pits in the space between ridges, were observed.*

Keywords: Fish scale chromatophore; metal; SEM; Sidoarjo mud; suspended solid

ABSTRAK

Lumpur Sidoarjo adalah fenomena pertama daripada gunung lumpur yang berlaku di kawasan berpenghuni dan disalurkan terus ke dalam sungai. Tanah liat, aluminium dan besi dilaporkan sebagai bahan cemar dominan yang mungkin boleh bersentuhan dan berkumpul di permukaan badan ikan tempatan dan menyebabkan perubahan pada mikrostruktur sisik. Kajian ini bertujuan untuk menilai kesan pencemaran lumpur Sidoarjo pada jasad air menggunakan ketumpatan kromatofor dan perubahan bentuk mikrostruktur pada sisik ikan yang bertindak sebagai penanda biologi. Sampel sisik diperoleh daripada ikan tilapia (Oreochromis mossambicus) *yang dikurung di dalam sangkar dan diletak di hulu dan hilir paip efluen lumpur Sidoarjo. Ketumpatan kromatofor pada sisik ikan yang terdedah di kawasan hilir adalah jauh lebih rendah (<50 kromatofor/mm²) dibandingkan dengan ikan dari kawasan hulu atau kawalan (>100 kromatofor/mm²). Kajian ini mencadangkan bahawa ketumpatan kromatofor berkait rapat dengan kepekatan pepejal terampai (*r*=0.69) yang mungkin dipertingkatkan oleh kepekatan besi (*r*=0.56). Berdasarkan analisis mikrostruktur menggunakan mikroskop imbasan elektron (SEM), beberapa perubahan bentuk dijumpai, iaitu ketidakteraturan bentuk sferul dan peningkatan lubang pada permukaan sisik di antara rabung.*

Kata kunci: Kromatofor sisik ikan; logam; lumpur Sidoarjo; pepejal terampai; SEM

INTRODUCTION

Drilling activities in gas exploration work in the Sidoarjo regency of East Java, Indonesia in 2006 triggered the eruption of a mud volcano, named as the Sidoarjo mud flow or Lumpur Sidoarjo (abbreviated LUSI). Huge volume of mud approximately 90,000 m^3/day (Istadi et al. 2009) was emitted. Due to the constant mud discharge, the Sidoarjo mud was channelled into the adjacent water body in the Sidoarjo regency, the Porong River, without any particular process, at an average volume of 50,000 m^3/day (Sukresno et al. 2008). Information on the effect of the mud discharge on the local aquatic life is lacking and the Sidoarjo mud could represent a new phenomenon of a mud volcano discharge in urban and rural water systems. The dry weight of the Sidoarjo mud was 54-90% and the particle size was found to be dominated by particles less than 10 µm. Aluminium and iron were reported as the dominant metal species (Plumlee et al. 2008). Accordingly, the high concentrations of suspended solids, aluminium and iron in the Porong River were considered to be contaminant species from the Sidoarjo mud.

The Mozambique tilapia *(Oreochromis mossambicus)* is covered by cycloid scales (Talwar & Jhingran 1992). The cycloid scale has a focus that divides the scale into anterior, posterior and lateral parts. The focus is surrounded by the linear elevations of the bony layer that excesses calcium salts called ridges or circuli (Esmaeli et al. 2007). In the anterior and lateral parts, the ridges are partitioned by deep and narrow grooves that run radially towards the focus, namely radii. Chromatophores are found in the posterior part (Esmaeli et al. 2012, 2007). Due to their location on the surface of fishes, scales are considered a suitable biomarker when they come into contact with water and its pollutants (Rishi & Jain 1998).

The surface layer, such as the cuticle, provides an important site for aluminium uptake in the crustacean *Asellus aquaticus* (Elangovan et al. 1999). Because the fish scale has similar composition to the crustacean cuticle, i.e. collagen, organic matrix and calcium (Kapoor & Khanna 2004; Promwikorn et al. 2005), similar responses of the scale to aluminium are possible. Skin bleaching syndrome was found in lake trout (*Salvelinus namaycush*) near a large iron-ore contaminated lake in Labrador (Payne et al. 2001). Several *in vitro* studies on the effect of heavy metals on the scale chromatophores indicated that cadmium and arsenic had adverse effects on scale morphology as well as on the number and mobility of melanophores. All of these data suggest that chromatophores can be used as a quick and reliable biomarker of aquatic metal pollution (Allen et al. 2004; Radhakrishnan et al. 2000; Rishi & Jain 1998; Shikha & Sushma 2011; Tang et al. 1997).

Pigmentation usually depends on environmental conditions, such as turbidity, that affect the light spectrum and thus visibility in the water. The perch (*Perca fluviatilis*) from dark environments (coloured water caused by algal blooms) shows dark body pigmentation, whereas the perch from turbid (water turbidity maintained using clay in the experiment) is duller/paler than other fishes (Gusen 2010). A scale consists of a plate of collagenous tissue, with superficial mineralization, surrounded by scleroblasts and fibroblasts (Kapoor & Khanna 2004). The bony layer is formed by osteoblastic cells on its outer surface in a manner similar to the formation of dermal bone (Dietrich 1953). Histological examination indicated that when aluminium was deposited diffusely on aluminium-treated bones, the bone formation and mineralization rates were markedly decreased and osteoblastic activities were also inhibited (Zhu et al. 1990).

