Evaluating stress caused by natural contamination in a volcanic island (Deception Island, Antarctica) using immobilized *Phaeodactylum tricornutum* and laser-induced fluorescence

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Abstract Antarctica is still the most remote and pristine place on the planet. However, volcanic activity is very important in providing natural sources of trace elements, mainly Mercury (Hg). The island of Deception, belonging to the South Shetland Archipelago, is a volcanic island, where high levels of trace elements can be found in surface waters. The trace elements enter the Antarctic marine food webs via phytoplankton, which are at the base of these webs. Physiological changes in photosynthetic phytoplankton process triggered by trace elements, can be used to assess contamination.

This study analyzes the stress caused by natural contamination in five different locations on the island of Deception: Colatina Beach, Whalers Bay, Fumarole Bay, Pendulum Cove e Morature Point. For this, cells of Phaeodactylum tricornutum were exposed to the waters for the sampling points. Later on, it was accessed the concentrations in the cells of the elements: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), mercury (Hg) and zinc (Zn). Physiological changes in the photosynthetic process in cells of *P. tricornutum* were also analyzer by laser induced fluorescence technique (LIF). Additionally, the concentrations of trace elements Zn, Cd, Pb, Cu, and Hg were determined in the water samples from the sites of exposure.

The concentrations of the elements in cells in the case of Cr, Cu, Zn and Hg were significant different from the control samples in all exposure sites, thus suggesting that those elements are bioavailable in Port Foster water from. For all the sampling sites, the LIF spectra of the treated cells showed changes in fluorescence intensity, resulting in an increased ratio F685 / F735, more accentuated in Morature Point. These changes suggest changes in the metabolic process of photosynthesis and are indicative that the chlorophyll molecules were damaged or that its biosynthesis was affected.The results obtained from the samples *in situ* in conjunction with tests carried out in controlled laboratory conditions showed that Hg causes changes in the photosynthetic process. They also showed that LIF technique can be used as time-saving, highly sensitive and effective tool for the detection of Hg stress, with potential for remote sensing and Hg contamination screening in polar areas.

Keywords: LIF, Trace elements, Chlorophyll, Stress.

1. Introduction

Deception Island (62°57'S, 60°38'W), one of the most active volcanoes in Antarctica, is located in the South Shetland Islands (Rey et al. 1995). Deception Island is a unique place in Antarctica with unusual natural, scientific, historical, educational, aesthetic and wilderness values. Deception Island has also had a long history of human activity, of which scientific research has been one of the dominant in recent decades.

Deception has a large flood caldera named Port Foster. It was the center of the earliest fur seals hunting commercial activity in Antarctica during 19th century and, nearly a century later, it was the most extensive anchorage used by the non-pelagic whale processing ship factories (Dibbern 2009). A young volcano in this archipelago, Deception Island, erupted before 1843, in the 1920s and then again in 1967, 1969 and 1970.

In aquatic systems we find a lot of elements of the periodic table in various fractions (dissolved,

colloidal particles), chemical forms (ions, organic compounds and inorganic) and oxidation states (Donat & Dryden 2010). Trace elements are those that present in nature or biota, concentrations in the range or below the part per billion (ppb). (Adriano 2001)

Phytoplankton constitute the base of marine webs, efficiently scavenging metals (González-Dávila, 1995), and rapidly responding to changes on metal availability in the environment (Cabrita et al. 2014).

Once incorporated into phytoplankton cells, trace elements may cause disturbance of cell membrane permeability, reduction of photosynthetic electron transport and carbon fixation, degradation and biosynthesis inhibition of photosynthetic pigments, inhibition of enzyme reactions or protein synthesis, among other cellular functions (Sunda and Huntsman 1998; Küpper et al., 2002). Inevitably, these alterations have clear repercussions on phytoplankton cell growth (Thomas et al. 1980) and photosynthetic performance (Cid et al., 1995). Responses of phytoplankton to trace elements at the photosynthetic level may thus be potentially used for metal contamination screening in marine ecosystems.

Chlorophyll a (Chl a) fluorescence is a sensitive indicator of the status of photochemical reactions (Buschmann, 2007). In vivo Chl a fluorescence spectra of plants typically include two maxima, one in the red (685 to 690 nm) and other in the far-red (710 to 740 nm) regions, which are primarily dependent on the concentration of Chl a (Buschmann, 2007). Most of this emission is linked to the pigment-protein complex photosystem II (PS II)-associated Chl a (Govindjee, 1995). Additionally, some fluorescence of the photosystem I (PS I), which is highest around 730 nm also contributes to the fluorescence emitted at the far-red band (Govindjee, 1995). Alterations in the fluorescence intensity of Chl a indicate variations in Chl a concentration, and have been pointed as one of the earliest indicators of physiological status in microalgae (Baker, 2008). Therefore, interest in the practical application of Chl a fluorescence as a rapid and sensitive bioindicator of stress in response to different contaminants, including trace elements (Kumar et al., 2014). Previous studies showed the red to far-red fluorescence ratio (F685/F735) as a promising indicator of different types of stress in plants (Lichtenthaler and Rinderle 1988; Schuerger et al. 2003; Buschmann 2007).

