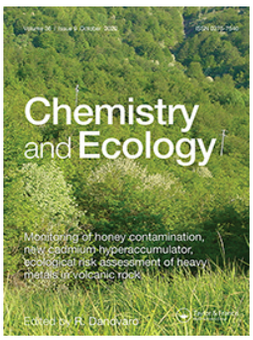

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RESEARCH ARTICLE

Oxidative responses of macro-invertebrates in relation to environmental variables in rivers of East Kalimantan, Indonesia

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ABSTRACT

The condition of river water quality can be considered as a potential source of oxidative stress of river organisms. The objective of this study was to evaluate the activities of superoxide dismutase (SOD), catalase (CAT) and the levels of malondialdehyde (MDA) in three genera of aquatic insects namely *Chrironominae*, *Gomphus* and *Lestes* collected from Karang Mumus River, Pampang River, and Nabah River of East Kalimantan, Indonesia. Aquatic insects were collected using Ekman-Grab, Surber net and Kick net. Determinations of SOD, CAT and MDA were conducted in all aquatic insect samples. Physical-chemical parameters of environmental quality were also measured in all rivers. Visual observation showed that the turbidities of water were in the order: Karang Mumus River>Pampang River> Nabah River. The highest SOD, CAT and MDA were recorded in *Chironominae* and *Gomphus* live in the Karang Mumus River, and the lowest recorded in Nabah River. The SOD and CAT activities in *Lestes* were not significantly different in all rivers, however *Lestes* in Nabah showed the highest MDA. The oxidative responses of *Chironominae* and *Gomphus* most likely affected by the water turbidity. Meanwhile, the high level of MDA in *Lestes* most probably related to the metals' levels both in sediment and water.

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KEYWORDS
Antioxidant enzyme; water quality; oxidative stress; aquatic insect; river; East Kalimantan

1. Introduction

Water quality of a river is influenced by several parameters like land use, settlement patterns, farming and industrial activities around that river [1,2]. Patang et al. [1] reported that rivers in East Kalimantan Province of Indonesia are also facing the problems with human activities like coal mines construction, harbour and oil-palm plantations exactly along the river banks. These activities destroy the water quality of rivers and consequently lead to a certain change in the benthic macroinvertebrates community structure [1]. During their study, three co-dominant genera of macroinvertebrate fauna were found in all studied rivers in East Kalimantan, i.e. *Chironominae*, *Gomphus* and *Lestes* [1]. These faunas,

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therefore interesting to be further studied in order to know their oxidative responses to the water quality of several rivers in East Kalimantan.

Change of water quality in river can be considered as a potential source of oxidative stress of river organisms [3–5]. In organisms, reactive oxygen species (ROS) are continuously produced and eliminated to maintain a steady-state ROS concentration. This dynamic equilibrium can be disturbed by water quality change leading to enhanced ROS level and damage to cellular constituents referred to as oxidative stress [6,7]. ROS that include superoxide radical (O_2^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen (O_1) can damage the cells by causing the oxidation of unsaturated lipids as well as amino and nucleic acids [6,8]. This generation of ROS can be positively correlated with environmental levels of DO [9,10]. Thus very high supersaturated concentrations of DO can be detrimental to both plants and animals [10,11]. Although counter intuitive, the production of ROS may also be stimulated by hypoxic conditions [8,9]. In addition, the generation of ROS can be stimulated by various pollutants such as organochlorine pesticides, chlorophenols, polychlorobiphenyls and heavy metals [12,13].

ROS are generally under tight control of antioxidant defense system [13]. The antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), are considered vital first-line defenses against ROS toxicity. When the rate of ROS generation exceeds the antioxidant capacity of cells, excessive ROS can provoke oxidative stress, which causes the damage of polyunsaturated fatty acid chains in cells and leads to lipid peroxidation (LPO) [14]. Malondialdehyde (MDA) as one of the final products of LPO is responsible for cell membrane damage [6,15,16]. Using this fact, we envisioned research on oxidative stress on some benthic macroinvertebrates in three rivers of East Kalimantan. In our study, we evaluated the activity of SOD, CAT and the level of MDA in three genera of aquatic insects.

2. Materials and methods

2.1. Study area

The study was conducted mainly on three rivers of East Kalimantan (Figure 1) following the study of Patang et al. [1], especially for Karang Mumus River and Pampang River [1], and Nabah River. The selection of the sampling stations was based on the pollutant loads and the magnitude of human activities along the rivers. Detailed location information of the sampling sites is presented in Table 1.

