

Improved sensitivity to heavy metals in the bioluminescence assay using alternative salt solutions

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Introduction

The marine bacterium *Vibrio fischeri* is widely used for the toxicity assessment of environmental samples. The toxicity is measured in a medium containing 2% NaCl. When the bacteria are exposed to toxic compounds, the light production is inhibited. Toxicity values are obtained after 15 and 30 minutes exposure and expressed as EC50 values, the effective concentration causing 50% loss in light emission. Because of the general presence of metals in our environment, study of their toxicity is important. In chloride containing media, many metals form chlorocomplexes resulting in an increase of the EC50 values.

In order to improve the sensitivity of the test, especially for online monitoring using the iTOXcontrol (Figure 1), alternative salt mixtures were tested: sucrose, NaClO₄ and the ProMet buffer of microLAN.

Experiments

Comparison of the four salt solutions

Dilution series of CdCl₂ and CuSO₄ were prepared in 2% NaCl, 20.4% sucrose, 4.2% NaClO₄ and ProMetal solution of microLAN respectively. To mimic the test conditions of the iTOXcontrol, bacteria were freshly prepared by adding 50 µl of a luminescent culture to 10 ml of each salt solution. The test was performed according to the ISO method 11348-3. Bacteria were mixed with each dilution in a 1:1 ratio. The bacteria were exposed for 30 minutes at 15°C to the metal. The light emission was measured using the TOXmini luminometer (microLAN) and EC50 values calculated.



Figure 1. iTOXcontrol: automated online biomonitor using light emitting bacteria to measure the toxicity of water samples.

