***J. Ocean Univ. China*** (Oceanic and Coastal Sea Research)
https://doi.org/10.1007/s11802-018-3593-8
ISSN 1672-5182, 2018 17 (6): 1487-1491
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# Trace Metals Analysis Along the Fildes Peninsula Coastline Using Two Red Algae, *Rhodymenia antarctica* and *Iridaea cordata*, as Monitors

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(Received August 28, 2017; revised December 18, 2017; accepted August 29, 2018)


**Abstract** Two red algae, *Rhodymenia antarctica* and *Iridaea cordata* (Rhodophyta), were employed to investigate the pollution situation along Fildes Peninsula coastline, King George Island. Ten sites from east and west coastlines were investigated, and the concentrations of Cu, Pb, Zn, Cd, Cr, As and Hg were determined. The metal pollution index (MPI) was used to evaluate the overall pollution level represented by the investigated sites and for interregional comparisons. The two algae exhibited different preference to special trace metal. *R. antarctica* could accumulate more Cd (0.63 mg $kg^{-1}$) and Hg (0.026 mg $kg^{-1}$) than *I. cordata* (Cd 0.34 mg $kg^{-1}$, Hg 0.019 mg $kg^{-1}$). *I. cordata* could accumulate more As (15.53 mg $kg^{-1}$) than *R. antarctica* (10.11 mg $kg^{-1}$). There was no significant difference in accumulating Cu, Pb, Zn and Cr between the two algae. *R. antarctica* could be used to monitor Cd and Hg. *I. cordata* would be more appropriate for monitoring As. MPI monitored by the two algae were from 1.02 to 2.26 (*R. antarctica*), and 1.03 to 1.25 (*I. cordata*), respectively. Pollution situation of Fildes Peninsula was becoming serious, especially of the east coastline. The objective of this research was to gather the baseline information for trace metals investigation in Antarctic.

**Key words** Fildes Peninsula; *Rhodymenia antarctica*; *Iridaea cordata*; trace metal; MPI

## 1 Introduction

In the study of trace metal pollution, many living aquatic organisms have been extensively used as natural monitors for their ability of absorbing the trace metals. Macroalgae, responding basically to metals present in water, are often used as biomonitors, which are considered as the uptake of trace metals by an organism directly from the abiotic environment with several orders of magnitude above the environmental levels (Majer *et al*., 2014; Chakraborty *et al*., 2014). Algae are considered as the optimum ones because of their wide distribution, the integration of the global variations of environment, as well as faster response to the environment and easier detected than organisms at higher trophic levels (Chakraborty *et al*., 2014). *Ulva lactuca*, *Ulva intestinalis*, *Padina gymnospora*, *Dictyota bartayresiana* among others have been proved to be used as potential biomonitors for trace metal pollution detection (Chakraborty *et al*., 2014). Pollution index MPI (Metal Pollution Index), evaluating the global pollution level, was highly effective for the comparison between different species and between different sites (El-Din *et al*., 2014).

Antarctica used to be perceived as a symbol of the last great wilderness untouched by human disturbance, but cannot escape the local and global anthropogenic pollution (Moreno *et al*., 1997; Jerez *et al*., 2013; Majer *et al*., 2014). The Fildes Peninsula locates on the southwest part of the King George Island, South Shetland Islands, and experiences a sub-Antarctic cold, moist, maritime climate with a mean annual air temperature of −2.2℃ and a mean summer air temperature above 0℃ for up to four months (Liu *et al*., 1992). The region is the largest ice-free and accessible area on the island and attracts more and more scientists and tourists. Five permanent research stations and one airport exist in this region (Hughes *et al*., 2013). The east of Fildes Peninsula faces the Maxwell Bay, an important shipping channel for research stations and tourists. And a continuous contamination does exist due to human intensive and extensive activities, such as shipping, boating, travelling, scientific researching among others (Moreno *et al*., 1997). The trace metals (*e.g*., Cu, Pb, Zn, Cd, Cr, As and Hg) may be continuously released to the marine coastline environment. Thus it is imperative to monitor the situation of trace metal pollution in Fildes

