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The Spatial Distribution of Sulfate-Reducing Bacteria and Its Environmental Implication in Sediment from Zhanjiang Bay and Leizhou Bay, China

Xiang-yun Zeng[1], Feng Li[1,3]*, Yan-yan Liang[1], Jin-qin Lin[2], Hua-yang Gan[2], Xiao-lin Long[1], Kai Liang[2], Xin Liu[2], Zhen-hai Huo[2], Jia-sheng Wen[1], Jia-yu Yang[4], Wen-rou Huang[1] and Jue-ting Mo[1]

[1]School of Civil Engineering, South China University of Technology, Guangzhou - 510641, China.
[2]Guangzhou Marine Geological Survey, Guangzhou - 510760, China.
[3]Environmental Monitoring Center Station of Dongguan City, Dongguan - 523009, China.
[4]School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou - 510006, China.

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The spatial distribution and environmental implication of sulfate-reducing bacteria (SRB) were investigated in the coastal sediments from Zhanjiang Bay and Leizhou Bay, China. The SRB existed in all over the sediments from Zhanjiang Bay and Leizhou Bay, and showed a high spatial variation. The counts of SRB in Zhanjiang Bay are much more than those in Leizhou Bay because Zhanjiang Bay was much more affected by human activity, whereas Leizhou Bay was protected as habitats. SRB counts increase from the outer area to inner in Zhanjiang Bay, mainly resulting from the fact that the inner area are influenced by anthropogenic frequency and has a low oxidation-reduction potential (E*h*), whereas outer area is little influenced by anthropogenic and has a high E*h*. Total organic carbon (TOC) content was not significantly positively correlated with SRB, but the tendency in the whole was that where there existed the higher TOC content, there would be the more counts of SRB. There was no significant relationship between SRB and particle size distribution (PSD) and E*h*, whereas acid volatile sulfide (AVS) and the concentration of Fe (II) were significant positive correlated with SRB. It can be concluded that the distribution of SRB closely related to the concentration of Fe (II) and is an important cause for the spatial distribution of AVS, which is regarded as one of the key indicator of heavy metal bioavailability.

Key word: Spatial Distribution; Sulfate Reducing Bacteria; Acid Volatile Sulfide (AVS); Environmental Implication; Geochemical Factors.

Sulfate-reducing bacteria (SRB) were firstly discovered by Beijerinck in 1895. Then, the research on SRB from many perspectives attracted lots of scientists[1-2]. As the kind of anaerobic bacteria, SRB can use sulfate as an electron acceptor to produce high concentration of hydrogen sulfide in the metabolic process[3]. SRB participates in the biogeochemical cycles of sulfur and the degradation of organic matter, and many other important biogeochemical processing[4-5]. SRB also plays an important role in the biological migration and transformation of environmental toxic pollutants, such as methylation of mercury, degradation of benzene and the transformation of uranium[6-8]. In other ways, however, due to the existence of SRB, a large number of hydrogen sulfides or sulfides were produced, which have been the indicators of organic contamination and

* To whom all correspondence should be addressed.
E-mail: hjlifeng@scut.edu.cn

the anaerobic in the marine environment. In the aquaculture area, the pollution of hydrogen sulfide can result in deterioration, and it is a crucial cause for the frequent occurrence of aquatic disease[9-10]. All in all, SRB play important roles in affecting the environment surrounding. In addition, the reproduction and biogeochemical activities of SRB were influenced by various geochemical factors. Therefore, it is important to discuss the relationship among them and its environmental implication in coastal sediments.

Acid volatile sulfide (AVS), derived primarily from the activities of SRB, is operationally defined as the amount of sulfide that can be volatilized during a cold 1N HCl extraction[11]. AVS plays important roles in controlling the distribution of heavy metals in the sediment - pore water systems and the chemical activity and bioavailability of heavy metals in sediment. AVS-SEM (simultaneously extraction metals) model has been a standard by USEPA for predicting the toxicity of heavy metals in sediments[12]. SRB, a main producer of AVS, constantly affects the environment through its metabolites. Therefore, it is important to investigate the distribution of SRB and AVS, and the relationship between them.