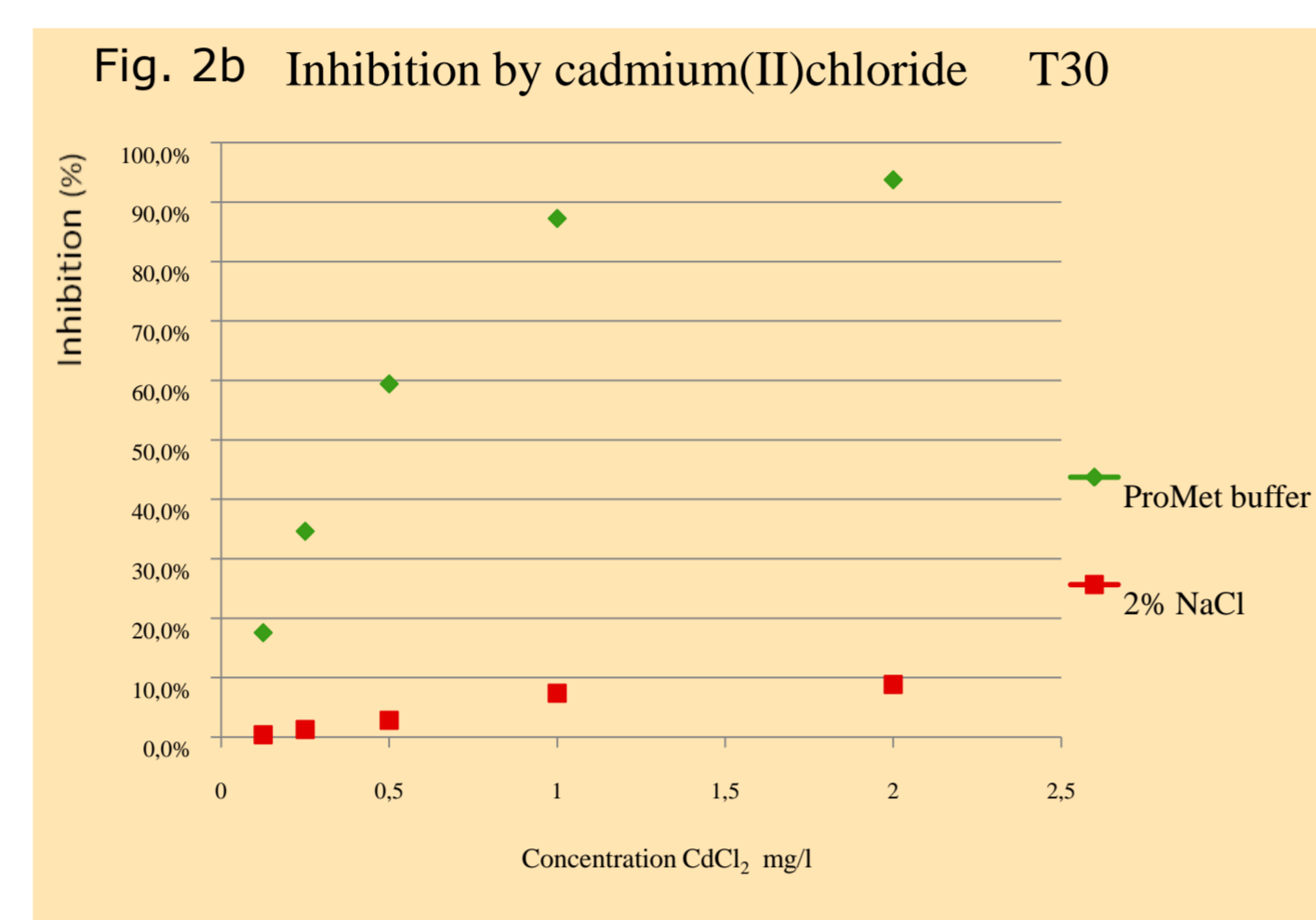