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Peninsula. Some works have been conducted to analyze the pollution of trace metals in Antarctic continent using macroalgae as monitors. Farías *et al*. (2002) analyzed the trace metals of 11 macroalgae in Potter Cove, King George Island and found *Monostroma hariotii* could be used as an adequate monitor for further study. Runcie and Riddle (2004) employed 8 algae to evaluate the contamination of Brown Bay and compared with that far from the source of contamination. Moreno *et al*. (1997) finished a comprehensive survey to evaluate the levels of essential and non-essential trace metals in Antarctic organisms, and 7 species of algae (*Durvillea antarctica*, *Adenocystis* sp., *Ascoseira* sp., *Cytosphaera* sp., *Desmarestia* sp., *Leptosomia simplex* and *I. cordata*) from King George Island were employed as monitors. However, no research has been conducted to analyze the pollution of coastline along Fildes Peninsula using macroalgae as monitors, especially using *R. antarctica*.

The aim of present work was to use two common red algae, *R. antarctica* and *I. cordata*, living in intertidal zone of coastline along Fildes Peninsula, as potential biomonitors to assess the pollution situation. MPI was used to compare the pollution level in this area with other areas in Antarctic, as well as to evaluate the extent of trace metals contamination in the selected studying sites which would serve as a baseline for future studies.

## 2 Materials and Methods

### 2.1 Studying Area and Sample Collection

Five sites from east coastline of Fildes Peninsula including Norma Cove, Ardley Island, Great Wall Cove, Wanglong Rock and Jiuquan River were selected and named as E1, E2, E3, E4 and E5 and five sites from west coastline including Xingfu Cove, Fengbao Cove, Biologist Cove, Horatio Cove and Geography Cove were named as W1, W2, W3, W4 and W5 were chosen to collect the algae.

Two red algae, *R. antarctica* and *I. cordata*, were employed as biomonitors of trace metal pollution of Fildes Peninsula. Samples were collected in intertidal zones during the 2013 austral summer season. The detail of sample collection was shown in Fig.1.