The topic of SRB and its environmental implication has been raised for some years. For example, Kondo et al. has studied the abundance of SRB in fish farm sediments along the coast of Japan and South Korea[1]. Vertical distribution of SRB was investigated by Sass et al. in littoral and profundal sediments of the oligotrophic Lake Stechlin[13]. Shen *et al*. studied the correlations between SRB and chemical factors in sediments from Baihua Lake[14].

Zhanjiang Bay is a famous natural harbor. Leizhou Bay, north of Zhanjiang Bay, is important habitats for dolphins. Both of them are the most dynamic, complex and vulnerable marine environments in the South China Sea. In this area, however, there has not seen the relative report about SRB so far, which is an important environmental factor.

The main object of this study was to analyze the spatial distribution characteristics of SRB in marine sediment, to study the environmental implication of SRB in sediment from Zhanjiang Bay and Leizhou Bay, China, and to study the relationship between SRB and geochemical factors. This paper is expected to offer useful related biological information to conduct some deep research, such as sulfur cycle in the marine environment, the migration or transformation of acid rain, heavy metals and other environmental pollutions.

MATERIALS AND METHODS

Study area

Zhanjiang Bay (21°3' -21°21' N, 110°19' -110°36' E) and Leizhou Bay (20°28' -21°06' N,110°10' -110°39' E), two adjacent sheltered Bays, are divided by Donghai Island, which is located on the east of Leizhou peninsula, southwest of Guangdong province, China. Great capability and a strong force of tides, expanse of water areas, stable benches and water troughs are obvious features in these two bays. Due to the safe water quality and owning a biggest natural harbor in Leizhou Peninsula, Leizhou Bay is a very suitable habitat for dolphins. Zhanjiang Bay, a semi-enclosed estuary and a natural deep water harbor in southern China, is one of the closest harbors in China to Southeast Asia, Oceania, Europe and Africa. Therefore, it is an important logistics and transport center. Zhanjiang Bay undertakes most services, such as cargoes handling in the Port, aquaculture, and sewage treatment. The seas from Leizhou Bay to Zhanjiang Bay are important habitats for Sousa Chinensis, a Chinese State - level wildlife.

Sample collecting

The sampling collection was undertaken in May 2012 and lasted for one week. Surface sediment (0 cm to 5 cm) samples were gathered by a Van Veen grab sampler from a small vessel in shallow water areas and a plastic spatula in beach areas. A total of 17 surface samples (Fig. 1) were collected in Zhanjiang Bay and others in Leizhou Bay. The surface sediment samples were immediately placed in plastic bags filled with N_2 after collection. The bags were sealed and then placed in a box filled with ice[15]. The samples were stored at -4 °C in the laboratory for further analysis.

Analysis and data processing

Counts of SRB were determined by the most probable number technique. The mineral medium for SRB contained per liter 0.1g KH_2PO_4, 0.1g NH_4Cl, 0.1g NaCl, 0.1g KCl, 0.2g $CaC1_2 \cdot 2H_2O$, 0.2g $MgC1_2 \cdot 6H_2O$, and 1ml trace element solution. Then, 3.69g/L $MgSO_4 \cdot 7H_2O$, 0.05g/L $FeSO_4 \cdot 7H_20$, 1 ml/L selenite-tungstate-solution, 1ml/L vitamin-solution, Na-DL-lactate, and Na-acetate (each 5mM end concentration) were added to the mineral medium. The dilution series of SRB were incubated under anaerobic operation box. All microplates were incubated in the dark at 28°C for 6 weeks. MPN and approximate 95% confidence intervals were calculated with the program of Klee (1993)[16-17].

AVS was determined via the purge-and-trap method[18]. The samples to measure Fe(II) were on site put into HCl until pH<1, and then were detected by phenanthroline spectrophotometry method (HJ/T345-2007) quickly[19].