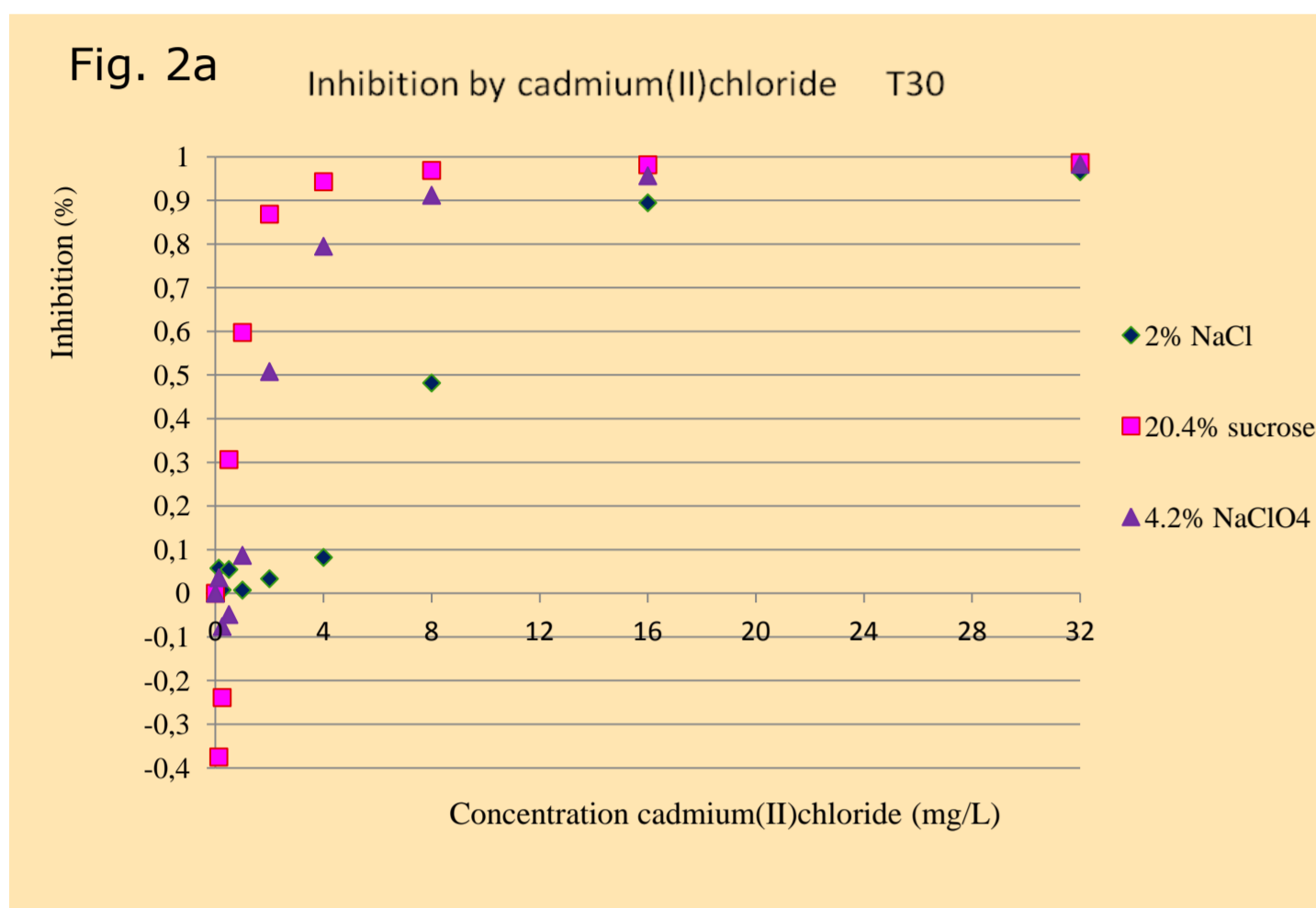