The Sidoarjo mud constituents in the water of Porong River has the potential to increase the suspended solid, aluminium and iron concentrations (Plumlee et al. 2008) and they can cause adverse effects, such as the disturbance of fish visibility in the water and stress to the fish, which would be expressed as decreasing chromatophore numbers. Moreover, it was hypothesized that the aluminium concentration in the Sidoarjo mud contributed to scale microstructure deformation. This investigation used scales as a biomarker that provide several advantages, among which are that they can be regularly, rapidly and non-lethally collected from fish (USGS 2011).

MATERIALS AND METHODS

SAMPLING

The Sidoarjo mud had been accumulating in huge basins protected by embankments to prevent the mud from flooding the villages. The excess watery mud has been channelled into the Porong River. Water sampling and experiments with caged fish (tilapia, *Oreochromis mossambicus*) were conducted at three stations downstream of the Sidoarjo mud spillway pipes (SWP) at P1 (7°32′ 41.8″S; 112°42′ 31.67″E),

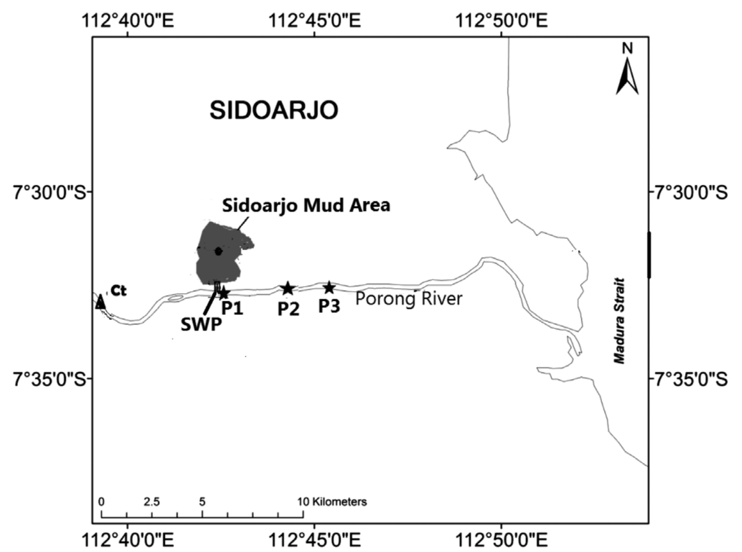

FIGURE 1. Map of mud volcano as the Sidoarjo mud source and mud reservoir pond area. The excessive watery mud was channelled into the Porong River through spillway pipe (SWP). The three sampling sites were located at downstream of the SWP at P1, P2 and P3. The control sampling site (Ct) was located at upstream of the SWP

which represents the area of initial discharge and at P2 (7°32′ 42.91″S; 112°43′ 49.53″E) and P3 (7°32′ 36.57″S; 112°44′ 35.06″E) located approximately 3 and 4 km from the SWP area, respectively (Figure 1). The fourth station, control area Ct (7°33′ 10.42″S; 112°39′ 30.32″E) located approximately 6 km upstream, represents a location free of Sidoarjo mud (Figure 1). The fishes were placed for a week in rectangular-shaped cages made from plastic and free of metal elements. Water sampling was carried out during the periods of June-July 2011 and January-February 2012, which represented the dry and wet seasons, respectively.

WATER QUALITY MEASUREMENT

The collected water samples were filtered immediately through a 0.45 μm membrane and the pH of the sample was adjusted to 2 with nitric acid for preservation. The aluminium and iron concentrations were then measured. The samples collected for total suspended solids (TSS) test were kept at 4°C in cool boxes. For each preserved water sample, an extraction was performed using the acid-mixture procedure prior to measurement of the aluminium and iron concentrations using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) according to EPA Method 200.7 (USEPA 1994). The TSS was measured using Gravimetric methods, adopted from US EPA Method 160.2 (USEPA 1971).

The Quality Ratio (QR) was calculated in order to determine the water quality among the sampling sites over time. The QR was obtained by comparing the observed value to the reference value and the ratio was standardized in the range from 0 to 1 (the measurement closer to the reference value indicates better water quality) (Schmedtje 2001; Swedish EPA 2010).