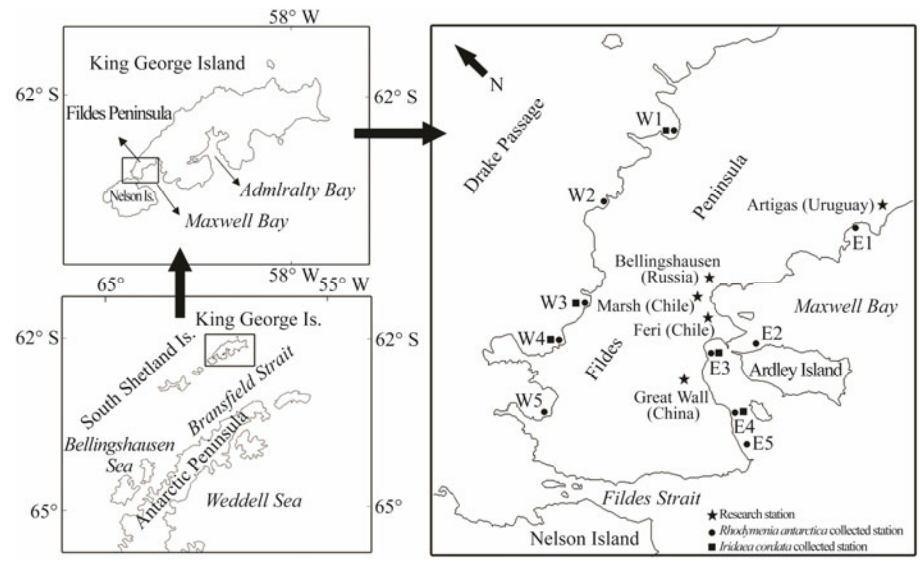


Fig.1 Sampling sites along Fildes Peninsula, King George Island, South Shetland Islands. E1, Norma Cove (62˚11.328´S, 58˚55.005´W); E2, Ardley Island (62˚12.550´S, 58˚56.586´W); E3, Great Wall Cove (62˚13.037´S, 58˚57.513´W); E4, Wanglong Rock (62˚13.217´S, 58˚57.129´W); E5, Jiuquan River (62˚13.476´S, 58˚57.146´W); W1, Xingfu Cove (62˚10.430´S, 58˚58.217´W); W2, Fengbao Cove (62˚11.166´S, 58˚59.634´W); W3, Biologist Cove (62˚12.117´S, 58˚59.908´W); W4, Horatio Cove (62˚12.481´S, 59˚00.432´W); W5, Geography Cove (62˚13.216´S, 59˚00.691´W).

The whole body of algae was picked up and transported to the laboratory in seawater. Epiphyta and sediments were then removed from the algae using nylon brushes under tap water. Then the algal materials were rinsed several times using ultrapure water very quickly (to minimize possible metal loss), inserted into polyethylene bags, and stored at −20℃ until use.

### 2.2 Sample Digestion and Analysis

To analyze the concentration of trace metals, samples were freezing-dried, ground to fine power in agate mortar. Microwave digestion machine was employed to digest the samples. There replicate solutions of each sample were prepared by MW-assist acid digestion. A 0.5 g portion of each sample was weighed directly into a digestion teflon tube, then 5 mL nitric acid (ultrapure grade) and 2 mL liquor hydrogen peroxide (ultrapure grade) were added. Mixture was left overnight until it was subjected to a microwave digestion. Digestion blanks were also carried out to the same procedure. The details of the MW procedure were in Table 1.

The digestion was carried out till the solution became completely clear and homogeneous. Then the whole solution was transferred to a teflon crucible and slowly evaporated to near dryness. The residue was allowed to cool down and diluted to a final volume of 50 mL with ultrapure water. The trace metal concentrations of algae were determined by Atomic Absorption Spectrometry (AAS) and Atomic Fluorescence Spectrometer (AFS). Flame AAS was used for Zn, graphite furnace AAS was used for Cu, Pb, Cd and Cr. The concentrations of As and Hg were confirmed by AFS.

Table 1 Procedure of digesting algal samples with MW (Farías *et al*., 2002)

| Parameter | Value |
|---|---|
| Sample weight | 0.5 g |
| Reagents | 5 mL $HNO_3$ + 2 mL $H_2O_2$ |
| Digestion procedure (digestion pressure and time of duration) | 0.5 MPa, 5 min<br>1.0 MPa, 5 min<br>1.5 MPa, 5 min<br>2.0 MPa, 10 min |
| Final volume | 50 mL |

To assess the accuracy and precision of previous method, the certified reference material (CRM) GBW10023 (National Center for Standard Materials, China) was performed in parallel with the corresponding analyzing process. All the wares used in this experiment had to be soaked in 10% nitric acid (ultrapure grade) for more than 24 h.

To compare the total content of metals at different sites along Fildes Peninsula, the Metal Pollution Index (MPI) (Usero *et al*., 1997) was employed and the equation was:

$$MPI = (Cf_1 \times Cf_2 \times \ldots \times Cf_n)^{1/n},$$

where $Cf_n$ was the concentration of trace metal and $n$ was the metal number. Comparison of the concentrations of trace metals or *MPI* between the algae or the sampling sites was performed by one-way analysis of variance (ANOVA).