Figure 2. Dose response curves of *V. fischeri* exposed to cadmium

In sucrose a EC50 of 0.22 mg/l could be obtained (Fig. 2a). In 2% NaCl the EC50 was 8.3 mg/l. The ProMet buffer (Fig. 2b) showed a EC50 of 0.3 mg/l. Note the difference in scale between Fig. 2a and 2b.

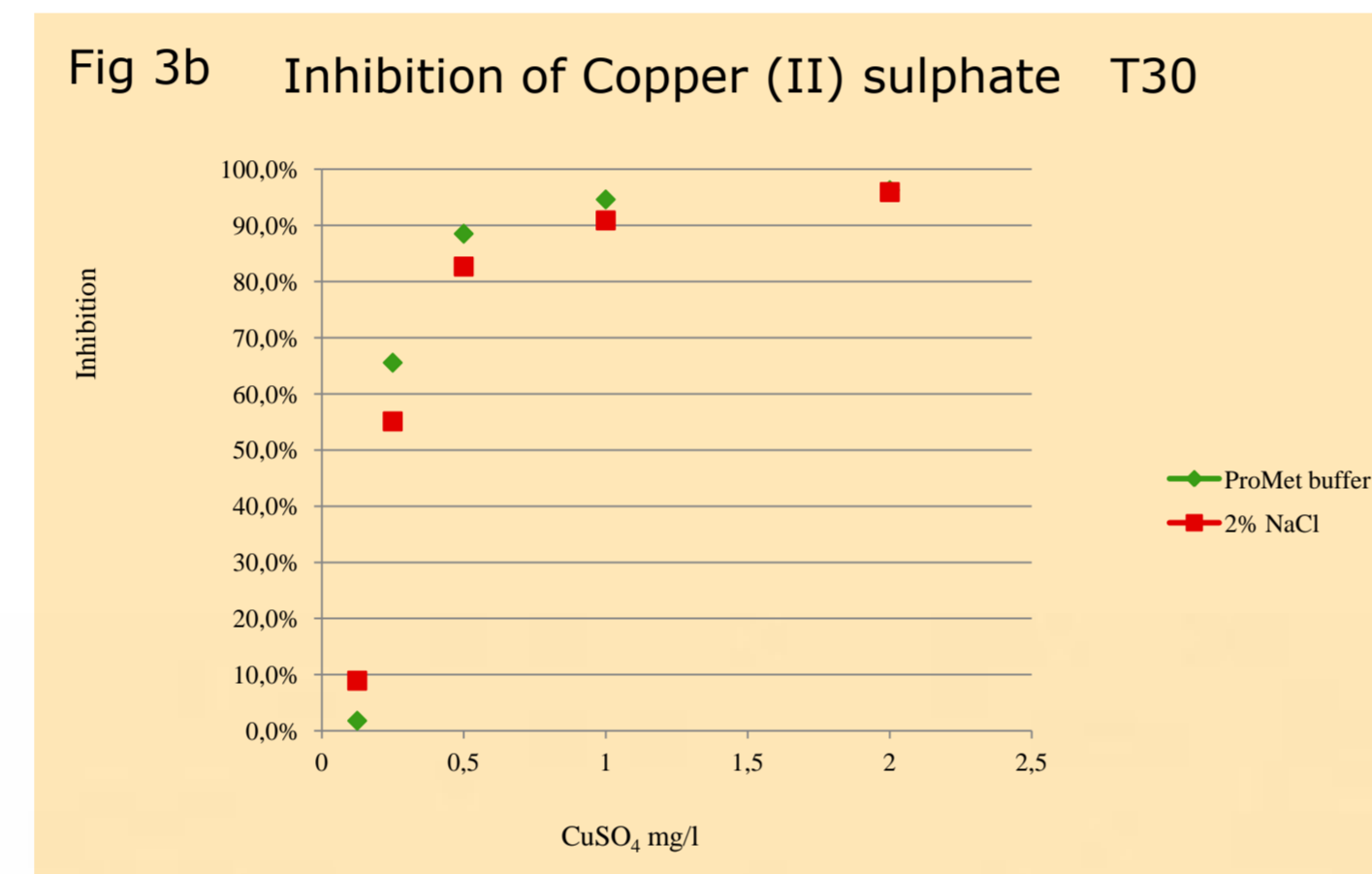
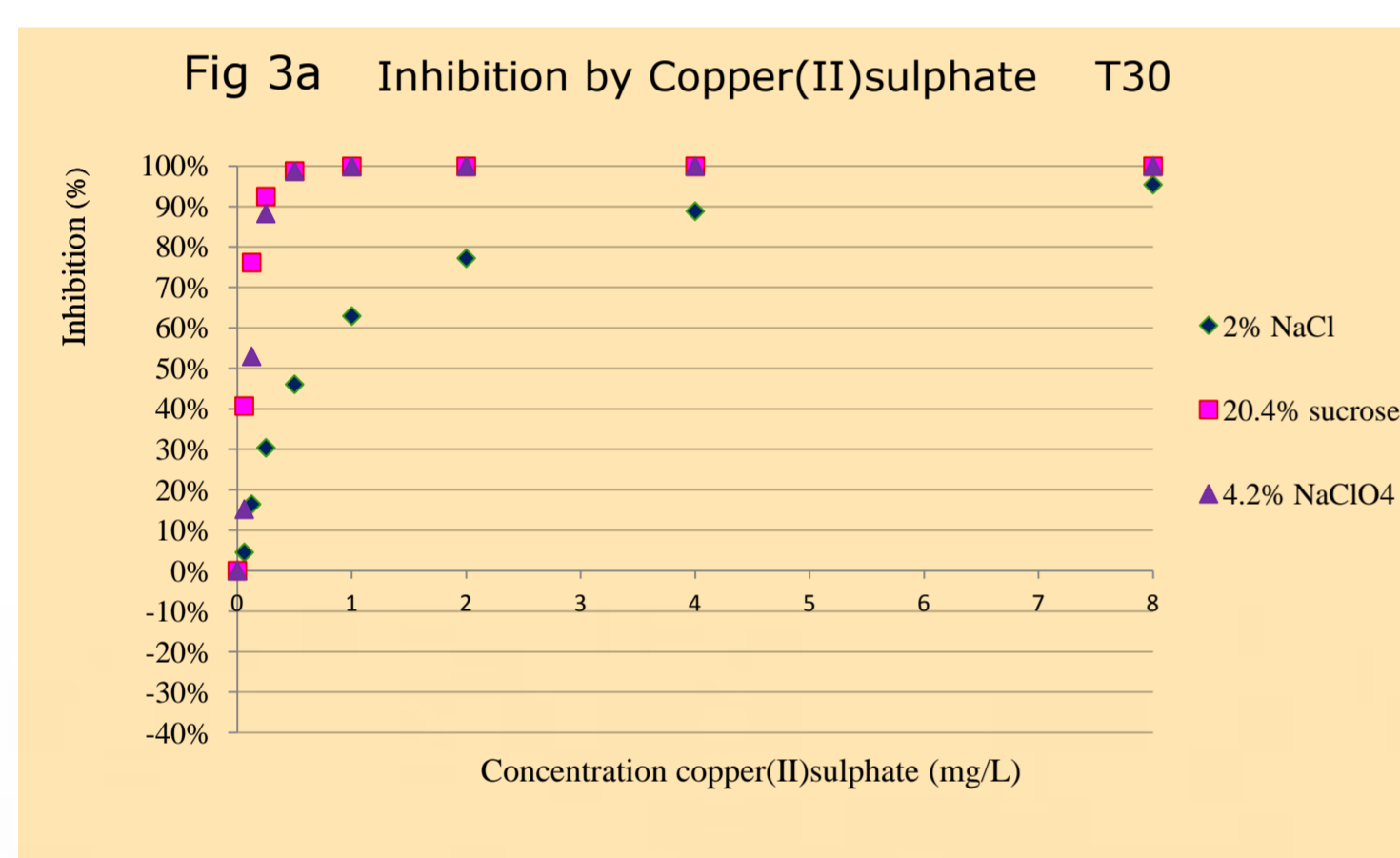