2.2. Sampling and identification of aquatic insects

Sampling of macroinvertebrates including aquatic insects was conducted in June to July 2016. Samples of benthic macroinvertebrate were collected using Ekman-Grab (25×25 cm^2) for muddy substrate, Surber net (30×30 cm^2) for rocky substrate and Kick net for habitat containing dense aquatic plants. Collected organisms were rinsed with water, separated from debris and sediment using forceps, and preserved in 70% ethanol. The macroinvertebrates were identified to the family level using [17–23].

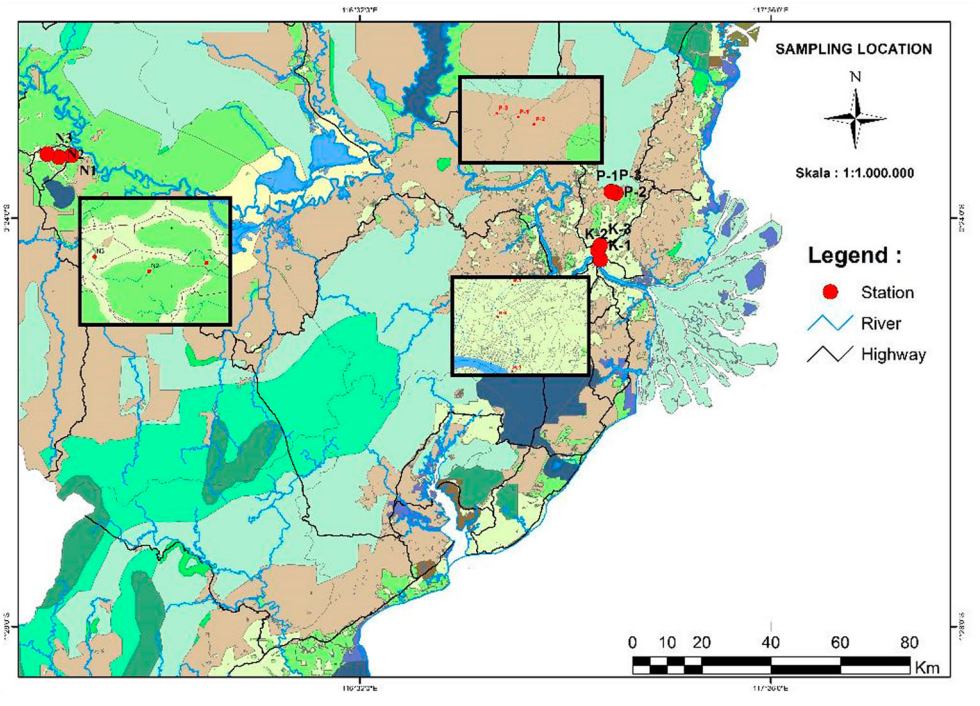

Figure 1 . Sampling locations of benthic macroinvertebrate and water quality parameters.

2.3. Water quality measurement

A wide range of water quality parameters were measured at all sampling locations, with key parameters being measured were dissolved oxygen (DO), pH, biological oxygen demand (BOD), temperature, total phosphate, nitrate, turbidity, total dissolved solid (TDS), hardness, and salinity. Measurement of heavy metal was conducted both in water and sediment. The analytical methods of water and sediment quality parameters were followed by Standard Method for the Examination of Water and Wastewater [24].

2.4. Determination of SOD

Due to the small size of animal analysed, we used pooled sample. One pooled sample contains approximately 40 individuals of *Chironominae* larvae, 20 individuals of *Gomphus*

Table 1. Detailed information of sampling stations.

Rivers's name	Sampling station	Geographical position	Surrounding environment
KarangMumus [1]	K-1	00°30.491′S and 117°09.426′E	Populated area, population density: 12,785 people/km^2, harbour with motor ship activities, usage of river for bathing, washing, and latrines
	K-2	00°29.073′S and 117°09.064′E	
	K-3	00°28.238′S and 117°09.442′E	
Pampang [1]	*P*-1	00°19.927′S and 117°11.555′E	Natural habitat, activity of oil-palm plantation, population density: 66 people/km^2
	P-2	00°20.076′S and 117°11.874′E	
	P-3	00°19.850′S and 117°11.132′E	
Nabah	N-1	00°14.191′S and 115°47.185′E	Natural habitat
	N-2	00°14.473′S and 115°45.237′E	
	N-3	00°13.976′S and 115°43.384′E	