Among the various fluorescence techniques, LIF technique is a powerful non-destructive tool to determine the total amount of Chl a fluorescence resulting from a very small fraction (only 1 or 2%) of total absorbed light. The high sensitivity and resolution of LIF has been shown to be well suited for reliable estimation of plant Chl a concentrations and photosynthetic efficiency changes due to various plant environmental stressors (Dahn et al. 1992; Apostol et al. 2003; Lavrov et al. 2012), including metals (Schuerger et al. 2003; Maurya and Gopal 2008), and therefore has potential as an alternative tool for detecting signs of metal stress in phytoplankton. This technique has been used in controlled laboratory conditions and in the field, with promising application to remote assessments.

This study examines the presence and bioavailability of trace elements (As, Cr, Cu, Cd, Zn, Ni and Hg) in Port Foster surface waters, by determination of these elements. Also, examines how trace elements bioavailable affect: (1) the biomass by cell growth and (2) the physiological state of the cells associated with *P. tricornutum* photosynthesis applying the technique of LIF (Laser Induced Fluorescence).

2. Materials and methods

2.1. Field work

The sampling work was been done during the campaign of research project CONTANTARC-4, between 6 and 9 February 2015 on the island of Deception. It was selected five sampling sites in the bay of Port Foster: Morature Point, Pendulum Cove, Fumarole Bay, Whalers Bay and Colatina Beach, in order to cover the places of possible natural contamination.

Sampling locations are shown in Figure 1 and also some landmarks such as the location of the scientific basis of the island Deception.

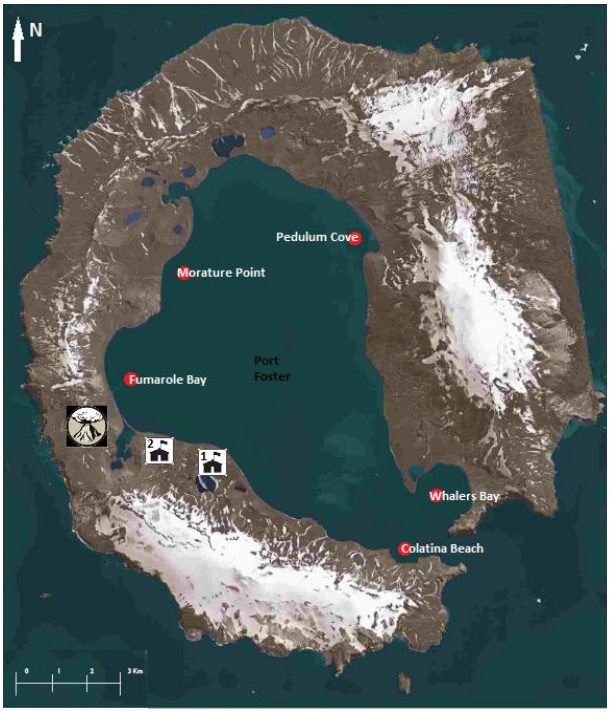

Figure 1_ Location of sampling

2.2. Sampling and analytical techniques

In Deception island bioavailability tests were performed using *P. tricornutum* cells previously immobilized in alginate.

The immobilized cells were placed in tubes, in f/2 culture (water supplemented with vitamins appropriate for the growth of the diatom) and kept in the dark at 4ºC until the beginning of the experiments.

For bioavailability tests, were prepared bags with control and test. In the control bags were placed the contents of the tubes (alginate with microalgae and the culture). Test bags were perforated to allow the entry and circulation of the water collected at the sampling sites. In these bags were introduced to only the immobilized algae.

The bags were removed after 24 hours of exposure to water of each location. After exposure immobilized cells were analyzed in IPMA and CQE-IST. In IPMA laboratory algae were disbanded into tri-sodium citrate solution 3%, according to the method described by Moreira et al. (2006) for cell count, determination of living cells and the concentration of trace elements incorporated in cells. In Lisbon, these cells were demobilized. A fraction of these cells were used in experiments LIF. The remaining fraction, part was used after acid digestion for the determination of trace elements and other (non-digested) for determining the content of Hg. In both cases the concentrations determined correspond to trace metals absorbed by the cells of the diatom and walls of cells.