FISH SCALE ANALYSIS

Fish scales were removed using tweezers from the left side of the body between the dorsal fin and lateral line. The scales were cleaned with sterile water and dehydrated in a gradient of 30, 50, 70 and 90% ethanol and dried on filter paper (Esmaeili et al. 2012). The clean and dried scales were observed using the light microscope and scanning electron microscope (SEM) Zeiss Evo Series for chromatophore and microstructure analysis, respectively. Chromatophore analysis refers to the melanophore density that may be found in the lateral body scales of *O. mossambicus* (Acharya & Ovais 2007). By ignoring the shape, melanophore populations were observed in the posterior part of the scale using the light microscope and quantified as the number of chromatophores per mm^2 (chr/mm^2). The size of surface scales was measured using a calibrated eyepiece graticule. Prior to SEM analysis, the scale was mounted on an aluminium stub and coated with gold in a sputter coater. The scale microstructure observed included the spherule on the ridge and the number of pits between ridges or circuli (Figure 2).

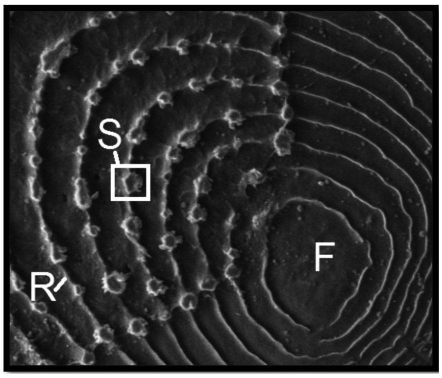

FIGURE 2. SEM image of a fish scale. The focus (F) is surrounded by the linear elevations of the bony layer, namely ridges (R). Along the ridges are urchin like spherules (S). The description of SEM scale microstructure is adopted from Sire (1988) and Sire and Akimenko (2004)

STATISTICAL ANALYSIS

The mean differences in chromatophore density and observed water quality parameters (TSS, aluminium and iron concentration) were analysed by ANOVA and Tukey's post-test at a significant level of 0.05. The correlation between observed water quality and scale density was determined by Pearson's correlation analysis at significant levels of 0.01 and 0.05.

RESULTS AND DISCUSSION

The observed water samples exhibited an average temperature of 28-30°C and a pH range of 6.0-8.4 (average 7.2), which were well within the safe limit for drinking water according to the WHO standard and the standard reference of the Indonesian Government's Regulations for water quality of class III (PPRI 2001; WHO 1996). The concentrations of aluminium, iron and TSS at the downstream sampling sites, P1 and P2, located near the mud spillway pipes, primarily in the dry season, were extremely higher than those stipulated under the water quality guidelines (WQGs) according the British Columbia criteria (Canadian WQGs 1998; Phippen et al. 2008) and Indonesian Government Regulation on WQGs for class III (PPRI 2001) (Table 1).

It was observed that the mud composition, which was dominated by clay, comprised SiO_2 (48.15-53.89%), Al_2O_3 (17.08-18.95%) and Fe_2O_3 (5.81-6.67%) (Zaenuddin et al. 2010). According to the observation by Hardjito et al. (2012), SiO_2 (31%), Al_2O_3 (5.6%) and Fe_2O_3 (42.8%) were responsible for the concentrations of TSS, aluminium and iron, primarily in the downstream areas near the mud discharge pipes. The highest quality ratio (QR) of the observed parameters upstream (control Ct) was at level 1, which indicated that they were within the reference values (Figure 3) and categorized as high quality for wildlife, irrigation and livestock (WQC class III). On the contrary, the mean QR value at the downstream stations,

TABLE 1. Concentrations of aluminium, iron and TSS and pH at the sampling sites. Concentrations of aluminium and iron in the water samples likely represent substantial colloidal contributions, as the samples were filtered only to 0.45 µm prior to analysis (Plumlee et al. 2008)

Sampling site	Sampling period	Aluminium (mg/L)	Iron (mg/L)	TSS (mg/L)	pH
Control	dry season	2.24±0.05	0.31±0.01	133.0±63.20	7.5±0.5
Control	dry season	3.14±0.01	0.29±0.02	380.5±58.01	7.5±0.5
Control	wet season	0.37±0.07	0.72±0.63	122.2±48.06	7.0±0.0
Control	wet season	2.08±0.50	1.38±0.60	40.0±12.00	6.9±0.2
P1	dry season	22.45±7.38	4.11±1.02	4176.8±1233.0	7.0±0.0
P1	dry season	23.70±8.97	6.17±1.67	1163.0±169.0	7.0±0.0
P1	wet season	11.70±1.93	6.60±1.10	672.0±116.0	7.3±0.3
P1	wet season	6.61±1.81	3.56±1.04	676.0±81.0	6.8±0.1
P2	dry season	23.41±3.18	4.52±0.36	1146.4±328.5	6.0±0.0
P2	dry season	12.82±2.02	3.19±0.18	459. 0±105.3	6.0±0.0
P2	wet season	13.53±5.86	6.97±2.85	1241.2±59.6	7.1±0.1
P2	wet season	2.31±0.65	1.97±0.15	582.0±95.3	7.3±0.1
P3	dry season	3.68±0.07	1.10±0.03	926.6±81.6	7.0±0.0
P3	dry season	3.70±0.09	1.09±0.03	709.0±189.4	7.0±0.0
P3	wet season	4.47±1.45	3.29±1.88	1013.3±179.0	7.0±0.1
P3	wet season	2.86±1.03	2.74±1.45	751.7±74.5	7.0±0.0
WQC		5*	1.7*	400**	6-9**