The E*h* was measured *in situ* with a portable redox device. The particle size distribution in all samples was determined by sieve and laser particle size analysis. Fine grain samples were directly analyzed by a Mastersizer 1 2000 laser particle analyzer (Malvern Instruments Ltd., UK), which allowed measurements from 0.02µm to 2000µm and had a repeatability error of <3%. The total organic carbon (TOC) content was determined using the potassium dichromate oxidation–ferrous sulfate titrimetric method[20].

The data were processed through SPSS for Windows ver.11.0 and Excel 2007. Pearson correlation analysis was used to assess the relationship between SRB and geochemical factors.

RESULTS AND DISCUSSION

General characteristics of sediments

In this study area, the mineral fraction of particles less than 4µm (clay) varied from 0 to 33.1% with an average of 17.8%. The fraction of 4-63µm (silt) particles varied from 0.75% to 65.9% with an average of 39.4%. Sand content (>63µm) varied from 1.76% to 99.3% with an average of 42.8%. As shown in Fig.2a, it can be concluded that the sediments in this study were mainly composed of fine grain (clay + silt) with an average value of 57.2%. This result verified that the marine sediments are usually fine, the same with Huang et al. findings from the study on the distribution and sources of PAHs in sediments from Zhanjiang Bay and Leizhou Bay[21].

TOC and Eh values are shown in Fig.2b and Fig.2c. The TOC contents in sediments were varied from 0.031% to 1.35% with an average of 0.71% in Zhanjiang Bay, and varied from 0.11% to 0.74% with an average of 0.33% in Leizhou Bay. The results were identified with the findings by Huang et al. that the contents of TOC in Zhanjiang Bay were ranged from 0.03% to 1.04% and from 0.04% to 0.62% in Leizhou Bay[21]. The highest TOC content was in station BS12D190, where there was an estuary mainly influenced by sewage. The stations of TOC content more than 1% are all located around the Techeng Island.

E*h* varied from -204mv to 129mv with an average of -136mv. The E*h* were all lower than 0mv except station BS12D244, and most of the stations were lower than -100mv (except station BS12D173, BS12D220, BS12D246 and BS12D223), which indicated that this study area is mainly in strong anaerobic conditions. The E*h* of the two highest stations (BS12D246 and BS12D244) was located in a space closer to high sea. Dramatic ocean dynamics caused a large number of oxygen dissolve into water, thus increased the values of E*h*.

Table 1. The relationship between SRB and the measured paremeters of surface sediment samples

	SRB	AVS	Fe(II)	TOC	E*h*
SRB	1				
AVS	0.447*	1			
Fe(II)	0.606*	0.811**	1		
TOC	0.427	0.660**	0.772**	1	
E*h*	-0.321	-0.262	-0.375	-0.578**	1
Sand	-0.333	-0.435	-0.601**	-0.819**	0.541*
Silt	0.288	0.395	0.572**	0.779**	-0.550*
Clay	0.402	0.490*	0.628**	0.856**	-0.498*

**.p<0.01 *. p<0.05

The concentration of AVS and Fe (II)

Fig.3 was drawn through inverse distance weighted interpolation algorithm by ARCGIS. The AVS mean concentration in the sediment was 6.902μmol/g and ranged from 0.299 to 46.22μmol/g (Fig.3a). AVS concentrations in Leizhou Bay were obviously lower than those in Zhanjiang Bay. Zhanjiang Bay is favor for the formation of AVS due to its high TOC content and low Eh, which were together contributed by large numbers anthropogenic pollutants discharged into Zhanjiang Bay, shipping and aquaculture, whereas Leizhou Bay has been protected for being a dolphin habitats and fewer pollutants. The highest value of AVS concentration was located in BS12D190, which suffered from the combined pollution of shipping, sewage and aquaculture. The lowest AVS concentration was located in BS12D173, where the water quality has always been well. The AVS concentrations increased from the outer area to inner in Zhanjiang Bay, mainly because the inner area is influenced by anthropogenic frequency and has a low oxidation-reduction potential (E*h*), whereas the outer area are little influenced by anthropogenic and has a high E*h* mainly caused by strong ocean dynamics.