The determination of Cd, Pb, As, Cr, Cu, Ni in the *P. tricornutum* cells was done by atomic absorption spectroscopy in the graphite furnace (GFAAS) and determination of Zn existing in higher concentration, was performed by atomic absorption spectrophotometry by flame.

Water samples collected at each sampling site were analyzed for determination of trace elements Zn, Cd, Pb and Cu by anodic stripping voltammetry (ASV). The determination of concentration of Hg was held at the University of Trent Canada by cold vapor-atomic fluorescence spectroscopy (CV-AFS).

For validation and comparison with the results obtained in the field it was done bioavailability tests on samples exposed in laboratory (controlled atmosphere). Also these tests were conducted in a controlled environment in order to determine the EC50 for each element.

To do this cultures of *P. tricornutum* cell were prepared and in exponential growth phase were exposed to selected trace elements Hg, Cr, Cu and Zn. Concentration gradients were created for each trace element, 0.010; 0.10; 1.0 and 10 µg/L. Cells were also exposed to a mix of elements being present all trace elements with a selected concentration of each of 0.010 ug/L. The cultures were maintained for 96 hours and proceeded to be taken of samples after 24 and 96 hours.

To perform cell count and calculating the growth rate of the cultured samples were collected after 24 and

96 hours and preserved with Lugol's solution immediately after harvest. The cell count was performed in a Neubauer chamber through an inverted microscope (Zeiss, Germany). Growth was evaluated by the average growth rate per day, calculated from the difference between the initial and final cell densities divided by the exposure period (Nyholm and Källqvist, 1989).

2.3. Quality assurance procedures

To assess the accuracy and precision of the obtained concentrations of trace elements were prepared two samples of blanks for each analyzed digestion procedure and certified reference materials (CRMs), under the same conditions used for the samples. Were chosen one marine sediments (MESS-3) and two biological matrix (279- Plankton and 414- Ulva). Duplicate samples were also conducted.

Differences between control and metal exposed *P. tricornutum* cells were evaluated using Kruskal-Wallis non-parametric test. Results yielding $p < 0.05$ were considered statistically significant. All statistical analyses were performed with Statistica 6.1 Software (StatSoft, Inc.).

3. Results and discussion

3.1. Water

In table 1 are show the results of the labile reactive concentration of Zn, Cd, Pb and Cu by ASV and the concentration of Hg in CV-AFS in water samples. Detection limit (LD µg/L) and Quantification limit (LQ µg/L) of the analytical techniques used are also showed. After UV irradiation no significant differences were fond for the trace elements concentrations, so that the total concentration does not differ from reactive labile fraction. This result may be related to the low concentration of dissolved organic matter (TOC ≤1 ppm).

In Colatina Beach it was not possible to quantify the presence of zinc due to a possible contamination of the sample. The standard deviations associated with this element are relatively high (average- 33.6%). The highest concentration was determined in the sample from Pendulum Cove.

It was not possible to quantify the concentration of Cu in the sample Fumarole Bay, due to a contamination occurring during the analysis. In the case of the concentration of Cd, the levels were below the detection limit (DL).

The values of Hg determined in dissolved fraction in different locations of Deception island ranged between 2.9 and 9.6 ng / L. These concentration values are very high compared to the values obtained in other studies, open ocean and in the southern Ocean. Compared to concentrations obtained by the study conducted by Mão de Ferro *et al.* (2014) it is noted a significant increase in Fumarole Bay, as well as Morature Point and Colatina Beach.

Table 1_ Concentration (mean ± SD) of the trace elements Zn, Cd, Pb and Cu (µg / L) and Hg (ng / L) in water samples from the sampling sites.

Sample	**Zn (µg/L)**	**Cd (µg/L)**	**Pb (µg/L)**	**Cu (µg/L)**	**Hg (ng/L)**
Whalers Bay	14 ± 8	0,32 ± 0,05	1,0 ± 0,5	3,9 ± 0,1	3,9 ±0,2
Colatina Beach	-	<LD	0,91 ± 0,08	0,65±0,05	2,9 ±0,1
Fumarole Bay	22 ± 5	<LD	0,33 ± 0,05	-	5,5 ±0,2
Morature Point	15 ± 8	<LD	0,51 ± 0,01	1,9 ± 0,5	9,6 ±0,3
Pendulum Cove	$(2,2 \pm 0,3)\times10^2$	0,9 ± 0,3	4,0 ± 0,8	0,53 ± 0,07	5,8 ±0,2
Detection Limit (LD)	8	0,1	0,2	0,2	0,01
Quantification limit (LQ)	24	0,4	0,7	0,5	0,03

The high presence of Hg has been attributed to the active volcanism of the island (Siegel et al, 1980;. Bargagli et al., 1993).