larvae and 20 individuals of *Lestes* larvae, respectively. Three pooled samples were analysed for each station. All reagents used for SOD analysis were supplied by EnzyChrom TM (ESOD-100, BioAssay Systems, Hayward, CA, USA). After collection, the samples were rinsed thoroughly in ice-cold phosphate-buffered saline (PBS, 0.01 M, pH = 7.4), and stored at −20°C until analysis (≤5 days). 100 mg of each pooled animal tissue was weighed and homogenised at 5 mL/g in cold lysis buffer (50mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100) with a motorised mini handheld homogeniser. The homogenates were then centrifuged at 12000*g* for 5 min at 4°C to obtain the supernatant.

To measure the activities of SOD, all of the standards and the samples experienced the following treatments: 20 µL of standard and 20 µL sample were added to each well, then immediately 160 µL of assay buffer, 5 µL of Xanthine and 5 µL of WST-1 were added to each well and mixed well. Then quickly added 20 µL xanthine oxidase (XO) enzyme to each assay well, mixed well and immediately read their optical density (OD) using an automatic micro plate reader (Bio-Rad, model iMark, Japan) at 440 nm. The plate was then incubated for 60 min at 25°C in the dark, and read again their OD using the same automatic micro plate reader at 440 nm. The activities of SOD were determined using the appropriate standard curves and data were expressed as U/mL. Afterwards, all data should be normalised to the weight of the tissues.

2.5. Determination of CAT

All reagents used for CAT analysis were supplied by EnzyChrom TM (ECAT-100, BioAssay Systems, Hayward, CA, USA). After general preparation, 100 mg of each pooled tissues was weighed and homogenised at 200 µL of cold PBS, then centrifuged at 14000*g* for 10 min at 4°C to obtain the supernatant. Ten (10) µL of sample was transferred into well. In addition of each assay run, one sample blank well was prepared and filled with 10 µL of assay buffer.

Approximately 400 µL of assay buffer was added into positive control tube and mixed well. Then 10 µL of the reconstituted positive control was transferred into separate wells. Approximately 1 µL of the 4.8 mM H_2O_2 (by mixing 5 µL 3% H_2O_2 and 914 µL dH_2O) with 95 µL assay buffer was added into each well, then added 90 µL of the 50 µM substrate into these wells, mixed well and incubate for 30 min at room temperature to initiate the catalase reaction. Then 100 µL of detection reagent was added into each well, mixed well, incubate for 10 min and then read their OD using the automatic microplate reader (Bio-Rad, model iMark, Japan) at 470 nm. The activities of CAT were determined using the appropriate standard curves and data were expressed as U/L. Then all data should be normalised to the weight of the tissues.

2.6. Determination of MDA

MDA assay was conducted in accordance with the procedure of Bioxytech, MDA-586 TM (Oxis Research TM, Portland, USA). Approximately 10 µL of probucol was added into each assay tube, then added 200 µL of sample or standard to the respective assay tubes. After that 640 µL of diluted R1 solution (*N*-methyl-2-phenylindole in acetonitrile) was added into each tube and mixed well with vortex. Further added 150 µL of R2 solution (concentrated hypo chloric acid) as stopper into each tube, mixed well using vortex and

incubated at 45°C for 60 min. Furthermore, the samples were centrifuged at 10000*g* for 10 min to obtain a clear supernatant. These supernatants then were transferred into cuvettes to measure their OD at 586 nm using an automatic microplate reader. The levels of MDA were determined using the appropriate standard curves and data were expressed as nM.

2.7. Statistical analysis

The data were tested for distribution using the Kolmogorov–Smirnov test. Subsequently, the data were subject to one-way ANOVA to compare the activities of SOD, CAT and the levels of MDA in each benthic organism among sampling locations, respectively. Tukey's test was employed for multiple means comparisons at a significance level of 0.05 when significant differences were detected ($p < 0.05$). A Principal Component Analysis (PCA) was used to examine which environmental components (dissolved oxygen (DO), pH, biological oxygen demand (BOD), temperature, total phosphate, nitrate, turbidity, total dissolved solid (TDS), hardness, salinity, heavy metals both in water and sediment) play a dominant role in grouping of sampling sites. The PCA was carried out using the open-source software PASTprogramVersion3b7 [1].