* Water quality criteria (WQC) of total aluminium and total iron for wild life, irrigation and livestock according to the British Columbia criteria (Canadian WQGs 1998; Phippen et al. 2008); ** Indonesian Government Regulation of Water Quality Criteria (WQC) for class III (PPRI 2001)

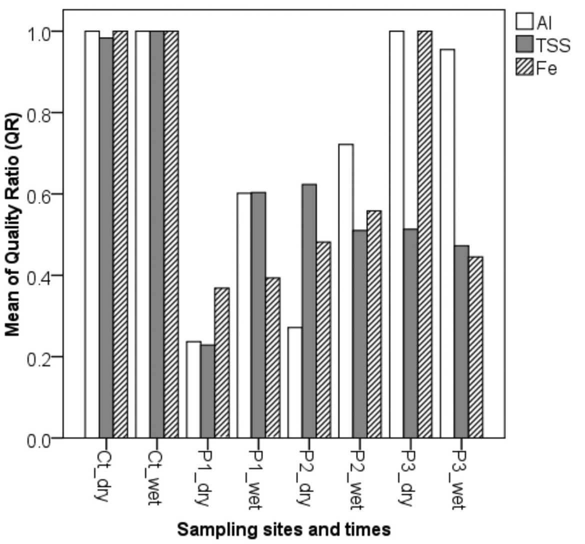

FIGURE 3. The quality ratio (QR) of observed parameters, total suspended solids (TSS), aluminium and iron at different sampling sites and times (dry and wet season). Interpretation of QR value is based on Schmedtje (2001): high (>0.95-100), good (>0.8-0.95), moderate (>0.6-0.8), poor (>0.3-0.6) and bad (0-0.3)

P1 and P2, were found to be between 0.23 and 0.72, which showed significant differences compared with the standard reference, with water body status between bad and moderate for wildlife, irrigation and livestock. Based on ANOVA and Tukey's post-test, there were significant differences in the QR values of aluminium between the control and stations P1 and P2 (p<0.05), while no significance was observed for P3 (Figure 1). The QR values of aluminium in P1 and P2 between the dry ([P1 = 0.24], [P2 = 0.27]) and wet seasons ([P1 = 0.60], [P2 = 0.72]) were found to be significant, whereas the dry season showed a lower QR value (Figure 3), indicating a higher concentration of aluminium contaminants compared to that of the wet season.

The mean QR values of iron found in station P1 (dry = 0.37; wet = -0.38) and P2 (dry = 0.48; wet = 0.56) were not found to be seasonally different. However, it was significantly lower than that of the control station (1.00). The QR value of TSS in the control station (0.98-1.00) was significantly lower than those of all the downstream stations (0.23-0.62).

Microscopic observation of fish scales showed a lower density of melanophores in the scales of fish from Porong river compared with fish scales from the control site, <50 chr/mm^2 (Figures 4 and 5). Plotting the mean of melanonophore density in scales of downstream fish exposed to higher suspended solids, aluminium and iron were significantly lower (5-31 chr/mm^2) compared with fish from the control upstream station (184 chr/mm^2) (Figure 5). However, there were no statistically significant spatial or seasonal differences in chromatophore density among the downstream stations. The Pearson correlation and scatter plot (Figure 6) demonstrate that the correlations of chromatophore density with the QR of TSS, iron and Al were on the level of r = 0.69; r = 0.56 and r = 0.35, respectively.

The results suggested that the low density of melanophores in the scales of caged fish at downstream sites was closely related to TSS, which was possibly enhanced by iron. The TSS concentration at Porong stations

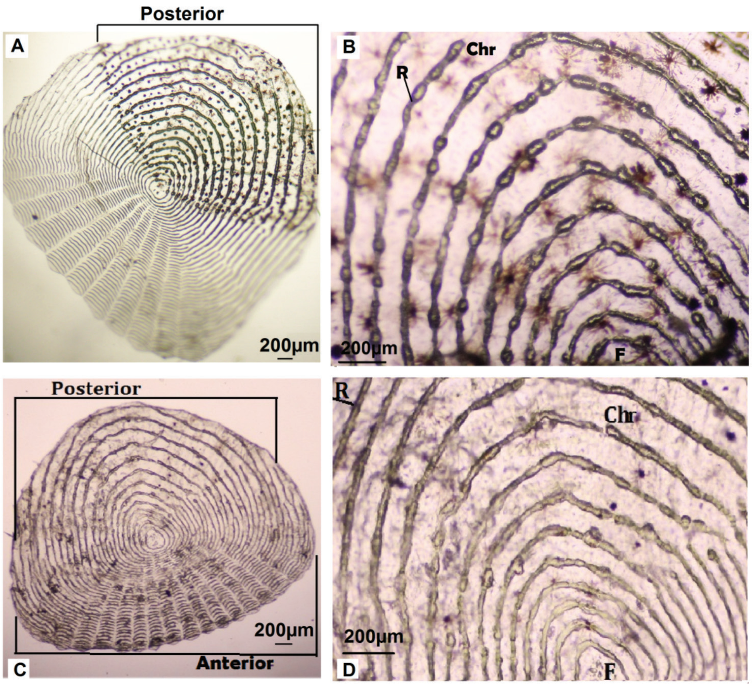

FIGURE 4. Light microscopic observation of chromatophores (Chr) that are localized at the posterior part of a tilapia scale (A). The density of chromatophores in the scale of caged fish from control site (A and B) was higher than P2 site (C and D). R = ridge (circulus) and F = Focus

