



Preparation of a filter for the removal of traces of atrazine and simazine from water employing porcine Odorant Binding Protein as a retention element.

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INTRODUCTION

1 - "An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" European Commission - Environment –

Endocrine Disruptors
Atrazine
Simazine

Pollute



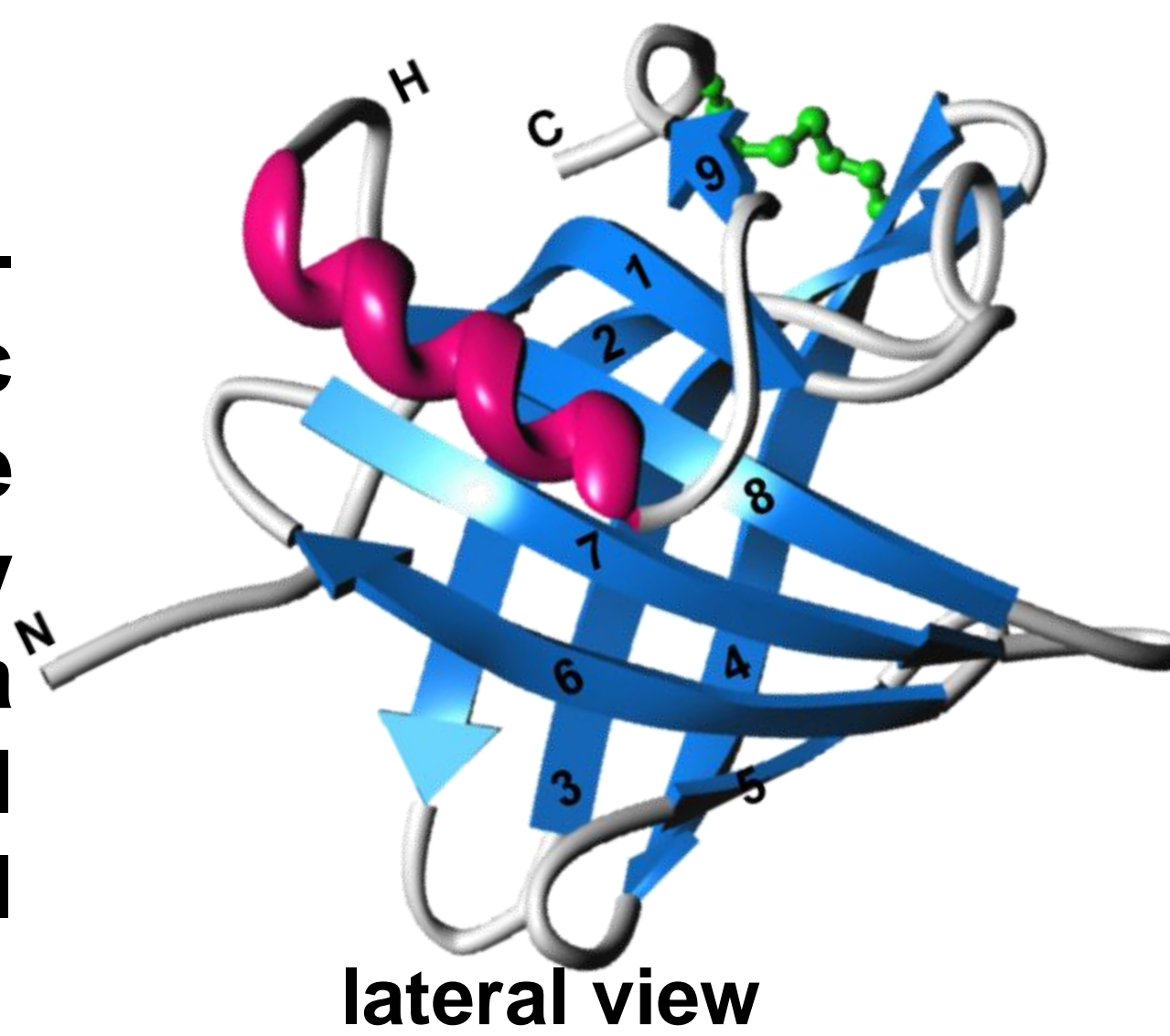
Effects on