3. Results and discussion

The activities of SOD, CAT and the concentration of MDA in three genera of benthic-macroinvertebrates in three rivers of East Kalimantan are presented in Figures 2–4 respectively. The lowest activity of SOD in *Chironominae* is presented in Nabah, meanwhile the highest one recorded in Karang Mumus. In *Lestes*, the highest of SOD activity was presented in Nabah, while the activities of SOD in Karang Mumus and Pampang were significantly different. In contrary, Nabah presented the lowest SOD in *Gomphus*, whereas the SOD activities in Karang Mumus and Pampang were not significantly different (Figure 2).

The lowest activities of CAT in *Chironominae* and *Gomphus* were noted in Nabah, while Karang Mumus and Pampang presented the similar values. The activities of CAT in *Lestes* were not significantly different in all locations (Figure 3).

The highest level of MDA in *Chironominae* was presented in Karang Mumus, meanwhile the levels of MDA in Pampang and Nabah were not significantly different (Figure 4). Nabah presented the highest level of MDA in *Lestes*, while the levels of MDA in Karang Mumus and Pampang were not significantly different. The highest value of MDA in *Gomphus* was recorded in Karang Mumus, meanwhile the lowest level of MDA was noted in Nabah.

The physical and chemical parameters of water and sediment quality of the sampling locations are presented in Table 2. According to the PCA, sampling sites N-1, N-2 and N-3 (Nabah) show a strong affinity to high Cd and Cu in sediment, Fe, Cu and Zn in water, transparency and substrate type. Sampling sites P-1, P-2 and P-3 (Pampang) demonstrate a strong affinity to high conductivity, pH, TDS, hardness, Mn, Cd and Pb waters and Mn sediment, while sampling sites K-1, K-2 and K-3 (Karang Mumus) present a strong affinity to high values of BOD, DO, turbidity, salinity, temperature, Zn, Pb and Fe sediments (Figure 5). Visual observation showed that the turbidities of water were in the order: Karang Mumus River > Pampang River > Nabah River.

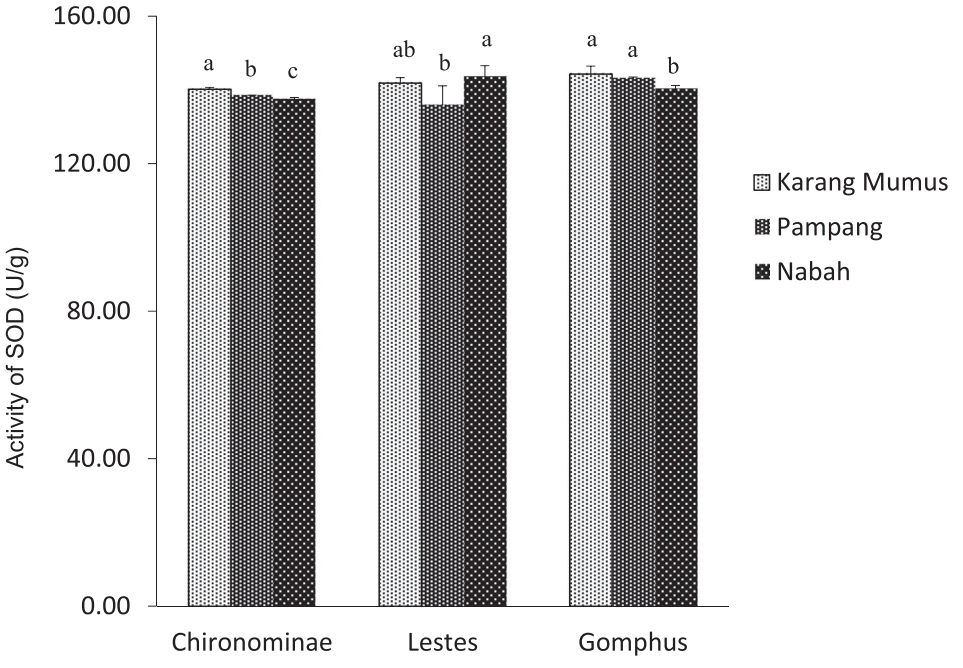

Figure 2. The activities of SOD of some benthic macroinvertebrates ($P < 0.05$, lowercase alphabet (a > b > c) explain the significant differences).

