Evidence of a hormetic effect in growth inhibition algal test

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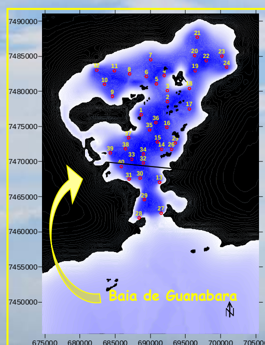
ABSTRACT

Elutriation process was applied on sediments of Guanabara Bay (Rio de Janeiro, Brazil), a very polluted coastal ecosystem, in order to determine their potential toxicity. Elutriates were tested using *Skeletonema costatum*, an eurialin Diatom. Several ratios of elutriate/growth medium (v/v) were assayed (1:10, 1:4, and undiluted) as cultural medium for exponentially growing algae, carrying out a blank control for each replicate. Tests were performed for five days, controlling algal growth by manual cell counting by microscope, in order to verify the *status* of the organisms every assay's day. This experimental design was proved able to highlight a particular phenomenon, *i.e.* hormesis. Regarding some sites in the southern part of the Guanabara Bay growth curves, relative to lower sediment elutriate dilutions, showed, in fact, a so called toxic stimulation typical of hormetic event. On the contrary undiluted elutriates never permitted algal growth. Obtained results could help furthermore to explain the particular eutrophic situation of the water body, in which there is a high superficial eutrophization and absolute life deficiency in the deeper water column.

INTRODUCTION

Guanabara Bay (Rio de Janeiro, Brazil) is the research area, a very polluted coastal tropical ecosystem. It is studying directly o by elutriation process, in order to determine their potential toxicity. *On the right*, localization of the 40 sediment sampling sites.



Here we highlight results obtained applying as biological tool *Skeletonema costatum* (Diatom Algae) growth inhibition test, using several ratios (v/v) of elutriate/growth *medium* (1:10, 1:4, and undiluted) as cultural *medium*, carrying out a blank control for each replicate. This test is been assayed in order to verify it as suitable component of a battery test.

Guanabara Bay sediment studied by

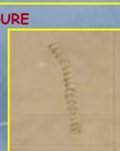
- geo-chemical tools (See poster WE1/EV/C15)
- biological *tools*:
  - microbiological aspects
  - algal growth test
  - micro- and macro-benthos community analysis
  - metallothionein in mussels (see poster TH1/MI/P10)
  - frozen mitochondrial test (see poster WE1/EV/P28)
  - mussel air survival test
  - mussel active and passive biomonitoring
  - heavy metal and PAH bioaccumulation in mussels (See poster TH1/MI/P10)

Algal growth inhibition test was choose because:

- algae are the first level of trophic web
- algae have an essential role on water body oxygenation processes
- this test shows an ecosystemic importance
- it is a chronic test able to highlight medium-long period potential effects.

MATERIALS & METHOSD

Skeletonema costatum GROWTH TEST PROCEDURE



- Guillard growth colture medium:
  - gentle mixed
  - forced aeration
- thermostatic chamber conditions:
  - T = 20 °C ±1
  - light: ✓ $380 \le \lambda \le 780$ nm
    ✓ 5000 lux light intensity
    ✓ 16:8/light:dark photoperiod
- 24h monitored growth (algal cells number/Vol)
- *end point* : growth inhibition or stimulation, *i.e.* coltured algal cells differential growth with respect to different testing matrix concentrations *versus* reference blank sample
- algal glass tube inhoculation: 10^4 cells mL^{-1} (log phase)
- test replication: 2/elutriate concentration
- test period: 5 days (120 h)
- elutriate concentration count number: 4/24 h
- test validation criteria (U.S.EPA,1996a)
  - pH monitoring (UNI EN ISO, 2000)
  - log phase in 96 h, *i.e.* growth rate 0,04 cells/h
  - reference toxic compound test: $K_2Cr_2O_7$ (1.11 10^{-4} M, 2.78 10^{-4} M, 5.55 10^{-4} M, 1.11 10^{-3} M).

ELUTRIATION METHOD (U.S.-EPA, 2001)

- ✓ 100 volumes of sediment + 400 volumes of artificial seawater
- ✓ ultrasound bath for 40 min, at 20 °C
- ✓ 20 min centrifugation at 4,000 rpm (ALC centrifuge, mod. 3226, r_m=46 mm, α=30°)
- ✓ surnatant filtration with cellulose-acetate (0.45 µm cut off)
- ✓ elutriates immedietaly used for test

Test glass tube (*from left to right*): blank ref., dil. 1:10, dil.1:4, undilute.

Negative inhibition values show algal growth stimulation, instead positive a concentration decrease, as UNI EN ISO (2000) rules.

RESULTS

Fig.1

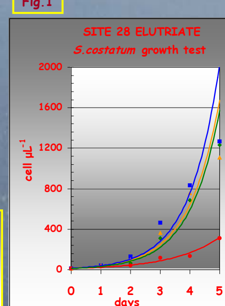


Fig.2

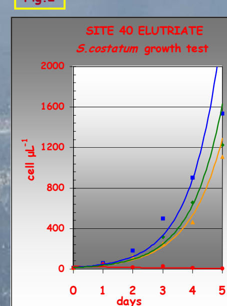
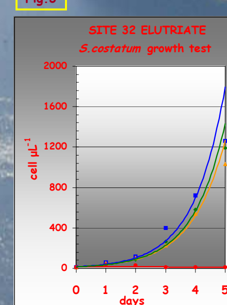


Fig.3



Dil. 1:10
Dil. 1:4
Undilute
Blank ref.