Reproductive System

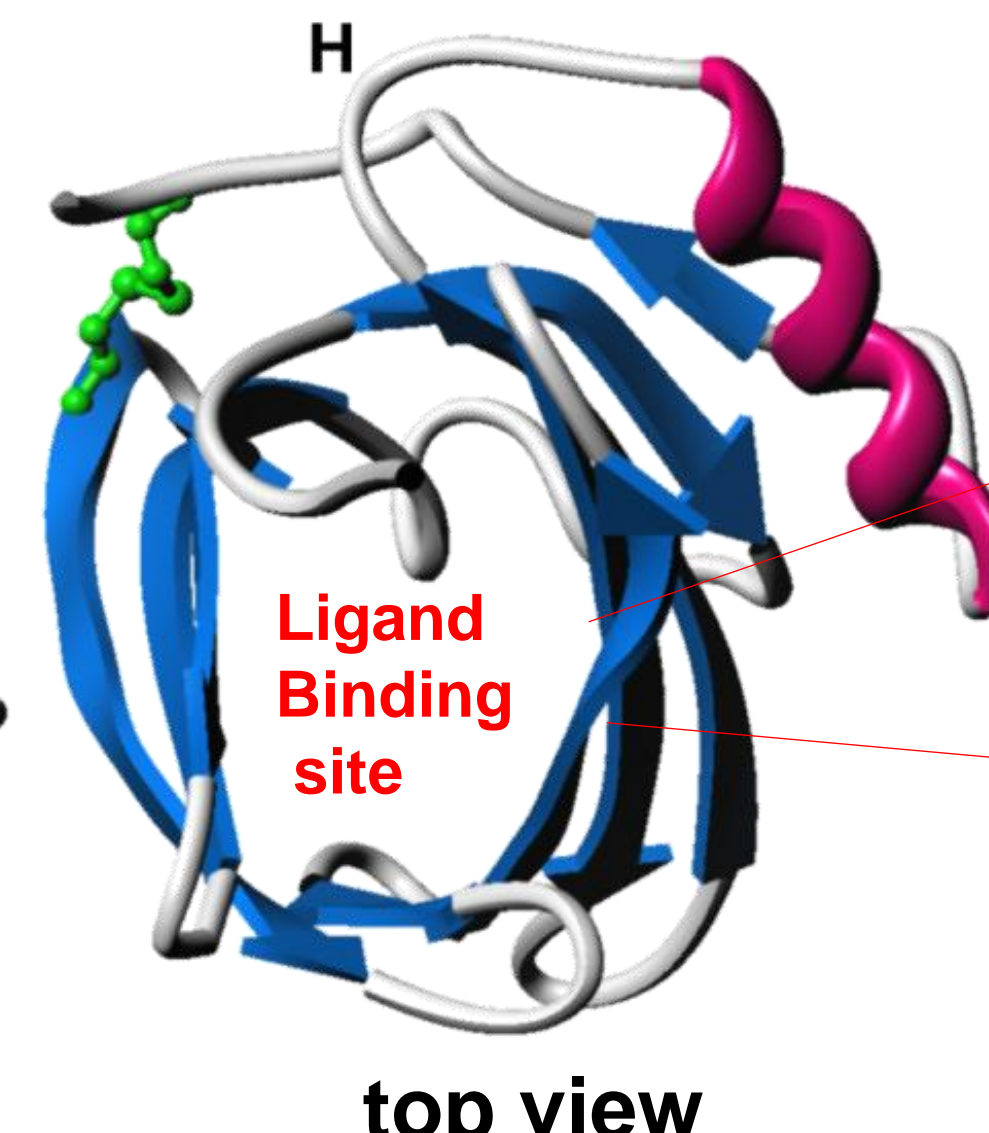
Central Nervous System

Immune System

2 - Odorant binding protein (OBP) is a multi-functional scavenger for small hydrophobic molecules dissolved in the mucus lining the nasal epithelia of mammals, characterized by broad ligand binding specificity towards a large number of structurally unrelated natural and synthetic molecules of different chemical classes.