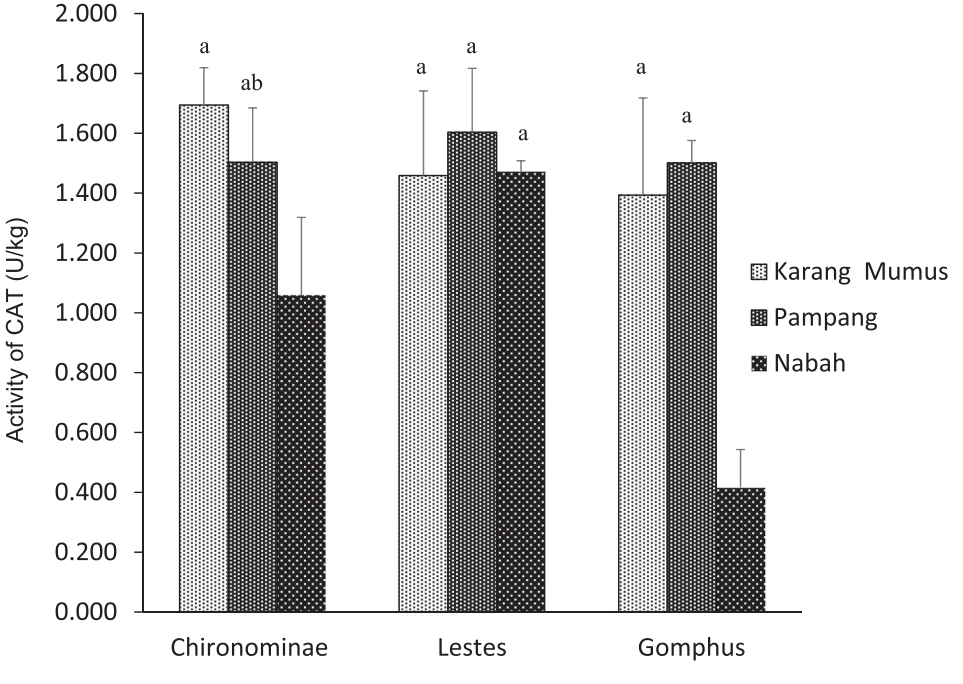

Figure 3. The activities of CAT of some benthic macroinvertebrates ($P < 0.05$, lowercase alphabet (a > b) explain the significant differences).

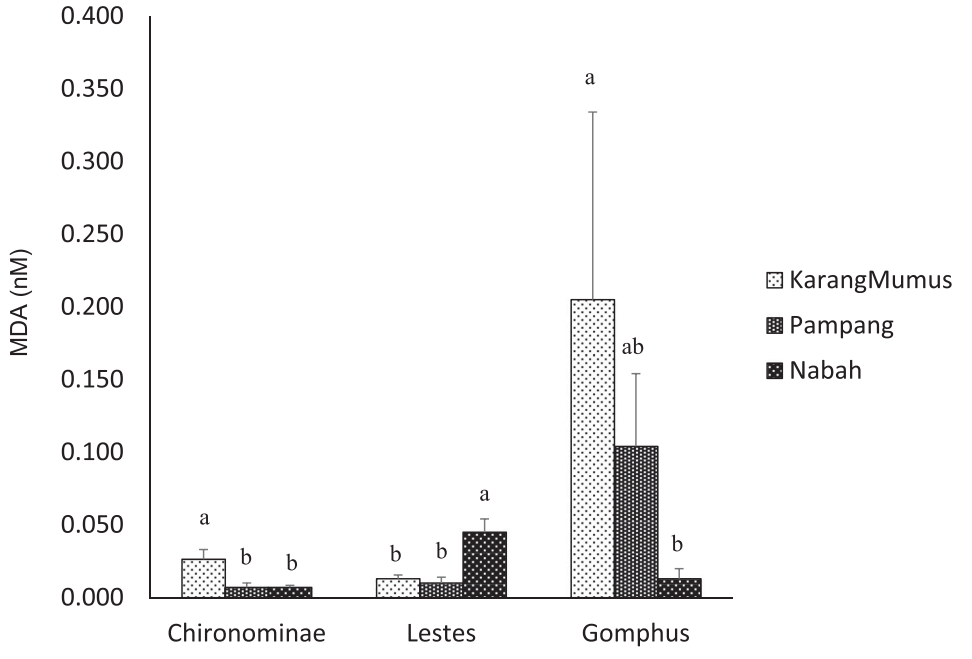

Figure 4. The levels of MDA of some benthic macroinvertebrates ($P < 0.05$, lowercase alphabet (a > b) explain the significant differences).