Figure 3. Dose response curves of *V. fischeri* exposed to copper

In Fig. 3a sucrose and NaClO₄ showed the best sensitivity. EC50 values of 0.08 and 0.11 mg/l respectively were obtained. The EC50 value for Cu in 2% NaCl was 0.7 mg/l in this test. Using the ProMet buffer, the EC50 was 0.35 mg/l (Fig. 3b).

Comparison of ProMet and 2% NaCl solutions

In a second series of experiments lead nitrate, iron(II)sulphate and silver nitrate in 2% NaCl were compared to the ProMet buffer only. Bacteria and dilution series were prepared as described above.

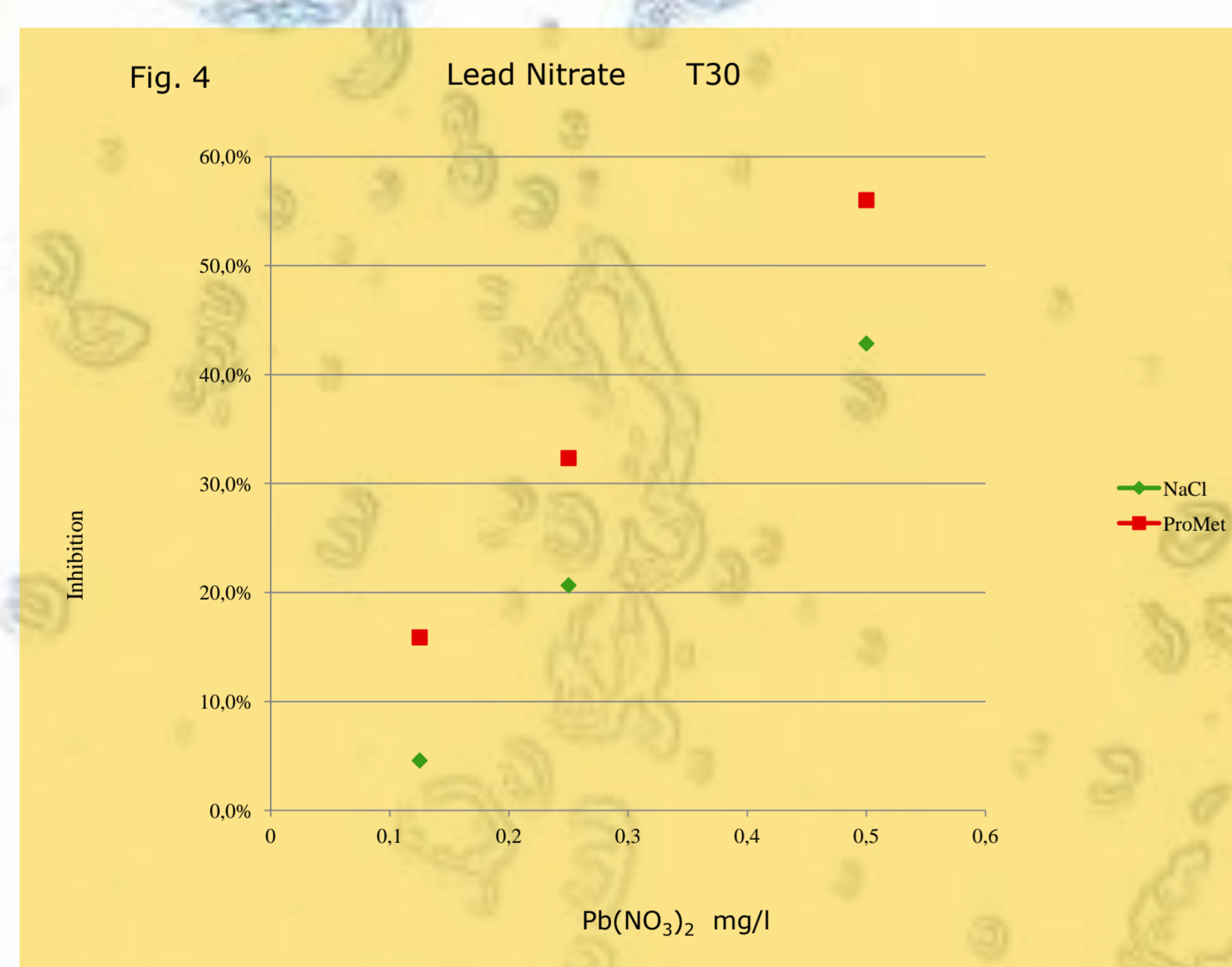


Figure 4. Lead nitrate

The ProMet buffer improves only slightly the sensitivity of the bacteria for this metal. At higher Pb levels, the toxicity stabilizes or decreases for both solutions (not shown).