Fig. 4

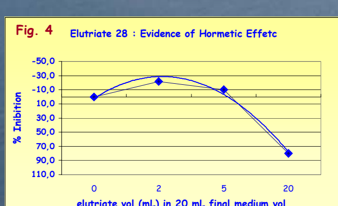


Fig. 5

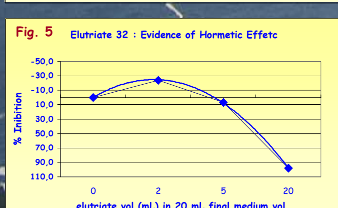
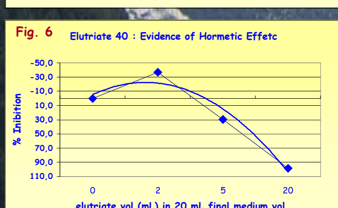


Fig. 6



- How you can see more in detail in the geo-chemical results reported in Poster WE1/EV/C15, entire Guanabara Bay area sediments are part of a really polluted coastal ecosystem. Therefore, elutriates from these sediments were expected generally to show high toxicity level when tested.
- In fact, *Skeletonema c.* growth was finally almost completely inhibited, when undiluted elutriate from each sampling sites was used as culture *medium* for the algae.

We have here reported only few examples, relative to elutriates from 28 (Fig. 1), 40 (Fig. 2) and 32 (Fig. 3) sampling sites sediment.

- But with diluted volumes of elutriates (1:10, particularly, and 1:4 dilution), instead, one can assist at growth stimulation phenomenon that it is often observable in aquatic ecotoxicology (Boiler & Oris, 1998; Cedergreen *et al.*, 2004; Ahlf & Heise, 2005; Sano *et al.*, 2005; Tuerker Saçan & Balcioglu, 2005; Devez *et al.*, 2005), *i.e.* a hormetic dose-response relationship.
- The most common form of the hormetic (characterized by a low-dose stimulatory and high-dose inhibitory response) dose-response curve could be depicted with the so named β- or *inverted U-shaped curve*. Normally, endpoints displaying this kind of curve include growth, fecundity and longevity.
- Then, it has been seen it [see reverse U-shaped curves in Fig. 4, Fig. 5 and Fig. 6, relative to same above mentioned elutriates from 28, 32 and 40 sampling sites sediment] for the sampling sites positioned where hydrous change was evident (i.e. generally in the southern and central zones, or everywhere - at this stage of our research - this is the riscontrata condition in the Guanabara Bay).
- The obtained results clearly highlight Guanabara Bay sediments as a very polluted matrix: natural sediment elutration events (following to resuspension or leaching or mixing at interface sediment/water column level, because of low/high tide, wave action, bioperturbation, drainage, etc.) seem able to carry out to strong acute toxicity, it has been confirmed by algal death after the first or the second day of contact in every undiluted elutriate sample tested [see red curves in Fig. 1, 2, 3].

CONCLUSIONS

- Using elutriate from polluted sediment as both total and partial cultured *medium* in order to assess *S. costatum* growth inhibition test, it has well showed the great capability of this approach, in order to verify sediment pollution impact to organism.
- Furthermore, this kind of approach has showed it is able to distinguish generically two different trends in Guanabara Bay zones, not as a strictly geographic but as a functional separation of area. Everywhere it is present a strong daily water turnover (and obviously in the same sites sediment have a sand texture that adsorb low polluted compounds concentration), the diluted 1:10 and 1:4 elutriate samples showed hormetic behaviour [see as example the blue and orange curves in Fig.1, 2, 3]. While in the areas where water flow tunrover are compromised (because of anthropogenic reasons), it is possible to observe a clear inhibition trend also in the dilute 1:10 and 1:4 elutriate samples [data not showed here].
- Diluted elutriate sample could virtually represent (Burton, ….; Manente, 2003) a dilution process in water column from deeper to higher layer of the water body. The obtained results of verifiyng hormesis could give partial reason of a particular local phenomenon, *i.e.* a strong eu-/dis-trophic situation into first parts of water column *versus* an atrophic condition "above" the sediment.

REFERENCES

- U.S.EPA,1996a
- UNI EN ISO, 2000
- Bailer & Oris (1998). *Human & Experimental Toxicology*, 17(5):247-250;
- Cedergreen *et al.* (2004). *Ecotoxicology and Environmental Safety*, 58:314-323;
- Ahlf & Heise (2005). *J. Soils & Sediments* 3(1):16-20;
- Sano *et al.* (2005). *Aquatic Toxicology* 71:283-296;
- Turker Saçan & Balcioglu (2005). *Ecotoxicology and Environmental Safety, In Press;*
- Devez *et al.* (2005). *Science of the Total Environment* 348:82-92.
- Burton (1998). AETE Project 3.2.2a Final Report December 1998.
- Manente (2003). *Environmental Sciences PhD Thesis*, Ca' Foscari University of Venice.

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