Fe (II) concentrations varied from 23.87 to 179.19μmol/g with an average of 73.78μmol/g. The lowest Fe (II) concentration was located in BS12D244, a space closer to high sea barely influenced by human activities. The highest Fe (II) concentration was located in BS12D190, which was mainly influenced by shipping and sewage, and a highest AVS concentration was also detected in this station. The tendency of the spatial distribution of Fe (II) was the same as the distribution of AVS (Fig.3b).

The spatial distribution of SRB

As shown in Fig.4, it is drawn through inverse distance weighted interpolation algorithm by ARCGIS. The SRB throughout all of the sediments in Zhanjiang Bay and Leizhou Bay and the spatial distribution of SRB show a significant difference. The range of the SRB counts was from 89 to 8.71×10^5 CFU/g with an average of 1.76×10^5 CFU/g in the sediments. The counts of SRB in this study area are higher than in Zhejiang-North Fujian shelf research by Chen et al. with an average of 436 CFU/g [22], but lower than those in fish farm sediments along the coast of Japan and South Korea with a range from 4.6×10^8 to 4.9×10^{10} CFU/g dry sediment[1].

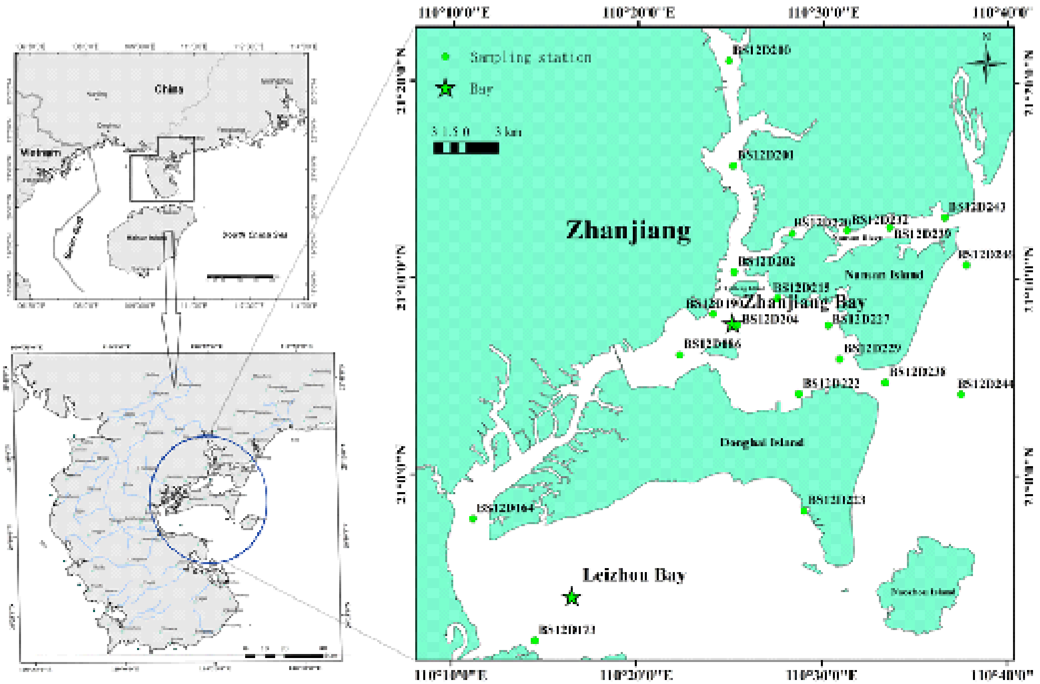

Fig. 1. Locations of sampling sites in Zhanjiang Bay and Leizhou Bay, China

The counts of SRB in Zhanjiang Bay are much more than those in Leizhou Bay. SRB is sensitive to the living environment. In general, SRB will be quite active in the conditions that are anaerobic and rich in organic matter which provides the necessary carbon and nitrogen sources[14]. There are higher TOC content and lower E*h* in Zhanjiang Bay than those in Leizhou Bay, which indicated that the conditions in Zhanjiang Bay are more suitable for SRB increase. The counts of SRB in Nansan River and the area around Techeng Island are more than those in other areas in Zhanjiang Bay. The up to two stations of SRB counts are located in