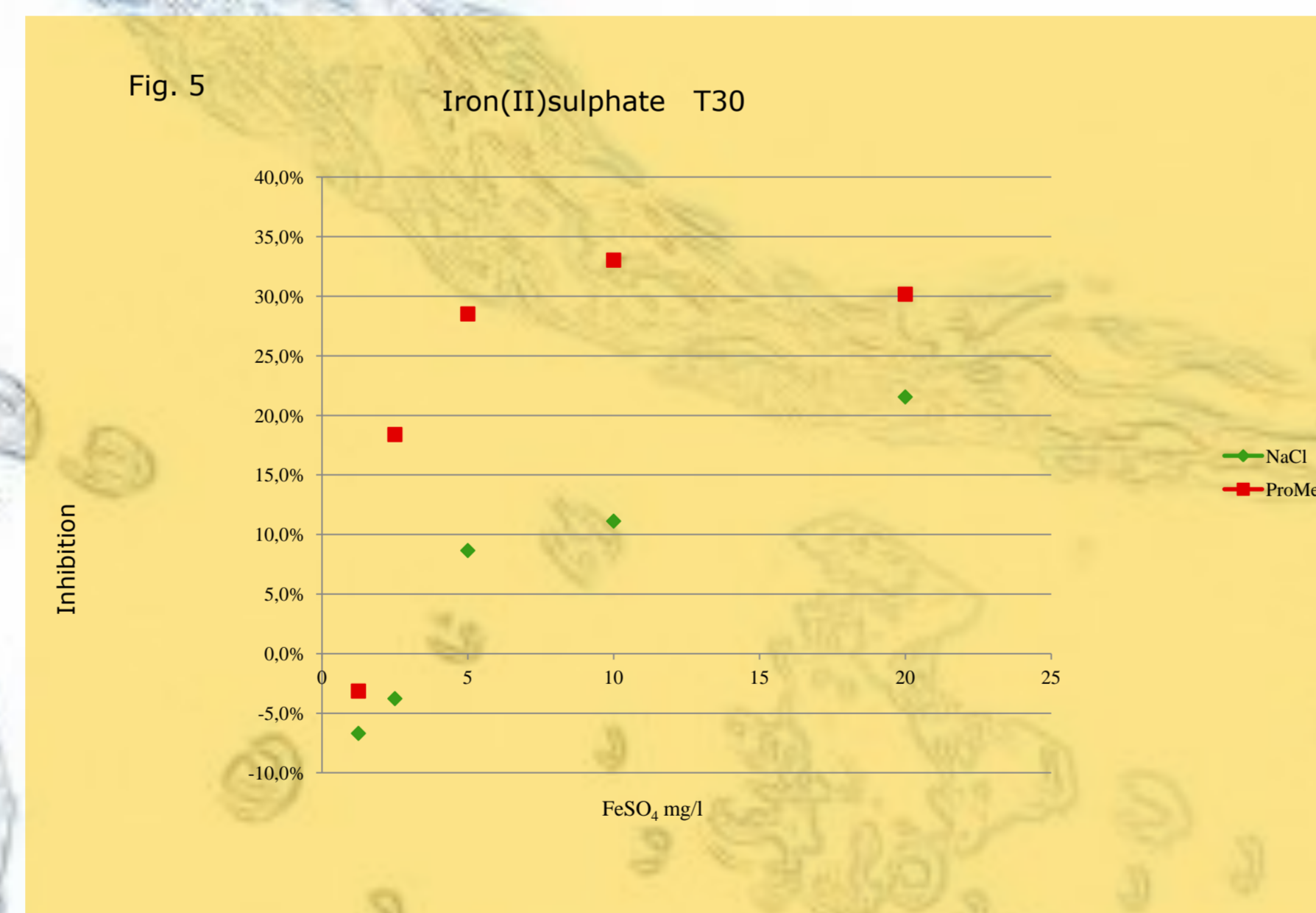


Figure 5. Iron Sulphate

The response for iron is faster using the ProMet buffer as compared to 2% NaCl. At higher concentrations of iron the effect becomes less pronounced.