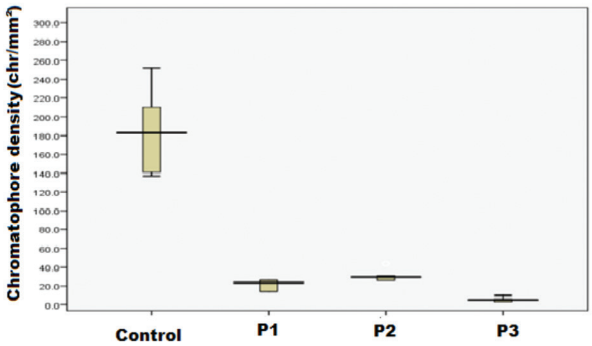

FIGURE 5. Chromatophore density comparison among the sampling sites

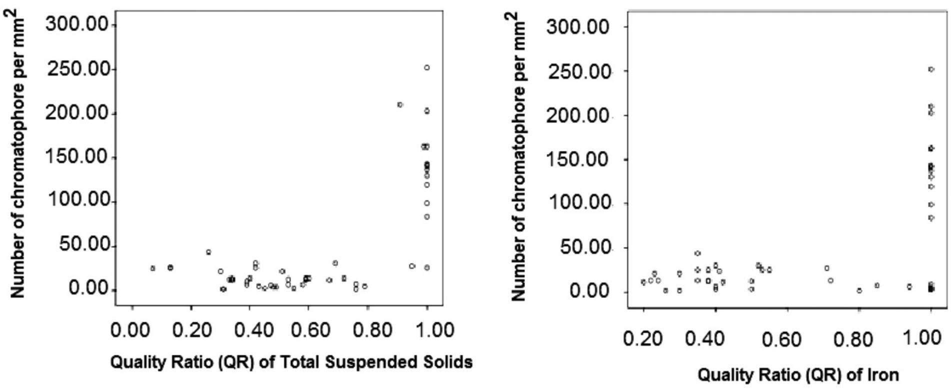

FIGURE 6. Relationship between chromatophore density and each of quality ratio (QR) of total suspended solids (TSS) and iron. QR of TSS and iron falling within the level of 1.00 (water quality was close to the standard reference) showed higher chromatophore density

at downstream (Table 1) contributed to the increase in turbidity, which partially scatters light and constrains the visual conditions (Gusen 2010). These disturbances potentially inhibit the optical sensory input from the hypothalamus to the melanotrope cells in the pituitary, which are responsible for secreting the alpha melanocyte stimulating hormone (α-MSH) (Dulcis & Spitzer 2008; Leclercq 2010; Vazquez-Martinez et al. 2001). Lower concentrations of α-MSH in *O. mossambicus* were shown to decrease melanophore motility (van Eys & Peters 1981). A further disturbance in motility led melanophores to undergo apoptosis (Sugimoto 2002), hence decreasing their density.

Moreover, the high concentration of iron in the downstream stations might be accumulated in the fish body. The excess of iron increasing the redox-active material in cells has the potential to cause oxidative damage to cellular constituents, which generates skin bleaching syndrome (Payne et al. 2001). Iron excess also leads to the degeneration and death of neuronal cells (Udipi et al. 2012). As previously described, a disturbance in neurons will affect pigmentation control, which ends with melanophore death (Sugimoto 2002). Hence, it was shown that the major pollutants in Sidoarjo mud, namely TSS and iron, had a negative influence on the melanophore population in fish scales.

The fish scales of the control scale microstructure (Figure 7) showed the usual normal shape of mineralized spherules, which have a structurally spherical shape and extensive bone material secretion at the peripheral site, hence their morphology is similar to that of a sea urchin (Kapoor & Khanna 2004; Sire 1988; Sire & Akimenko 2004). Spherules were located on the upper ridges of scales. The SEM structure of scale damage was highlighted by the irregularities of spherule shape, decrease in bone material secretion and increase in number of pits in the space between the ridges. The highest number of pits (27 pits per 0.1 mm^2 of scale surface) was found on scales from station P2 during the dry season (Figure 9). A smaller number of pits (3 pits per 0.1 mm^2 of scale surface) was found on scale samples from stations P1 and P3. However, scales from P1 demonstrated the abnormal spherule form (Figure 8), while the scale surface of controls had a rare appearance of pits (1 pit per 0.1 mm^2 of scale surface).