In summary, the values determined in this study Zn, Cd, Cu, Pb and Hg concentrations on five sampling locations on the island of Deception, are generally higher than those already reported in the literature for various local Antarctica. In the case of mercury the highest value was determined Morature Point, while the higher Cu concentration was determined at Whalers Bay. For the other elements the highest concentrations were found in Pendulum Cove.

3.2. Bioavailability tests on the samples of the field

Cell growth

In all sampling sites, the number of *P. tricornutum* cells decrease compared to the control (Figure 2). The most marked decrease was found in Morature Point, most probably due to the presence of toxic trace elements that cause cell death in cultures.

Trace elements in *P. Tricornutum* cells

Table 2 shows the average and standard deviation of the of Zn, Cu, Cd, Ni, Cr and Pb concentration in *P. tricornutum* cells exposed to water collected at the sampling sites as well as the respective control. The trace elements Zn, Cu, Cr, As and Hg present in all studied locations have, the most significant differences. Cells exposed in Pendulum Cove, show higher concentrations of Zn, Cu, Ni, Cr and Pb, which is consistent with the higher concentrations of Zn and Pb found in water the same does not happen in the case of Cu, Cd and Hg.

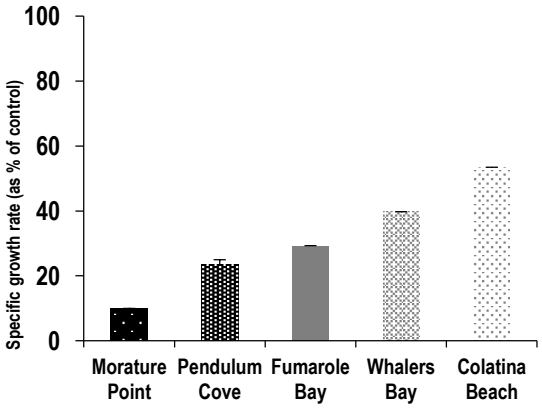

Figure 2_ Specific growth rate of cells at different sampling locations relative to the control.

Table 2_ Concentration of trace element (µg/g) in *P. tricornutum* cells from the bioavailability tests on sample sites.

		Zn	As	Cu	Cd	Ni	Cr	Pb	Hg
Colatina Beach	Control	3,2 ±0,9	27±4	1,1±0,2	0,1±0,1	<LD	67±8	<LD	<LD
	Exposed	10,4±0,2	30,3±0,2	2,1±0,6	0,4±0,1	8±1	115±2	<LD	0,31±0,02
Whalers Bay	Control	9±6	23±2	1,3±0,6	<LD	<LD	<LD	<LD	<LD
	Exposed	24±3	24±2	4±1	0,14±0,03	5,8±0,6	4,1±0,5	<LD	0,29±0,01
Fumarole Bay	Control	13±5	23±2	2±1	<LD	<LD	<LD	<LD	<LD
	Exposed	24±4	23±2	7±1	<LD	6,2±0,4	30,8±0,3	<LD	0,42±0,01
Pendulum Bay	Control	2,6±0,1	24±2	1,08±0,02	<LD	<LD	<LD	<LD	<LD
	Exposed	14±2	26±2	3,11±0,04	0,4±0,1	2,4±0,4	24±2	2,2±0,3	0,51±0,01
Morature Point	Control	6,2±0,4	22±1	0,984±0,002	0,13±0,04	<LD	<LD	<LD	<LD
	Exposed	12±1	26±2	5±2	0,21±0,04	0,41±0,03	2,3±0,1	0,21±0,01	1,2±0,2

LIF

As can be seen in Figure3, the LIF spectre show two zones of maximum intensity, the first in the visible range in red (685 to 690 nm) and a second in the far-red (710 to 740 nm). It is evident that there is a major change of *P. tricornutum* spectra exposed in situ relative to the control, which evidences the negative impact that exposed cells suffered.

The most affected cells were exposed in Morature point, as can be concluded by the marked differences across the Morature spectrum compared to the control spectrum. The change is so significant that the fluorescence emission in the far-red zone is almost nil, and may be indicative of changes in the PSI and PSII.

The F685 / F735 ratio allows to highlight the changes in the spectrum caused by the *P. tricornutum* cells exposure in the sites.

From Table 3 we can see that there is an increase in the ratio in all the sampling locations, however, in the case Morature Point, this increase is more evident. This is mainly because of a decrease in fluorescence intensity at 735 nm. This demonstrates that the exposure causes changes in the PSII efficiency, but also affects the distribution of excitation energy between the two photosystems (Wollman, 2001). These results demonstrate that the ratio F685 / F735 is a good indicator for the early detection of stress, being in agreement with several studies showing that this ratio can be used to evaluate the concentration of Chl, photosynthetic efficiency (Lichtenthaler et al. , 1996) and stress tolerance in different plant species (D'Ambrosio et al, 1992;. Buschmann, 2007).