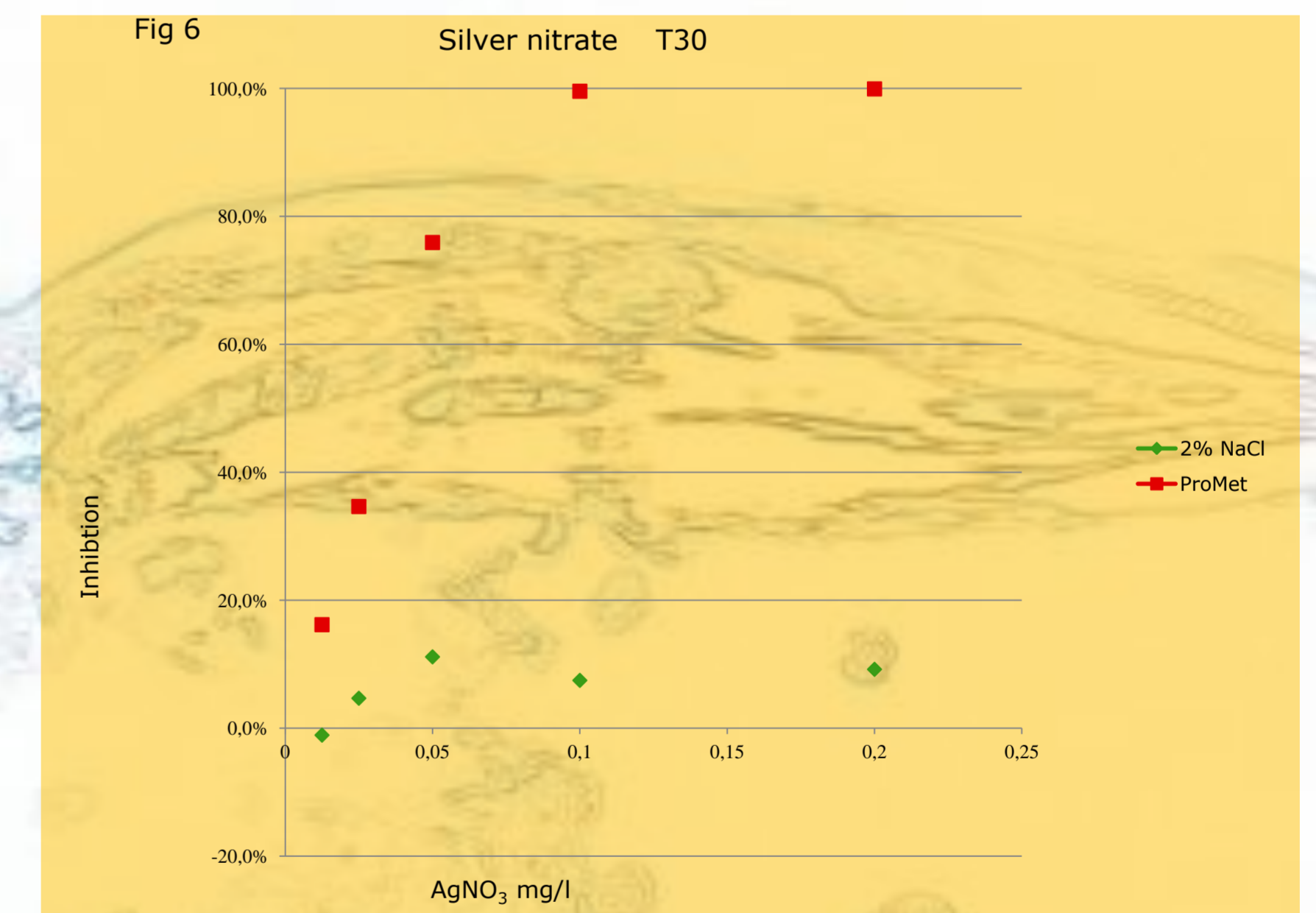


Figure 6. Silver nitrate

The ProMet buffer is superior to the 2% NaCl. In ProMet buffer the EC50 was 0.035 mg/l. At a concentration of 0.1 mg/l Ag, 100% inhibition of the light emission was measured. The EC50 for Ag in 2% NaCl was 1.7 mg/l.

Discussion

For practical reasons, the 20.4% sucrose solution is not suitable for online monitoring. It should be added as a solid to the sample and it is impossible to use it as a ready-to-use 10 times concentrated solution. It is also not obvious to use NaClO₄ for environmental analysis, because of the toxic properties of this compound. The new ProMet buffer of microLAN does not have these drawbacks and therefore it is an excellent alternative to improve the sensitivity of the bioluminescence test for metals like Cd, Ag, Ni, which are known to form chlorocomplexes. The buffer not only improves the sensitivity of test for a group of heavy metals, it can also be added as a 10x concentrated solution to adjust the osmotic value of the sample. This is a prerequisite for online monitoring using small sample volumes. From other metals like Zn and Pb it is known that chloride does not affect the toxicity.