# 3 Results

## 3.1 Trace metals in *R. antarctica* and *I. cordata*

Mean concentrations of 7 trace metals and MPI of *R. antarctica* are listed in Table 2. To the alga *R. antarctica*, collected from 10 sites, samples from E5 showed the maximum values for Pb (3.75 mg $kg^{-1}$), Zn (36.98 mg $kg^{-1}$), Cd (0.90 mg $kg^{-1}$) and Hg (0.058 mg $kg^{-1}$). The highest concentrations of Cu, As and Cr were found at W1 (2.43 mg $kg^{-1}$), W2 (12.65 mg $kg^{-1}$) and E4 (4.81 mg $kg^{-1}$), respectively. While the minimum values for Pb (0.16 mg $kg^{-1}$) and Zn (15.42 mg $kg^{-1}$) were found at W2. The minimum values for Cu (1.05 mg $kg^{-1}$), Cd (0.41 mg $kg^{-1}$), As (6.42 mg $kg^{-1}$), Cr (1.34 mg $kg^{-1}$) and Hg (0.022 mg $kg^{-1}$) were found at E4, W5, E1, W3 and W2, respectively. The descending order of MPI monitored using *R. antarctica* was E5 > E2 > E1 > E3 > E4 > W4 > W1 > W3 > W5 > W2.

Due to the influence of tide, *I. cordata* was collected at 5 sites including E3, E4, W1, W3 and W4. The samples from W3 showed the maximum values for Cu (2.64 mg $kg^{-1}$), Zn (25.86 mg $kg^{-1}$) and Cr (3.04 mg $kg^{-1}$) (Table 3). The minimum values for these three trace metals were found at E3 (Cu 1.02 mg $kg^{-1}$, Zn 20.17 mg $kg^{-1}$) and W4 (Cr 1.64 mg $kg^{-1}$). The maximum values for Cd (0.55 mg $kg^{-1}$) and Hg (0.023 mg $kg^{-1}$) were found at W1, while the minimum values were found at E3 (Cd 0.16 mg $kg^{-1}$, Hg 0.014 mg $kg^{-1}$). E4 and E3 showed the minimum and maximum values for Pb (0.12 and 3.25 mg $kg^{-1}$, respectively). To the trace metal As, the highest concentration (19.50 mg $kg^{-1}$) was found at W4 and the lowest (12.14 mg $kg^{-1}$) one was found at W1. The descending order of MPI monitored using *I. cordata* was E3 > W1 > E4 > W4 > W3.

Table 2 Mean concentrations (mg $kg^{-1}$ dry weight) of trace metals in *R. antarctica* and MPI monitored using *R. antarctica* along the Fildes Peninsula coastline, King George Island

| Sites | Cu | Pb | Zn | Cd | Cr | As | Hg | MPI |
|---|---|---|---|---|---|---|---|---|
| E1 (Norma Cove) | 1.09 ± 0.23 | 2.88 ± 0.17 | 30.10 ± 1.64 | 0.77 ± 0.14 | 1.48 ± 0.06 | 6.42 ± 1.16 | 0.032 ± 0.011 | 1.54 ± 0.17 |
| E2 (Ardley Island) | 1.17 ± 0.06 | 1.08 ± 0.01 | 29.40 ± 0.25 | 0.49 ± 0.04 | 4.45 ± 0.35 | 8.11 ± 0.68 | 0.049 ± 0.050 | 1.64 ± 0.04 |
| E3 (Great Wall Cove) | 1.58 ± 0.07 | 1.52 ± 0.47 | 24.54 ± 6.68 | 0.37 ± 0.02 | 3.49 ± 0.11 | 9.10 ± 0.81 | 0.027 ± 0.060 | 1.51 ± 0.14 |
| E4 (Wanglong Rock) | 1.05 ± 0.30 | 1.03 ± 0.17 | 20.76 ± 2.68 | 0.73 ± 0.03 | 4.81 ± 1.36 | 8.45 ± 0.70 | 0.026 ± 0.020 | 1.49 ± 0.04 |
| E5 (Jiuquan River) | 1.39 ± 0.17 | 3.75 ± 0.13 | 36.98 ± 4.19 | 0.90 ± 0.01 | 4.21 ± 1.35 | 7.58 ± 2.05 | 0.058 ± 0.008 | 2.26 ± 0.17 |
| W1 (Xingfu Cove) | 2.43 ± 0.87 | 0.83 ± 0.11 | 18.16 ± 0.59 | 0.71 ± 0.07 | 1.65 ± 0.05 | 10.79 ± 2.40 | 0.025 ± 0.030 | 1.41 ± 0.07 |
| W2 (Fengbao Cove) | 1.19 ± 0.09 | 0.16 ± 0.01 | 15.42 ± 0.53 | 0.69 ± 0.08 | 2.07 ± 0.19 | 12.65 ± 1.81 | 0.022 ± 0.020 | 1.02 ± 0.04 |
| W3 (Biologist Cove) | 1.41 ± 0.25 | 0.44 ± 0.05 | 27.38 ± 2.03 | 0.75 ± 0.06 | 1.34 ± 0.09 | 9.56 ± 0.51 | 0.026 ± 0.030 | 1.22 ± 0.06 |
| W4 (Horatio Cove) | 1.24 ± 0.42 | 1.19 ± 0.07 | 26.46 ± 3.43 | 0.60 ± 0.11 | 1.72 ± 0.16 | 12.64 ± 1.53 | 0.026 ± 0.030 | 1.43 ± 0.13 |
| W5 (Geography Cove) | 1.39 ± 0.25 | 0.35 ± 0.06 | 19.85 ± 3.77 | 0.41 ± 0.10 | 2.63 ± 0.16 | 8.03 ± 0.62 | 0.030 ± 0.03 | 1.13 ± 0.04 |