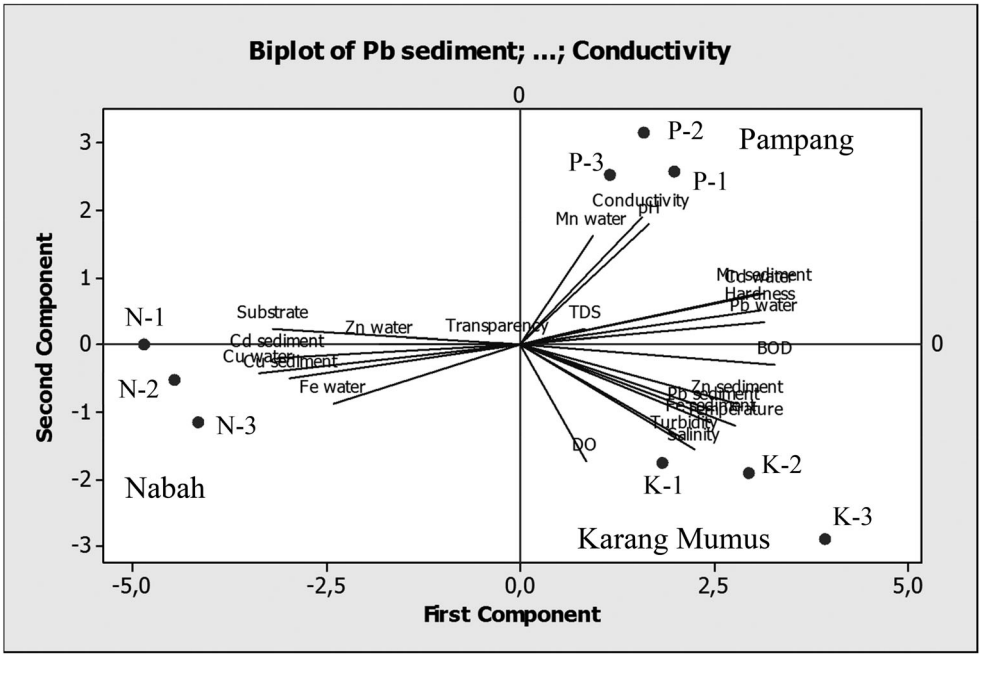

Figure 5. Diagram of sampling sites by Principal Component Analysis based on the physical–chemical variables in three rivers of East Kalimantan, Indonesia.

Table 2. Physical–chemical parameters in each sampling site of three rivers in East Kalimantan, Indonesia.