From the above results we can conclude that the place where the exposed cells were most affected was Morature Point. At the same time this is the sampling point where we observe a high Hg concentration in cells and in the water. All results indicate that Hg is responsible for the negative changes in *P. tricornutum* cells. In order to prove this conclusion, tests were done in a controlled environment (laboratory).

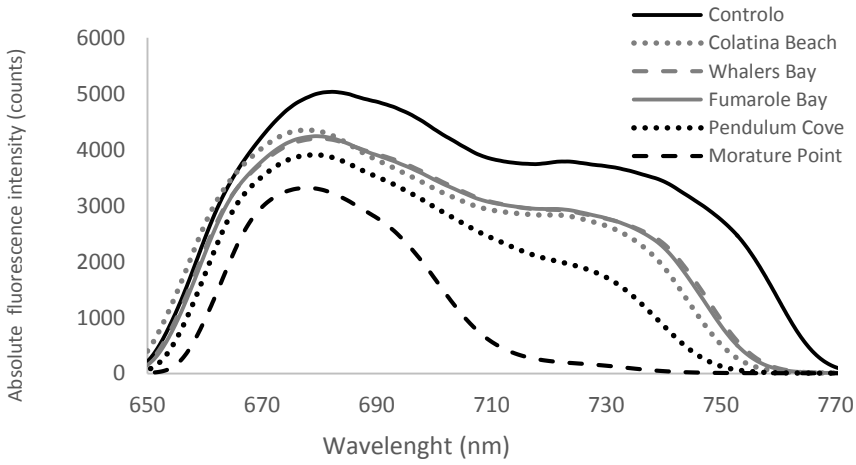

Figure 3_ Absolute intensity of fluorescence (counts) compared to bioavailability testing of cells in the sample sites.

Table 3_ F685 / F735 ratio (mean ± SD) related to sampling sites for cells of *P. tricornutum*.

	Control	***Colatina Beach***	***Whalers Bay***	***Fumarole Bay***	***Pendulum Cove***	***Morature Point***
F685/F735 ratio	1,21±0,04	1,54± 0,02	1,435±0,006	1,42±0,01	1,9±0,1	15,2±1,4

3.3. Bioavailability testing of laboratory samples

Cell growth

For all metal there was a decrease in cell growth of *P. tricornutum* after 24 and 96 h of exposure, more pronounced in the higher concentration of the trace elements in the medium culture. In the case of Hg this decrease is particularly pronounced, such for concentrations ≥1 µg/L cell mortality was 98%.

Trace elements in *P. Tricornutum* cells

Table 4 presents the average and standard deviation of the Hg concentration of in *P. tricornutum* cells laboratory prepared at different concentration levels as well as the respective control. In all selected concentrations (0.010, 0.10, 1.0 and 10 µg/L) Hg incorporation differences were found. It should be stressed that the higher the concentration in the medium the higher the mercury concentration found in the cells.

LIF

From the Hg LIF spectra shows in Figure 4, for concentrations ≥0,1 µg/L there are clear differences in the spectra obtained after 24 hours of exposure, being the decrease of fluorescence intensity more pronounced at the highest concentration. After 96 hours of exposure and the concentration of 10 µg/L there was no fluorescence detection and one can conclude that a change has occurred in the photosynthetic cell process. Compared the Mix situation there is a large decrease in fluorescence emission after 24 hours of exposure. Since in this case of Mix the concentrations of each component used were 0.01 µg / L and taken into account the previous results, this behavior cannot be assigned only to mercury but probably due to a synergetic effect of the toxicity of this element in the presence of other elements.

In agreement with the results obtained by several authors, Hg is a toxic element which causes an inhibition in the growth of *P. tricornutum* changes in the physiological processes (Hannan and Patouillet, 1972; Deng et al, 2013.). We can thus prove that the *P. tricornutum* photosynthetic efficiency decreases when it is under stress caused by trace elements. The concentrations of Cr, Cu and Zn used in this study have shown no significant effect on cell growth (figure 4), being maintained fluorescence intensity compared with the control cells. However, higher concentrations of these trace elements can have similar effects to the Hg as previously observed for this species and other species of diatoms (Sunda, 1989; Persic and Horvatić, 2007).

The fluorescence emission shifts were clearly evidenced by the ratios F685 / F735 presented in Table 5. The reason for the significant increase in the case of Hg and Mix compared with control is mainly due to a decrease in fluorescence intensity at 735 nm. These observations indicate that trace elements not only causes changes in the PSII efficiency, but also affect the distribution of excitation energy between the two photosystems, as reported by Wollman (2001). These results also show that the ratio F685 / F735 is a good indicator for the detection of stress caused by trace elements in *P. tricornutum* in accordance to others studies that demonstrated that this ratio can be used to evaluate the change of chlorophyll concentration and photosynthetic efficiency (Lichtenthaler et al., 1996) in response to different kinds of stress in different plant species (D'Ambrosio et al, 1992;. Buschmann, 2007).