In general, the fish scale consists of a thin layer of bone that is formed by osteoblastic cells on its outer surface in a manner similar to the formation of dermal bone. Excess bony material accumulates in ridges or circuli (Dietrich 1953). A scale consists of a plate of collagenous tissue, with superficial mineralization, surrounded by scleroblasts and fibroblasts. The region between the epidermis and scale surface consists of mineralized spherules that are formed by the accumulation of substances, probably synthesized by the epidermal basal cells. Urchin-like spherules are localized in the posterior region of the scale, which is covered by epidermis and visible at the epidermis-scale interface. These spherules incorporate the inner boundary of epidermis and the scale surface (Kapoor & Khanna 2004; Sire 1988; Sire & Akimenko 2004).

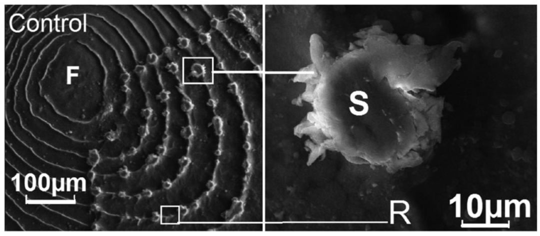

FIGURE 7. SEM images of a control fish scale. The scale surface showed the usual normal shape of urchin-like spherules (S) with a rare appearance of pit areas between the ridges (R)

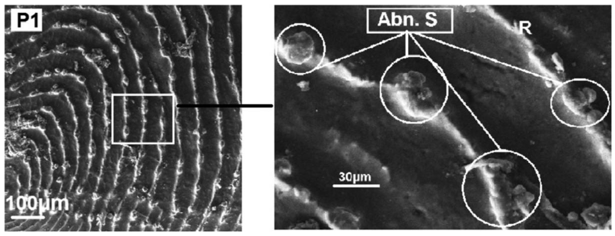

FIGURE 8. SEM images of a fish scale from station P1 exhibiting abnormal spherules (Abn. S) along the ridges (R)

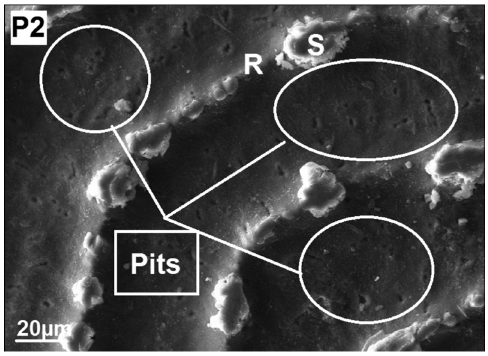

FIGURE 9. SEM image of a fish scale from station P2 marked by the high number of pits (circles) in the spaces between ridges (R)

Iron and aluminium might also act on scales. At neutral pH, the high concentrations of iron, aluminium and Sidoarjo mud particles (size <10 μm) that were found in the area of downstream stations might have accumulated on the scales (Elangovan et al. 1999; Plumlee et al. 2008), thus inhibiting scale formation, which could lead to scale microstructure deformation. These alterations were marked by the irregularity of spherule architectures (Figure 8).

The acidic condition observed at station P2 during the dry season (pH = 6.0) provides suitable conditions for aluminium and iron to dissolve, hence becoming bioavailable to the scale (Huang & Keller 1972). The high number of pits on the scale surface (Figure 9) might be related to the scale defect caused by aluminium and iron contamination. Aluminium potentially forms tight complexes with bone collagen causing cross linkage, destroying bone matrix and leading to mineralization defects similar to rickets (soft and weakened bones) (Malluche 2002; Zhu et al. 1990). Moreover, it was observed that bone iron content was increased in the iron-overloaded mice compared with the controls. Iron-induced inhibition of osteoblast activity is mediated by ferritin and its ferroxidase activity (Zarjou 2010).

It is therefore hypothesized that excessive concentration of pollutants in Sidoarjo mud, primarily iron and TSS, could be evaluated by the low chromatophore densities in fish scales. Furthermore, scale microstructure deformations, coupled with pH measurement, could be a potential biomarker for aquatic monitoring of aluminium and iron contaminations.

CONCLUSION

The findings of this study indicate that chromatophore density and microstructure deformation of fish scales could be used as biomarkers to assess the effect of the Sidoarjo mud on aquatic life. This study also showed that water contaminated by Sidoarjo mud had significantly adverse effects on aquatic life.

ACKNOWLEDGMENTS

This work was supported by "Dirjen Pendidikan Tinggi" National Education Ministry (Indonesia) for the doctoral program scholarship and School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM). The physico-chemical and biological analyses were conducted at Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Indonesia.

REFERENCES

Acharya, L.S.K. & Ovais, M. 2007. α1 and α2 adrenoceptor mediated melanosome aggregatory responses *in vitro* in *Oreochromis mossambicus* (Peters) melanophores. *Indian Journal of Experimental Biology* 45: 984-991.

Allen, T., Awasthi, A. & Rana, S.V.S. 2004. Fish chromatophores as biomarkers of arsenic exposure. *Environmental Biology of Fishes* 71(1): 7-11.