Parameter	Unit	K-1	K-2	K-3	Mean ± SD	*P*-1	*P*-2	*P*-3	Mean ± SD	N-1	N-2	N-3	Mean ± SD
Pb sediment	mg/kg	0.925	0.823	2.103	1.284±0.711	0.725	0.612	0.668	0.668±0.057	0.336	0.421	0.268	0.342±0.077
Cu sediment	mg/kg	0.009	0.009	0.009	0.009±0.000	0.009	0.009	0.009	0.009±0.000	0.75	0.801	1.674	1.075±0.519
Zn sediment	mg/kg	2.855	2.108	4.255	3.073±1.090	0.931	1.431	2.308	1.557±0.697	0.252	0.455	0.454	0.387±0.117
Mn sediment	mg/kg	9.363	7.266	7.781	8.137±1.093	10.325	9.336	9.821	9.827±0.495	0.252	0.455	0.454	0.387±0.117
Cd sediment	mg/kg	0.001	0.001	0.001	0.001±0.000	0.057	0.001	0.001	0.020±0.032	0.767	0.604	0.283	0.551±0.246
Fe sediment	mg/kg	12.244	37.891	25.621	25.252±12.827	5.211	7.641	4.426	5.759±1.676	0.541	0.411	0.53	0.494±0.072
Pb water	mg/L	0.556	0.562	0.552	0.557±0.005	0.696	0.501	0.458	0.552±0.127	0.281	0.185	0.279	0.248±0.055
Cu water	mg/L	0.008	0.008	0.008	0.008±0.000	0.008	0.008	0.008	0.008±0.000	0.009	0.009	0.009	0.009±0.000
Zn water	mg/L	0.001	0.001	0.001	0.001±0.000	0.001	0.001	0.001	0.001±0.000	0.207	0.001	0.001	0.070±0.119
Mn water	mg/L	0.002	0.002	0.002	0.002±0.000	0.455	0.828	0.028	0.437±0.400	0.007	0.002.	0.009	0.008±0.001
Cd water	mg/L	0.069	0.072	0.051	0.064±0.011	0.091	0.064	0.076	0.077±0.014	0.001	0.001	0.001	0.001±0.000
Fe water	mg/L	0.379	0.001	0.001	0.127±0.218	0.001	0.001	0.001	0.001±0.000	0.227	0.339	0.362	0.309±0.072
DO	mg/L	8.1	7.9	8.2	8.07±0.15	6.7	7.1	6.8	6.87±0.21	6.71	7.01	8.31	7.34±0.85
pH	-	7.01	7.05	7.01	7.17±0.28	7.5	7.35	7.4	7.42±0.08	7	6.8	7	6.93±0.12
BOD	mg/L	1.12	2.01	1.55	1.56±0.45	1.03	1.06	1.05	1.04±0.02	0.0121	0.0365	0.0365	0.03±0.01
Temperature	°C	30.5	31	31	30.8±0.29	29	27	27	27.67±1.15	26	26.5	27	26.50±0.50
Salinity	ppt	0.6	0.6	0.6	0.60±0.00	0.07	0.09	0.11	0.09±0.02	0.1	0	0.1	0.07±0.06
Transparency	cm	80	80	70	76.67±5.77	70	120	30	73.33±45.09	80	80	80	80.00±0.00
Turbidity	NTU	15.5	20.1	33	22.87±9.07	17	2.3	2.2	7.18±8.50	6.02	6.31	6.49	6.27±0.24
TDS	mg/L	16.1	18	17.1	17.07±0.95	12.5	26.5	12.5	17.33±7.94	12	19	14	15.00±3.61
Substrate	-	1	1	1	1±0	2	1	3	2±1	4	4	4	4±0
Hardness	mg/L	44	40	96	60.00±31.24	64	72	76	70.67±6.11	0	0	0	0.00±0.00
Conductivity	mS.cm	35	32	35	34.00±1.73	174.2	201.8	242	206.00±34.09	2.27	2.46	2.61	2.45±0.17

Note: Substrate, 1 = mud, 2 = sand, 3 = pebbles, 4 = gravel.

The levels of SOD and CAT in *Chironominae* and *Gomphus* in Karang Mumus as the more turbid river were higher than those in clearer river (Nabah), meanwhile *Lestes* demonstrated inconsistent levels of SOD and CAT. *Gomphus* and *Lestes* belong to Odonata order, however, these animals presented the different oxidative responses to environmental parameters. The different levels of SOD and CAT *Chironominae*, *Gomphus* and *Lestes* could be due to the different responses of these animals to the environmental parameters of the location. Karang Mumus was visually turbid, had the highest level of turbidity and the highest level of BOD (as an indicator of organic pollution) than the other rivers. Interestingly, although Karang Mumus had the high level of BOD, the level of DO was high. This situation is most probably caused by highly motor ship activities such as stirring and aerating the river water [1]. Sriariyanuwath et al. [25] reported that chironomid assemblage composition found at sites was affected by a heavy pollution gradient (especially with high turbidity and high suspended solid) in the Pong river, Thailand. Arslan et al. [26] found that by comparison with the chemical water quality grading, *Gomphus* found abundantly in slightly polluted or moderate water quality, while *Chironomus* found abundantly in poor or very poor water quality. The parameters of water quality measured during their study consisted of temperature, pH, DO, NO_2^-N, NO_3^-N, total phosphorus and total nitrogen which contribute the high turbidity of the waters. Luoto [27] reported that *Chironomus* were most successful in location with elevated nutrient conditions, low oxygen concentrations and clay turbid hyper-eutrophic lakes. Suzuki et al. [28] observed that the SOD and CAT of caddisfly (*Stenopsyche marmorata*) larvae increased significantly after exposed to 500 and 2000 mg/L suspended solid at both 10°C and 25°C. Moreover, the generation of ROS can be positively correlated with environmental levels of DO [9,10]. Ross et al. [10] demonstrated that antioxidant enzymes (SOD, CAT and glutathione peroxidase (GPx)) in fish increased significantly in site with the highest DO cycling and the most DO supersaturation. While Rifkind et al. [9] and Jones [8] suggested that the production of ROS may also be stimulated by hypoxic conditions.