Note: Data are given as mean ± standard deviation (three size groups analyzed).

Table 3 Mean concentrations (mg $kg^{-1}$ dry weight) of trace metals in *I. cordata* and MPI monitored using *I. cordata* along the Fildes Peninsula coastline, King George Island

| Sites | Cu | Pb | Zn | Cd | Cr | As | Hg | MPI |
|---|---|---|---|---|---|---|---|---|
| E3 (Great Wall Cove) | 1.02 ± 0.21 | 3.25 ± 0.39 | 20.17 ± 2.93 | 0.16 ± 0.03 | 2.21 ± 0.56 | 15.96 ± 0.67 | 0.014 ± 0.003 | 1.25 ± 0.10 |
| E4 (Wanglong Rock) | 2.43 ± 0.12 | 0.12 ± 0.02 | 21.39 ± 2.24 | 0.48 ± 0.80 | 2.14 ± 0.13 | 16.59 ± 0.11 | 0.020 ± 0.000 | 1.08 ± 0.03 |
| W1 (Xingfu Cove) | 1.99 ± 0.23 | 0.15 ± 0.01 | 25.54 ± 1.78 | 0.55 ± 0.01 | 2.62 ± 0.29 | 12.14 ± 2.09 | 0.023 ± 0.001 | 1.17 ± 0.05 |
| W3 (Biologist Cove) | 2.64 ± 0.39 | 0.16 ± 0.02 | 25.86 ± 2.71 | 0.17 ± 0.04 | 3.04 ± 0.74 | 13.46 ± 2.45 | 0.017 ± 0.004 | 1.03 ± 0.09 |
| W4 (Horatio Cove) | 1.63 ± 0.14 | 0.18 ± 0.01 | 23.51 ± 2.62 | 0.36 ± 0.08 | 1.64 ± 0.06 | 19.50 ± 1.91 | 0.019 ± 0.006 | 1.05 ± 0.08 |

Note: Data are given as mean ± standard deviation (three size groups analyzed).

### 3.2 Comparison of Trace Metals Uptake Ability Between Two Algae

A comparison of the concentrations of trace metals between two algal species collected at E3, E4, W1, W3 and W4 revealed that the two algae exhibited different preference to special trace metal (Fig.2). *R. antarctica* accumulated more Cd (0.63 *vs*. 0.34 mg kg$^{-1}$, $P=0.026$) and Hg (0.026 *vs*. 0.019 mg kg$^{-1}$, $P=0.001$) than *I. cordata*. However, *I. cordata* showed preference to As (15.53 *vs*. 10.11 mg kg$^{-1}$, $P=0.006$) than *R. antarctica*. To the trace metals Cu, Pb, Zn and Cr, there was no significant difference between two algae, thus *R. antarctica* accumulated more Cd and Hg and less As than *I. cordata*.