Nansan Riverÿwhich was an intensive aquaculture area. The counts of SRB decrease from west to east in Nansan River. This decrease tendency may be related to the velocity of flow and lower aquaculture in the outer area. A larger number of SRB were also discovered on the surroundings of Techeng Island. Two significant power plant discharge sewage into this region, together with many aquacultures, contribute to the growth of SRB. All in all, the counts of SRB gradually increase from the outer bay to inner, which has the same distribution of AVS and Fe (II) in Zhanjiang Bay. The station of sediment containing the minimal SRB counts is BS12D173, located in Leizhou Bay with a good water quality. It's worth noting that the lowest AVS concentration, lower TOC content and higher E*h* are also detected in this station.

SRB was considered to live only in strictly anaerobic conditions in previous studies. However, larger number of SRB was found in the hypoxia or oxygen-enriched water environment in current research. Both Sieburth and Andreas found that numbers of SRB live in the bottom water of hypoxia[24-25]. In this study, 120CFU/g SRB were detected in the sediment from station BS12D244, whereas the E*h* is reaching 129mv. Therefore, it was demonstrated again that SRB can not only live in the anaerobic conditions, but also adapt to the oxygen-rich environment in sediments.

The environmental implication of SRB

The characteristics of sediment grain size are the interactions among sediment provenance, hydrodynamic energy and other environmental factors. Grain size is an important matrix affecting the growth of microbial in the sediment. As shown in table 1, clay and silt showed a different level of positively correlative with SRB but not significant ($p<0.218$ and $p<0.079$, respectively), and sand showed a negative correlation with SRB but also not significant ($p<0.152$). Above all, it is indicated