SOD and CAT are the vital first-line defenses against oxidative damage oxygen toxicity. They are essential for the conversion of ROS to harmless metabolites [29]. The dismutation of O_2^{-2} is scavenged to H_2O_2 and O_2 by SOD, and H_2O_2 is decomposed to non-toxic H_2O and O_2 by CAT [29–33]. The higher levels of SOD and CAT in *Chironominae* and *Gomphus* in Karang Mumus River confirm that these animals able to decompose the high amount of ROS by producing high quantity of SOD and CAT as the antioxidant defense system. While *Lestes* showed indifferent levels of SOD and CAT both in more turbid and clearer rivers.

The increase of MDA with increasing the levels of SOD and CAT in *Chironominae* and *Gomphus* indicated that lipid peroxidation has occurred in these macroinvertebrates. Suzuki et al. [28] reported that CAT activity and lipid peroxidation were significantly increased after exposed to 500 and 2000 mg/L suspended solid at 25°C. Lipid peroxidation occurs when ROS attack polyunsaturated fatty acids in cell membranes [34]. Cruz et al. [35] reported that CAT activity is positively and significantly correlated with LPO levels. Production of H_2O_2 as a precursor of hydroxyl radical increases with CAT activity and may promote lipid peroxidation [36]. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues [37]. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases and injuries in animals [38].

Although there were no significantly difference on the levels of SOD and CAT in *Lestes* in all rivers, however, the levels of MDA in Nabah were higher than those in Karang Mumus and Pampang. This indicates that *Lestes* suffers cellular injury in the clearer (high transparency) river compared in the more turbid river. It is most likely the specific environmental parameters of the location affect this cellular injury.

Heavy metal content in the Nabah river sediment (Cd and Cu) and in river water (Cu, Zn and Fe) (Figure 5) most probably be the cause of increased levels of lipid peroxidation in *Lestes*. Girgin et al. [39] reported that six Odonata species including *Lestes* were found closely related to cadmium, boron, iron and total hardness in the Ankara Stream and its tributaries, Turkey. In the laboratory, Odonata larvae exhibited high tolerance to Pb (462 mg Pb/L) and Cd (250 mg Cd/L), at least in terms of survivability for 7 days, while only exposures to Cu demonstrated any effect on mortality at concentrations above 150 mg Cu/L [40]. All of the concentrations of Pb, Cu, or Cd that caused mortality [40], however, were well above any concentration to which Odonata larvae would be exposed in the field. Simon et al. [41] observed the Cu and Zn concentrations in Odonata collected from the Tisza River Hungary which is contaminated by communal wastewater. Haque et al. [42] reported that the enzymatic activities of CAT and SOD in polychaete were increased with increasing level of zinc pyrithione (ZnPT) and inorganic Zn ($ZnCl_2$), whereas the levels of glutathione (GSH), glutathione peroxidase (GPx) and glutathione were decreased. Furthermore, Haque et al. [42] noted that increasing the activities of CAT and SOD increased the level of DNA damage of polychaete. Barker et al. [43] demonstrated that the levels of lipid peroxidation product malondialdehyde (MDA) in liver and heart of African catfish (*Clarias gariepinus*) juveniles increased concomitant with dietary iron (Fe) dose indicating heightened oxidative stress in catfish consuming diets high in Fe. The high MDA in *Lestes* of the present study could be the response of toxic effect of animal to metals in their habitat. MDA can form covalent protein–DNA adducts that are mutagenic [42,44] and possibly carcinogenic [45]. Therefore, more studies are needed to know whether the MDA contained in these animals affect the mutagenicity and the carcinogenicity in these animals.

4. Conclusion

In conclusion, the highest activities of SOD and CAT, and the highest levels of MDA were recorded in *Chironominae* and *Gomphus* live in the Karang Mumus River (as most turbid rivers), followed by those living in Pampang River and the lowest recorded in Nabah River (as a clear river). The activities of SOD and CAT in *Lestes* were not significantly different in all rivers, however, *Lestes* in the clear river (Nabah) showed the highest level of MDA. The oxidative responses of *Chironominae* and *Gomphus* were most likely affected by the water turbidity. Meanwhile, the high level of MDA in *Lestes* is most probably related to the level metals both in sediment and water.

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Disclosure statement

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Fatmawati Patang received her Ph. D degree from Universitas Airlangga Indonesia and is currently working at the University of Mulawarman, Samarinda Indonesia as an associate professor. Her research interest is fresh water ecology.

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