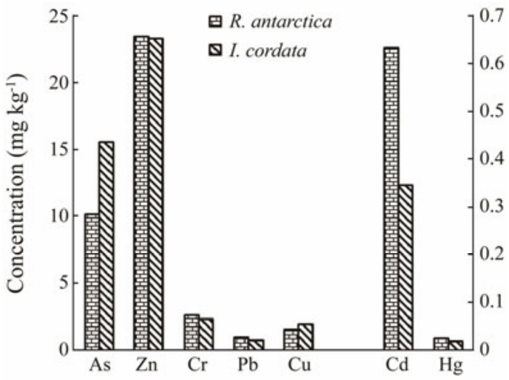


Fig.2 Comparison of trace metals concentrations in two algae collected at sites including E3, E4, W1, W3 and W4.

## 4 Discussion

Macroalgae could accumulate dissolved trace metals, reflecting the trace metal levels in seawater and sediment, thus they were considered as optimal tools to monitor the levels of trace metal contamination and environmental quality of investigated areas (Sawidis *et al*., 2001). As stated earlier, no much work had been done to investigate the pollution of trace metals in Antarctic using algae as monitors. The data set was compared with the values of other sites around Antarctica, especially those using red algae as monitors. Comparing *I. cordata* and *Leptosomia simplex* collected from Artigas Base, King George Island (Moreno *et al*., 1997), the same site named as E1 (Norma Cove) in this research, the concentrations of Cu, Zn and Cd were higher in this study (Table 4). Comparing with the alga *I. cordata* collected from Potter Cove (Farías *et al*., 2002) that was at the east of the E3 (Great Wall Cove) and was 15 kilometers away, the concentrations of Cu and Zn were higher, Pb, Cd, Cr and As were lower in this research. Research had been carried out to investigate the concentrations of Hg and Zn in Admiralty Bay, King George Island, using *Palmaria decipiens* (Rhodophyta) as monitor. It was found the concentrations of Hg and Zn were 0.020 mg kg$^{-1}$ and 37.8 mg kg$^{-1}$, respectively (Santos *et al*., 2005), and the concentration of Zn was higher than the result in this research. Riva *et al*. (2003) had investigated the contamination of Ross Sea, Antarctica, and found the concentrations of Hg in *I. cordata* and *Phyllophora antarctica* (Rhodophyta) were 0.03 mg kg$^{-1}$ and < 0.009 mg kg$^{-1}$, respectively; Cd were 5.95 mg kg$^{-1}$ and 4.01 mg kg$^{-1}$, respectively, and the concentrations of Cd in both algae were higher than the results detected in this research.

The previous comparison results indicated that the concentrations of each trace metal varied a great deal from specie to specie and from one site to another, being not able to suggest a clear pattern of pollution. Thus MPI was employed to evaluate the pollution level of the Fildes Peninsula. Using *I. cordata* as monitor, MPI was calculated with the data (calculated with mean concentrations of Cu, Zn and Cd) from Artigas Base (Moreno *et al*., 1997) was 0.67, lower than MPI (range from 1.46 to 3.03) calculated with the same three trace metals in this research. MPI (calculated with mean concentrations of Cu, Pb, Zn, Cd, Cr and As) monitored by *I. cordata* ranged from 1.93 to 4.17 in Fildes Peninsula, which was higher than the MPI (<1.13, using the same five trace metals) from Potter Cove. Thus the pollution of Fildes Peninsula was becoming more serious than that of the Potter Cove (data from 2002). MPI was also used to evaluate the pollution distinction between east and west coastlines along Fildes Peninsula. MPI of *R. antarctica* in east coastline was significantly higher ($P<0.05$) than that of the west coastline, and the highest one was found at E5 (Jiuquan River). However, MPI monitored by *I. cordata* showed