Table 4_ Incorporation (mean±SD) of Hg in cells exposed in laboratory.

		Concentrations (µg/L)			
		0,010	**0,10**	**1,0**	**10**
***Hg** (µg/g)*	24 h	3,4 ±0,2	19±6	(4,8±0,8)×10^2	(6±1)×10^2
	96 h	2,1±0,2	10,1±0,3	(7±1) ×10^2	(3,6 ±0,8) ×10^2

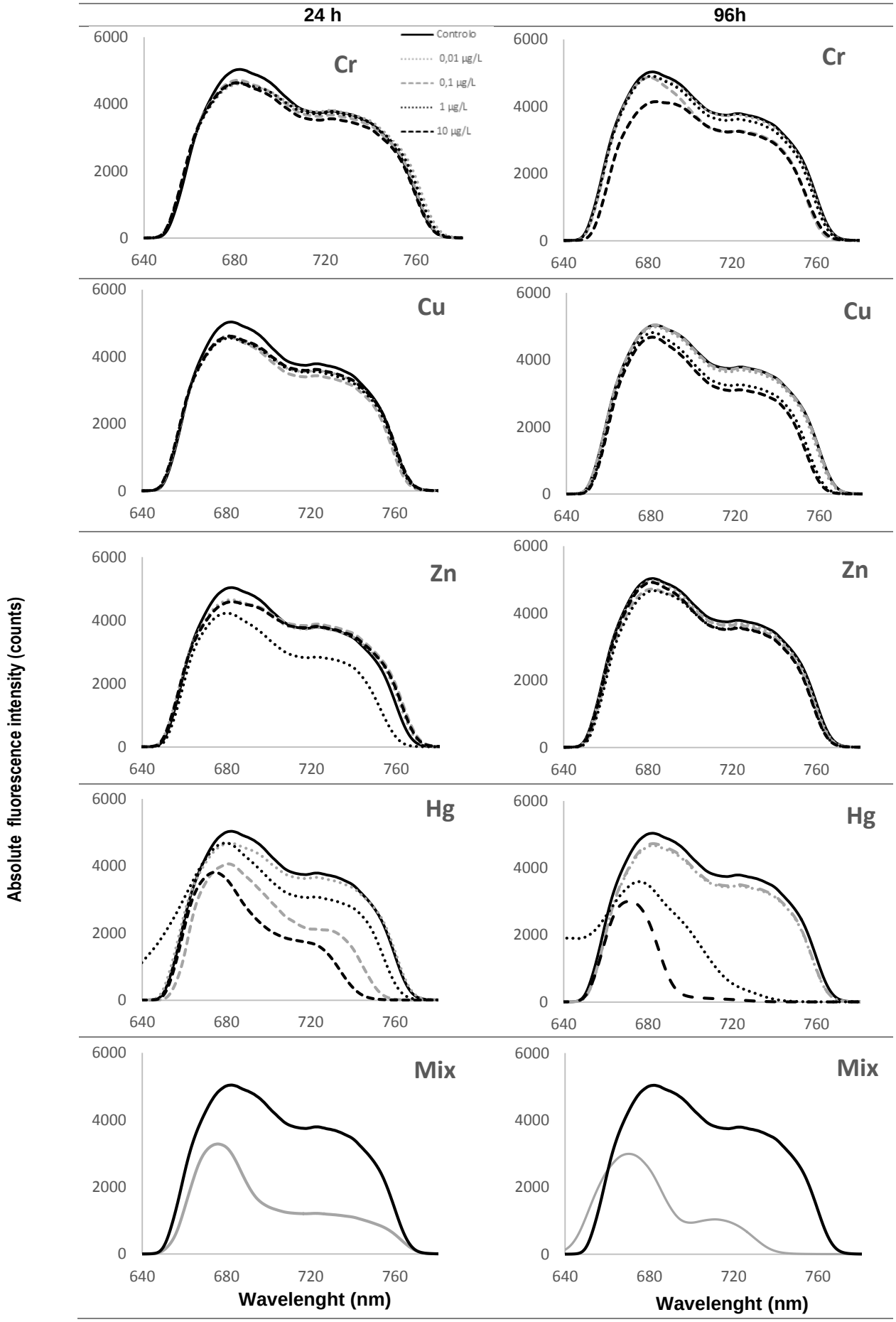

Figure 4_ Absolute intensity of fluorescence (counts) for the incubation period (24 h and 96 h) in relation to the bioavailability tests of cells exposed in the laboratory.

