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)

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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 (<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

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 m³/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 m³/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 exceeds 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](image-url)
which represents the area of initial discharge and at P2 (7°32' 42.91"S; 112°43' 30.32"E) located approximately 6 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 the shape, melanophore populations were observed in the chromatophore and microstructure analysis, respectively. 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$_2$O$_3$ (17.08-18.95%) and Fe$_2$O$_3$ (5.81-6.67%) (Zaenuddin et al. 2010). According to the observation by Hardjito et al. (2012), SiO$_2$ (31%), Al$_2$O$_3$ (5.6%) and Fe$_2$O$_3$ (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,
The significance was observed for P3 (Figure 1). The differences in the water body status between bad and moderate for wildlife, irrigation and livestock. 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 (<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/mm² (Figures 4 and 5). Plotting the mean of melanophore density in scales of downstream fish exposed to higher suspended solids, aluminium and iron were significantly lower (5-31 chr/mm²) compared with fish from the control upstream station (184 chr/mm²) (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

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)

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Sampling period</th>
<th>Aluminium (mg/L)</th>
<th>Iron (mg/L)</th>
<th>TSS (mg/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>dry season</td>
<td>2.24±0.05</td>
<td>0.31±0.01</td>
<td>133.0±63.20</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>Control</td>
<td>dry season</td>
<td>3.14±0.01</td>
<td>0.29±0.02</td>
<td>380.5±58.01</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>Control</td>
<td>wet season</td>
<td>0.37±0.07</td>
<td>0.72±0.63</td>
<td>122.2±48.06</td>
<td>7.0±0.0</td>
</tr>
<tr>
<td>Control</td>
<td>wet season</td>
<td>2.08±0.50</td>
<td>1.38±0.60</td>
<td>40.0±12.00</td>
<td>6.9±0.2</td>
</tr>
<tr>
<td>P1</td>
<td>dry season</td>
<td>22.45±7.38</td>
<td>4.11±1.02</td>
<td>4176.8±1233.0</td>
<td>7.0±0.0</td>
</tr>
<tr>
<td>P1</td>
<td>dry season</td>
<td>23.70±8.97</td>
<td>6.17±1.67</td>
<td>1163.0±169.0</td>
<td>7.0±0.0</td>
</tr>
<tr>
<td>P1</td>
<td>wet season</td>
<td>11.70±1.93</td>
<td>6.60±1.10</td>
<td>672.0±116.0</td>
<td>7.3±0.3</td>
</tr>
<tr>
<td>P1</td>
<td>wet season</td>
<td>6.61±1.81</td>
<td>3.56±1.04</td>
<td>676.0±81.0</td>
<td>6.8±0.1</td>
</tr>
<tr>
<td>P2</td>
<td>dry season</td>
<td>23.41±3.18</td>
<td>4.52±0.36</td>
<td>1146.4±328.5</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>P2</td>
<td>dry season</td>
<td>12.82±2.02</td>
<td>3.19±0.18</td>
<td>459.0±105.3</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>P2</td>
<td>wet season</td>
<td>13.53±5.86</td>
<td>6.97±2.85</td>
<td>1241.2±59.6</td>
<td>7.1±0.1</td>
</tr>
<tr>
<td>P2</td>
<td>wet season</td>
<td>2.31±0.65</td>
<td>1.97±0.15</td>
<td>582.0±95.3</td>
<td>7.3±0.1</td>
</tr>
<tr>
<td>P3</td>
<td>dry season</td>
<td>3.68±0.07</td>
<td>1.10±0.03</td>
<td>926.0±81.6</td>
<td>7.0±0.0</td>
</tr>
<tr>
<td>P3</td>
<td>dry season</td>
<td>3.70±0.09</td>
<td>1.09±0.03</td>
<td>709.0±189.4</td>
<td>7.0±0.0</td>
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<tr>
<td>P3</td>
<td>wet season</td>
<td>4.47±1.45</td>
<td>3.29±1.88</td>
<td>1013.0±179.0</td>
<td>7.0±0.1</td>
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<tr>
<td>P3</td>
<td>wet season</td>
<td>2.86±1.03</td>
<td>2.74±1.45</td>
<td>751.7±74.5</td>
<td>7.0±0.0</td>
</tr>
</tbody>
</table>

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 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 melanocyte 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 mm² 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 mm² 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 mm² 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).

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.

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