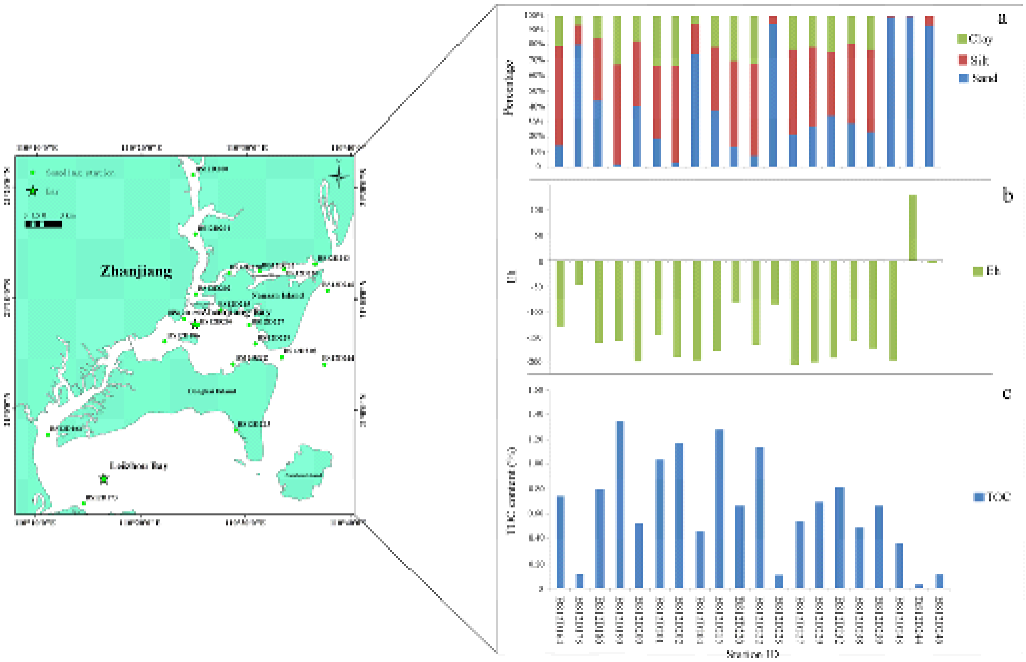

Fig. 2. The percentage of particle size, E*h* and TOC content in surface sediments from Zhanjiang Bay and Leizhou Bay

that the sediment grain size did not play a decisive role in the growth of SRB.

Shen *et al*. (2012) research on the correlation between SRB and TOC showed a significantly positive correlation in Baihua Lake [26]. The same result was the finding by Chen et al. (2000) research in Zhejiang-North Fujian shelf[20]. In this study, however, the TOC content is positively correlative with the counts of SRB but not significant. There are many other environmental elements influencing SRB counts, so when TOC improves the biological effectiveness of SRB in proper conditions, the SRB counts may increase. In other words, an increase of TOC content not always leads to SRB counts' growth[22]. For instance, in station BS12D239, the counts of SRB were 871640CFU/g but TOC content was only 0.11%, probably resulting from the TOC content in this station that has improved the biological effectiveness of SRB. By contrast, the same TOC content occurred in BS12D220 while the counts of SRB were only 96 CFU/g.

SRB can live under anaerobic condition but also in aerobic condition. In this study, SRB are also found for it exists in oxygen-rich sediments. The E*h* was negatively correlative with SRB counts but not significant ($p<0.168$) in this study.

The counts of SRB were significantly positive correlated with Fe (II) concentration in this study ($p<0.01$). Fe (II) was the active part of all kinds of enzymes in SRB cell, such as Cytochrome C_3, Ferredoxin, Rubredoxin and Catalase. Hence, the growth of SRB may be influenced by the concentration of Fe (II). Zhang *et al.* (1999) studied the laws of SRB growth in water injection wells of Oilfield noted that SRB will be inhibited by Fe (II) when the concentration of Fe (II) is less than 13-15mg/L[27]. Above all, it can be concluded that the distribution of SRB closely related to the concentration of Fe (II) and can help the growth of SRB.

AVS can only be formed when the sulfur supply is sufficiently large and the conditions within the sediment favor sulfate reduction, i.e., in the range from moderately to strongly reducing environments where SRB exist[28]. Furthermore, David *et al.* (2005) noted that most of the S (-II) in the sediments is produced by sulfate-reducing bacteria[11]. In addition, the main part of AVS is S (-II), and in anaerobic sediment the S (-II) is usually formed in the process of SO_4^{2-} reduction by organic carbon with participation of SRB[11,15]. Thus, it is reasonable to expect that the concentration of AVS is closely related to SRB. As the main producer of AVS, plenty of SRB exists in those areas where