Table 5_ Ratio F685 / F735 (mean ± SD) for trace elements Cr, Cu, Zn, Hg and mix in relation to exposed period (24h and 96h) for cells of *P. tricornutum*.

F685/F735 ratio		**Concentrations (µg/L)**				
		Control	**0,010**	**0,10**	**1,0**	**10**
Cr	24 h	1,311±0,001	1,252±0,004	1,238±0,005	1,24±0,01	1,33±0,04
	96 h		1,382±0,002	1,34±0,09	1,48±0,01	1,31±0,01
Cu	24 h		1,37±0,01	1,36±0,02	1,34±0,01	1,31±0,03
	96 h		1,51±0,04	1,47±0,04	1,36±0,02	1,32±0,03
Zn	24 h		1,26±0,02	1,188±0,002	1,5±0,1	1,248±0,006
	96 h		1,482±0,002	1,33±0,01	1,31±0,04	1,422±0,004
Hg	24 h		1,347±0,005	1,9±0,1	1,52±0,01	2,4±0,6
	96 h		1,43±0,01	1,32±0,02	7±1	40±4
Mix	24 h		2,74 ±0,01			
	96 h		3,21±0,01			

4. Conclusions

Throughout this study it was found that emissions from volcanic activity influence the concentrations of trace elements (Zn, Pb, Cu, Cd and Hg) particularly Hg in the water column of the Port Foster bay. The most affected sampling points were Morature Point followed by Pendulum Cove, Fumarole Bay, Whalers Bay and finally Colatina Beach. This result may be due to the fact that the existence of volcanic outcrops carrying trace elements also being associated with the existing recirculation Port Foster.

Bioavailability tests using *P. tricornutum* carried out on Deception Island show that those elements are in a bioavailable form, which can potentially be transferred to the marine food chain, primarily through its accumulation in the phytoplankton. This transfer to the biota may have affected primary productivity, as shown by the results obtained using the LIF technique, in addition to other possible effects on biodiversity of this remote area.

It was possible to conclude that Hg concentrations found in the bay of Port Foster, Deception Island were high enough to affect the physiological processes of *P. tricornutum*, the level of growth and photosynthesis physiology. The laboratory tests using similar levels dissolved Hg corroborate these results.

Regarding the elements Cr, Cu and Zn, it was concluded that the levels of these elements observed on surface water from the island of Deception, were not high enough to cause serious damage in *P. tricornutum*, which was also confirmed by laboratory tests.

Laboratory tests also allowed to determine the EC50 (concentration 1 µg/L) of Hg. The concentrations of other elements used in these tests, although reflecting the levels typically found in contaminated marine areas, did not allow to determine the EC50. To this end, it would be necessary to use higher concentrations, which, however, do not reflect the conditions in the island of Deception and even other contaminated coastal areas.

For the Mix exposure conditions where various elements were used in order, trying to reproduce the contamination that occurs in the natural environment, an EC50 value can not be determined in conceptual terms. However the cell mortality observed in the Mix solutions, may help to understand the effect of trace elements in future studies of evaluation of toxicity performed in situ.

This study also showed that the LIF is a highly reliable, sensitive technique, precise and effective tool for the detection of trace elements causing stress in phytoplankton communities. Thereby it is a great choice

for sensing and contamination screening in remote places such as the polar areas.

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5. References

Adriano, D. C. (2001). Trace Elements in Terrestrial Environments. USA: Springer Science+Business Media, LLC.

Apostol, S., Viau, A.A., Tremblay, N., Briantais, J.-M., Prasher, S., Parent, L.-E., & Moya, I. 19 (2003). Laser-induced fluorescence signatures as a tool for remote monitoring of water and 20 nitrogen stresses in plants. *Canadian Journal of Remote Sensing* 29(1), 57-65.

Baker, N.R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis *In Vivo*. *Annual* 22 *Review of Plant Biology* 59, 89-113.

Bargagli, R., Battisti, E., Focardi, S., Formichi, P. (1993). Preliminary data on environmental distribution of mercury in northern Victoria Land. Antarctica, Antarctic Science (5), 3-8.

Bargagli, R. (2008). Environmental contamination in Antarctic ecosystems. Science of The Total Environment 400 (1-3), 212-26.

Buschmann, C. (2007). Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves. *Photosynthesis Research* 92, 261-271.

Cabrita, M.T., Raimundo, J., Pereira, P., Vale, C., 2014. Immobilised *Phaeodactylum tricornutum* as biomonitor of trace element availability in the water column during dredging, Environ. Sci. Pollut. R. 21 (5), 3572-3581.

Cid, A., Herrero, C., Torres, E., & Abalde, J. (1995). Copper toxicity on the marine microalga *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. *Aquatic Toxicology* 31, 165-174.