Canadian WQGs. 1998. *Guidelines for Interpreting Water Quality (WQGs) Data Province of British Columbia, Ministry of Environment, Lands and Parks*. Accessed on January 5 2011. http://www.ilmb.gov.bc.ca/risc/pubs/ aquatic/interp/interp.htm.

Dietrich, M.A. 1953. A histological study of the development of the largemouth black bass (*Micropterus salmoides*). *Quarterly Journal of Microscopical Science* 94: 71-82.

Dulcis, D. & Spitzer, N.C. 2008. Illumination controls differentiation of dopamine neurons regulating behaviour. *Nature* 456: 195-201.

Elangovan, R., Balance, S., White, K.N., McCrohan, C.R. & Powell, J.J. 1999. Accumulation of aluminium by the freshwater crustacean *Asellus aquaticus* in neutral pH. *Environmental Pollution* 106(3): 257-263.

Esmaeili, H.R., Gholamifard, A., Zarei, N. & Arshadi, A. 2012. Scale structure of a cyprinid fish, *Garra rossica* (Nikol'skii, 1900) using scanning electron microscope (SEM). *Iranian Journal of Science and Technology* 4: 487-492.

Esmaeili, H.R., Teimory, A. & Hojat Ansari, T. 2007. Scale structure of cyprinid fish *Capoeta damascina* (Valenciennes in Cuvier & Valenciennes, 1842) using scanning electron microscope (SEM). *Iranian Journal of Science and Technology* 31(A3): 255-262.

Gusén, A. 2010. Environmental variation phenotypic plasticity: The effect of water visibility on body pigmentation in perch (*Perca fluviatilis* L.). Master Thesis. Disciplinary Domain of Science and Technology, Biology, Biology Education Centre, Uppsala University, Sweden (Unpublished).

Hardjito, D., Gunadi, A., Wibowo, M. & Christianto, D. 2012. Pozzolanic activity assessment of lusi (lumpur Sidoarjo) mud in semi high volume pozzolanic mortar. *Materials* 5: 1654-1660.

Huang, W.H. & Keller, W.D. 1972. Geochemical mechanics for the dissolution, transport, and deposition of aluminum in the zone of weathering. *Clay Minerals* 20: 69-74.

Istadi, B., Pramono, G.H., Sumintadireja, P. & Alam, S. 2009. Modelling study of growth and potential geohazard for LUSI mud volcano East Java, Indonesia. *Journal Marine and Petroleum Geology* 26: 1724-1739.

Kapoor, B.G. & Khanna, B. 2004. *Ichthyology Handbook*. 1st ed. Berlin, Heidelberg: Springer. pp. 55-58.

Leclercq, E., Taylor, J.F. & Migaud, H. 2010. Morphological skin colour changes in teleosts. *Fish and Fisheries* 11: 159-193.

Malluche, H. 2002. Aluminium and bone disease in chronic renal failure. *Nephrology Dialysis Transplantation* 17: 21-24.

Payne, J.F., French, B., Hamoutene, D., Yeats, P., Rahimtula, A., Scruton, D. & Andrews, C. 2001. Are metal mining effluent regulations adequate? Identification of a novel bleached fish syndrome in association with iron-ore mining effluents in Labrador, Newfoundland. *Aquatic Toxicology* 52: 311-317.

Phippen, B., Horvath, C., Nordin, R. & Nagpal, N. 2008. *Ambient Water Quality Guidelines for Iron*. Overview Report, Water Stewardship Division Ministry of Environment, Province of British Columbia.

Plumlee, G.S., Casadevall, T.J., Wibowo, H.T., Rosenbauer, R.J., Johnson, C.A., Breit, G.N., Lowers, H.A., Wolf, R.E., Hageman, P.L., Goldstein, H., Anthony, M.W., Berry, C.J., Fey, D.L., Meeker, G.P. & Morman, S.A. 2008. Preliminary analytical results for a mud sample collected from the lusi mud volcano, Sidoarjo, East Java, Indonesia. U.S. *Geological Survey Open-File Report 2008*.

PPRI (Peraturan Pemerintah Republik Indonesia). 2001. Indonesian government regulation for water quality, No. 82.

Promwikorn, W., Boonyoung, P. & Kirirat, P. 2005. Histological characterization of cuticular depositions throughout the moulting cycle of the black tiger shrimp (*Penaeus monodon*). *Songklanakarin Journal of Science and Technology* 27(3): 499-509.

Radhakrishnan, M.V., Hemalatha, S. & Paul, V.I. 2000. Effect of cadmium chloride on the melanophores of *Channa striatus* (Bloch). *Indian Journal of Fisheries* 47: 135-141.

Rishi, K.K. & Jain, M. 1998. Effect of toxicity of cadmium on scale morphology in *Cyprinus carpio* (Cyprinidae). *Bulletin of Environmental Contamination and Toxicology* 60: 323-328.