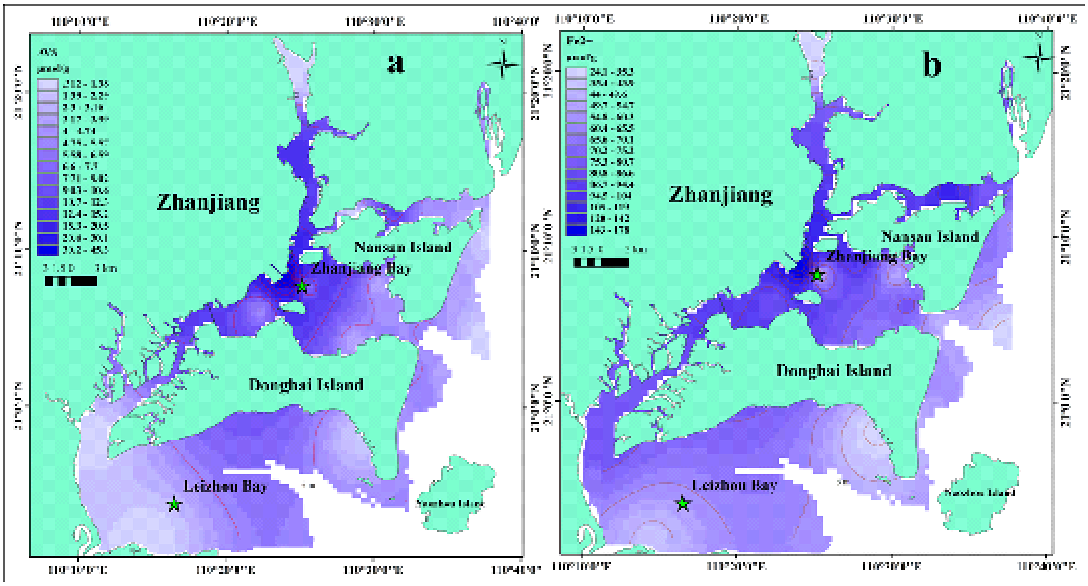

Fig. 3. The spatial distribution of AVS (acid-volatile sulfide) and Fe (II) concentration in surface sediments from Zhanjiang Bay and Leizhou Bay.

AVS concentration is high. Such as in this study, the AVS concentrations were significantly positive correlated with SRB counts ($p<0.05$), which demonstrated the above mentioned conclusion. In addition, the distribution feature of AVS is much the same with the distribution feature of SRB. A conclusion can be achieved that the distribution of SRB is a crucial cause for the spatial distribution of AVS.

CONCLUSIONS

According to this investigation, SRB existed in all over the sediments from Zhanjiang Bay and Leizhou Bay and showed a high spatial variation. The SRB counts in Zhanjiang Bay are much more than those in Leizhou Bay, where the water quality is always fine. The SRB counts in Nansan River and the area around Techeng Island are obviously more than other areas in Zhanjiang Bay, and decrease from the inner city to outer.

The correlation between SRB and PSD did not show a significant relationship, indicating that sediment grain size did not play a decisive role in the growth of SRB. TOC content was positively correlative with SRB but not significant. However, the tendency in the whole was that the higher TOC content was, the more counts of SRB

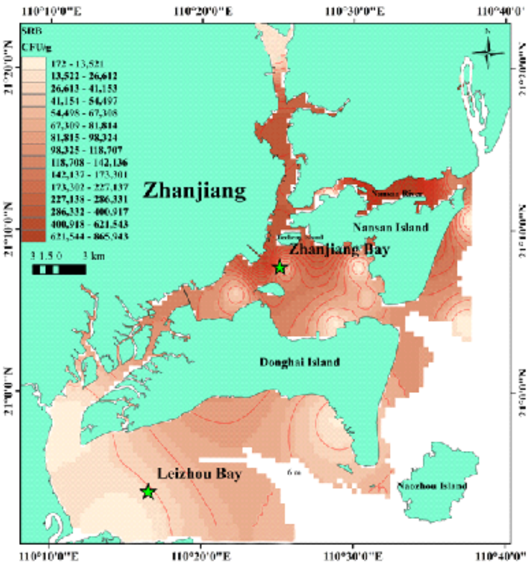

Fig. 4. The spatial distribution of SRB (sulfate reducing bacteria) in surface sediments from Zhanjiang Bay and Leizhou Bay

would. The E*h* was not significantly negatively correlated with SRB, because sometimes SRB can also live in aerobic condition. The correlation between SRB and Fe (II) was a significantly positive relationship, indicating that the distribution of SRB was closely related to the concentration of Fe (II), and demonstrating that Fe (II) can help the growth of SRB as the active part of all kinds of enzymes in SRB cell. AVS, as the main product of SRB, was significantly positive with SRB. A conclusion can be achieved that the distribution of SRB is an important cause for the spatial distribution of AVS.

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