Dahn, H.G., Günther, K.P., & Lüdeker, W. (1992). Characterization of drought stress of maize and wheat canopies by means of resolved laser induced fluorescence. *EARSel Advances in Remote Sensing* 1(2)-II, 12-19.

D’Ambrosio, N., Szábo, K., & Lichtenthaler, H.K. (1992). Increase of the chlorophyll fluorescence ratio F690/F735 during the autumnal chlorophyll breakdown. *Radiation and Environmental Biophysics* 31, 51-62.

Dibbern, J. S. (2009). Fur seals, whales and tourists: a commercial history of Deception Island, Antarctica. *Polar Record* 46 (03), 210-221.

Donat, J., Dryden, C. (2010).Transition Metals and Heavy Metal Speciation. *Marine Chemistry and Geochemistry*, edited by John H. Steele, Steve A. Thorpe, & Karl K. Turekian. Jamestown Road, London, UK: Academic Press, 72 – 80

González-Dávila, M. (1995). The role of phytoplankton cells on the control of heavy metal concentration in seawater. Marine Chemistry 48, 215-236.

Govindjee (1995). Sixty-three years since Kautsky: chlorophyll *a* fluorescence. *Australian Journal of Plant Physiology* 22, 131-160.

Kumar K.S., Dahms, H.-U., Lee, J.-S., Kim, H.C., Lee, W.C., & Shin, K.-H. (2014). Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. *Ecotoxicology and Environmental Safety* 104, 51-71.

Küpper, H., Setlik, I., Spiller, M., Küpper, F.C., & Prasil, O. (2002). Heavy-metal-induced inhibition of

photosynthesis: targets of in vivo heavy metal chlorophyll formation. *Journal of Phycology* 38, 429-441.

Lavrov, A., Utkin, A.B., Marques da Silva, J., Vilar, R., Santos, N.M., & Alves, B. (2012). Water stress assessment of cork oak leaves and maritime pine needles bases on LIF spectra. *Optics and Spectroscopy* 112, 271-279.

Lichtenthaler, H.K., & Rinderle, U. (1988). The role of chlorophyll fluorescence in the detection of stress conditions in plants. *CRC Critical Reviews in Analytical Chemistry* 19, S29-S85.

Lichtenthaler, H.K., Lang, M., Sowinska, M., Heisel, F., & Miehé, J.A. (1996). Detection of vegetation stress via a new high resolution fluorescence imaging system. *Journal of Plant Physiology* 148(5), 599-612.

Mão de Ferro, A., Canário, J., Mota, A. *(*2014). Pathways and speciation of mercury in the environmental compartments of Deception Island, Antarctica. Chemosphere 95, 227–233.

Moreira, M., Santos, M., Guilhermino, L., & Ribeiro, R. (2006). Immobilization of the marine microalga Phaeodactylum tricornutum in alginate for in situ experiments: bead stability and suitability. *Enzym Microbiol Technol, 38(1–2)*, 135–141.

Nyholm, N., & Källqvist, T. (1989). Methods for growth inhibition toxicity tests with freshwater 3 algae. *Environmental Toxicology and Chemistry* 8(8), 689-703.

Rantala, R., & Loring, D. (1977). A rapid determination of 10 elements in a marine suspended matter by atomic absorption spectrophotometry. *Atom. Absorp. Newsletter, 16*, 51-52.

Rey, J., Somoza, L,, Martinez-Frías, J. (1995). Tectonic, volcanic, and hydrothermal event sequence on Deception Island (Antarctica). *Geo-Marine Letters*.

Schuerger, A.C., Capelle, G.A., Di Benedetto, J.A., Mao, C., Chi, N., Mark, T., Evans, D., Richards, J.T., Blank, T.A., & Stryjewski, E.C. (2003). Comparison of two hyperspectral imaging and two laser-induced fluorescence instruments for the detection of zinc stress and chlorophyll concentration in bahia grass (*Paspalum notatum* Flugge.). *Remote Sensing of Environment* 84, 572-588.

Siegel, S. M., Siegel, B. Z.,McMurtry, G. (1980). Atmosphere-soil mercury distribution: The biotic fator, *Water. Air, and Soil Pollution* 13 (1), 109-12.

Sunda, W.G. (1989). Trace metal interactions with marine phytoplankton. *Biological Oceanography* 6(5-6), 411-442.

Sunda, W.G., & Huntsman, S.A. (1998). Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Science of the Total Environment* 219(2-3), 165-181.

Thomas, W.H., Hollibaugh, J.T., Seibert, D.L.R., & Wallace, G.T. Jr. (1980). Toxicity of a mixture of ten metals to phytoplankton. *Marine Ecology Progress Series* 2, 213-220.