Schmedtje, U. 2001. *Assessment of the Ecological Status and Classification of Surface Water Bodies*. UN-ECE Workshop, Approaches and Tools for River Basin Management.

Shikha, S. & Sushma, D. 2011. Effect of fly ash pollution on fish scales. *Research Journal of Chemical Sciences* 1: 24-28.

Sire, J.Y. 1988. Evidence that mineralised spherules are involved in the formation of the superficial layer of the elasmoid scale in the cichlids *Hemichromis bimaculatus* and *Cichlasoma octofasciatum* (Pisces, Teleostei): An epidermal active participation? *Cell Tissue Research* 253: 165-172.

Sire, J.Y. & Akimenko, M.A. 2004. Scale development in fish: A review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). *The International Journal of Developmental Biology* 48(2-3): 233-247.

Sugimoto, M. 2002. Morphological color changes in fish: Regulation of pigment cell density and morphology. *Microscopy Research and Technique* 58: 496-503.

Sukresno, B., Priyono, B., Zahrudin, D.A. & Subki, B.A. 2008. Investigation of total suspended matter in Porong region using aqua-modis satellite data and numerical model. *Research Report, Institute for Marine Research and Observation (IMRO)*.

Swedish EPA (Swedish Environmental Protection Agency). 2010. Status, potential and quality requiremenets for lakes, watercourses, coastal and transitional water. *A Handbook on How Quality Requirements in Bodies of Surface Water Can Be Determined and Monitored*. Naturvardsverket, Stockholm.

Talwar, P.K. & Jhingran, A.G. 1992. *Inland fishes of India and Adjacent Countries*. Vol-1 and Vol-2. Oxford and IBH Publishing Co. PVT. Ltd. New Delhi, Bombay and Calcutta. p. 1063.

Tang, S.M., Orlic, I., Yu, K.N., Sanchez, J.L., Thong, P.S.P., Watt, F. & Khoo, H.W. 1997. Nuclear microscopy study of fish scales. *Nuclear Instruments and Methods in Physics Research* 130: 396-401.

Udipi, S., Ghugre, P. & Gokhale, C. 2012. Iron, oxidative stress and health. In *Oxidative Stress - Molecular Mechanisms and Biological Effects*, edited by Lushchak, V.I. & Semchyshyn, H.M. http://www.intechopen.com/books/oxidative-stress-molecular-mechanisms-and-biological-effects/iron-oxidative-stress-and-health-authors.

USEPA (U.S. Environmental Protection Agency). 1971. *Method 160.2. Residue, Non-filterable (Gravimetric, Dried at 103-105°C)*. http://www.caslab.com/EPA-Methods/PDF/EPA-Method-160-2.pdf.

USEPA (U.S. Environmental Protection Agency). 1994. *Methods 200.7. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry.* Environmental Monitoring Systems Laboratory Office of Research and Development, U. S. Environmental Protection Agency Cincinnati, Ohio.

USGS. 2011. Water Science Glossary of Terms. U.S. Geological Survey. Accessed on February 1 2012. http://water.usgs.gov/edu/dictionary.html.

van Eys, G.J. & Peters, P.T. 1981. Evidence for a direct role of alpha-MSH in morphological background adaptation of the skin in *Sarotherodon mossambicus*. *Cell and Tissue Research* 217: 361-372 .

Vazquez-Martinez, R., Peinado, J.R., Gonzalez, J.L., Desrues, L., Tonon, M.C., Vaudry, H., Gracia-Navarro, F. & Malagon, M.M. 2001. Melanotrope cell plasticity: A key mechanism for the physiological adaptation to background color changes. *Endocrinology* 142: 3060-3067.

WHO (World Health Organization). 1996. *pH in Drinking Water, Originally Published by Guidelines for Drinking Water Quality*. 2nd ed. Volume 2. World Health Organization, Geneva.

Zaenuddin, A., Badri, I., Padmawidjaja, T., Humaida, H. & Sutaningsih, E. 2010. *Geological Phenomenon of Sidoarjo Mud Flow*. Geology Agency, Ministry of Energy and Mineral Resources.

Zarjou, A.V., Jeney, P., Arosio, M., Poli, E., Zavaczki, G. & Balla, J. 2010. Ferritin ferroxidase activity: A potent inhibitor of osteogenesis. *Journal of Bone and Mineral Research* 25(1): 164-172.

Zhu, J.M., Huffer, W. & Alfrey, A.C. 1990. Effect of aluminium on bone matrix inductive properties. *Kidney International* 38(6): 1141-1145.

Dewi Hidayati
Department of Biology, Faculty of Mathematics and Science (FMIPA)
Institut Teknologi Sepuluh Nopember
Surabaya-60111
Indonesia

Norela Sulaiman*, Ismail B.S & M. Shuhaimi-Othman
School of Environmental and Natural Resource Sciences
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor Darul Ehsan
Malaysia

Maria Elena de Bellard
Department of Biology
California State University, Northridge
USA

*Corresponding author; email: vozela@ukm.edu.my

Received: 10 October 2014
Accepted: 23 July 2016