Table 4 A comparison of the mean concentrations (mg kg$^{-1}$ dry weight) of trace metals determined in this research with the findings of other studies

| Locations | Species | Cu | Pb | Zn | Cd | Cr | As | Hg |
|---|---|---|---|---|---|---|---|---|
| This research | *R. antarctica* | 1.39±0.48 | 1.32±1.12 | 24.91±6.81 | 0.64±0.18 | 2.79±1.39 | 9.33±2.33 | 0.026±0.01 |
| | *I. cordata* | 1.94±0.63 | 0.77±1.29 | 23.29±3.13 | 0.34±0.17 | 2.33±0.62 | 15.53±3.02 | 0.018±0.00 |
| Artigas Base, King George Island (Moreno *et al*., 1997) | *I. cordata* | 0.91±0.03 | | 5.5±0.48 | 0.06±0.01 | | | |
| | *Leptosomia simplex* | 0.91±0.10 | | 6.67±2.20 | 0.08±0.01 | | | |
| Potter Cove, King George Island (Farías *et al*., 2002) | *I. cordata* | <0.20 | 1.60±0.08 | <0.1 | 0.55±0.02 | 4.26±0.21 | 27.7±1.5 | |
| Admiralty Bay, King George Island (Santos *et al*., 2005) | *Palmaria decipiens* | | | 37.8 | | | | 0.020 |
| Ross Sea, Antarctica (Riva *et al*., 2003) | *I. cordata* | | | | 5.95 | | | 0.03 |
| | *Phyllophora antarctica* | | | | 4.01 | | | <0.009 |

no significant difference ($P > 0.05$) between the two coastlines, while MPI of E3 (Great Wall Cove) was the highest. The progress of sampling algae found that the pollution of the east coastline should be more serious than the west. Amaro *et al*. (2015) found that the east coastline of Fildes Peninsula was subjected to a large environmental pressure. There were several reasons attributing to this fact. First, more than five research stations were located on the east coast of Fildes Peninsula, including Great Wall Station, Artigas Station, Bellingshausen Station, Marsh Station and Feri Station. Much household refuse were discharged into the sea, and building materials were piled up on the east coast. Thus the anthropogenic impact to the east coast was heavier than to the west. Additionally, the east coast was one part of Maxwell Bay. As the bay was an important waterway to the research stations, much shipping garbage were discharged into the bay. This result could also attribute to the geographic factor. The west coast faced the Drake Passage and trace metal can be easily transported by ocean current.

Not all the algae could be used as monitors. Various intrinsic factors (assimilation efficiency, efflux rate or growth rate) and extrinsic factors (turbidity, nutrient availability, light intensity, salt content or temperature) could influence metal uptake ability (Majer *et al*., 2014). The ability of accumulating trace metals varied a great deal from species to species. Comparing the trace metals uptake ability of *R. antarctica* and *I. cordata*, there was significant difference ($P<0.05$) except Cu, Pb, Zn and Cr. The two potential monitors exhibited different accumulation ability to the trace metals. Thus only special algae could be used to monitor target trace metal. *R. antarctica* could be used to monitor Cd and Hg, while *I. cordata* would be more preferable to monitor As.

In this study, the pollution status of the coastline along Fildes Peninsula was assessed using two red algae, *R. antarctica* and *I. cordata.* The global pollution situation of investigated locations was determined for the first time. The results showed that the pollution situation of Fildes Peninsula was becoming serious. However, due to the small number of species studied, the results must be viewed with caution, and more algal species must be analyzed to confirm the pollution situation of this area. Furthermore, more studies are necessary to investigate the trace metal concentration in the seawater or sediment where the algae live, and find the relationship between algae and the surrounding environment. Nevertheless, it must be strongly highlighted that the Antarctic pollution situation is becoming serious and strict environmental protocols should be implemented to prevent it.

## Acknowledgements

This work is financially supported by the Chinese Polar Environment Comprehensive Investigation and Assessment Program (Nos. CHINRE2015-02-01, CHINARE-04-01). The authors also thank the team members of 30th CHINAREs for helping collect the samples.

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**(Edited by Qiu Yantao)**