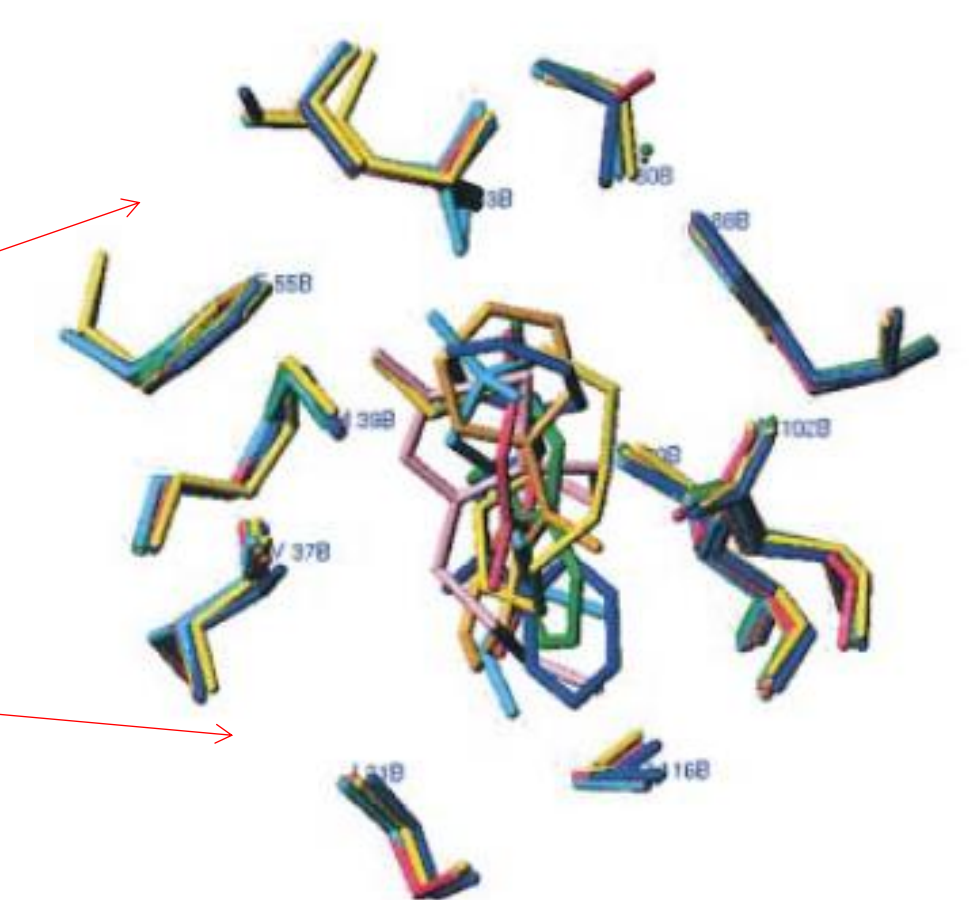


lateral view

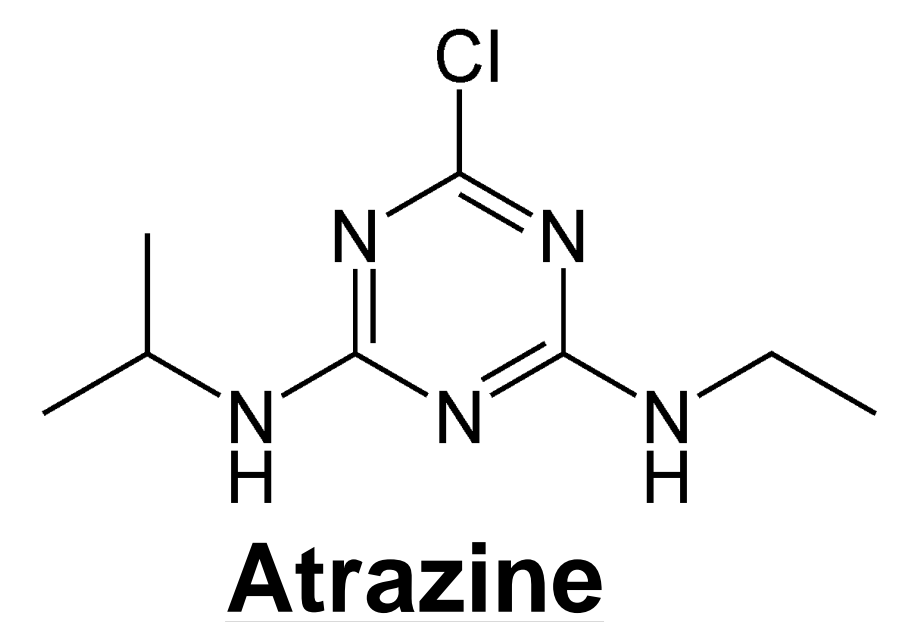


top view

porcine OBP 3D structure



The ligand binding site of porcine OBP with the superimposition of several ligands

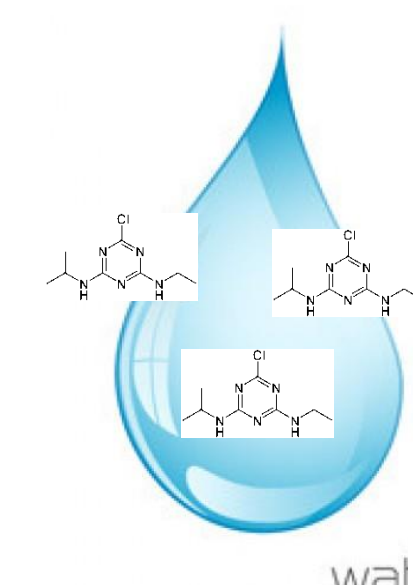


Atrazine

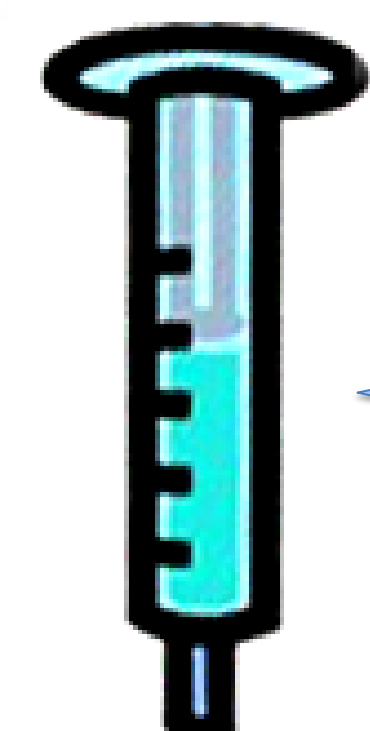
3 - Here we show, the application of porcine odorant binding protein as the retention element of an innovative filtering matrix for the removal from water samples of trace levels of environmental pollutants with endocrine disruptor activities such as the triazine herbicides Atrazine-ATRA and Symazine-SYM.

The filtering device, that was obtained by coupling histidine-tagged porcine OBP to a Ni-NTA agarose resin, was characterized in terms of retention capacity for the fluorescent OBP ligand 1-aminoanthracene and for the herbicides atrazine and simazine. Water solutions of the different compounds were loaded on the OBP-based filter and the amounts of the unretained substances were either visualized under the microscope (1 below) or quantitatively detected by GC-SIM-MS (2 below).

EXPERIMENTAL: the OBP based filter



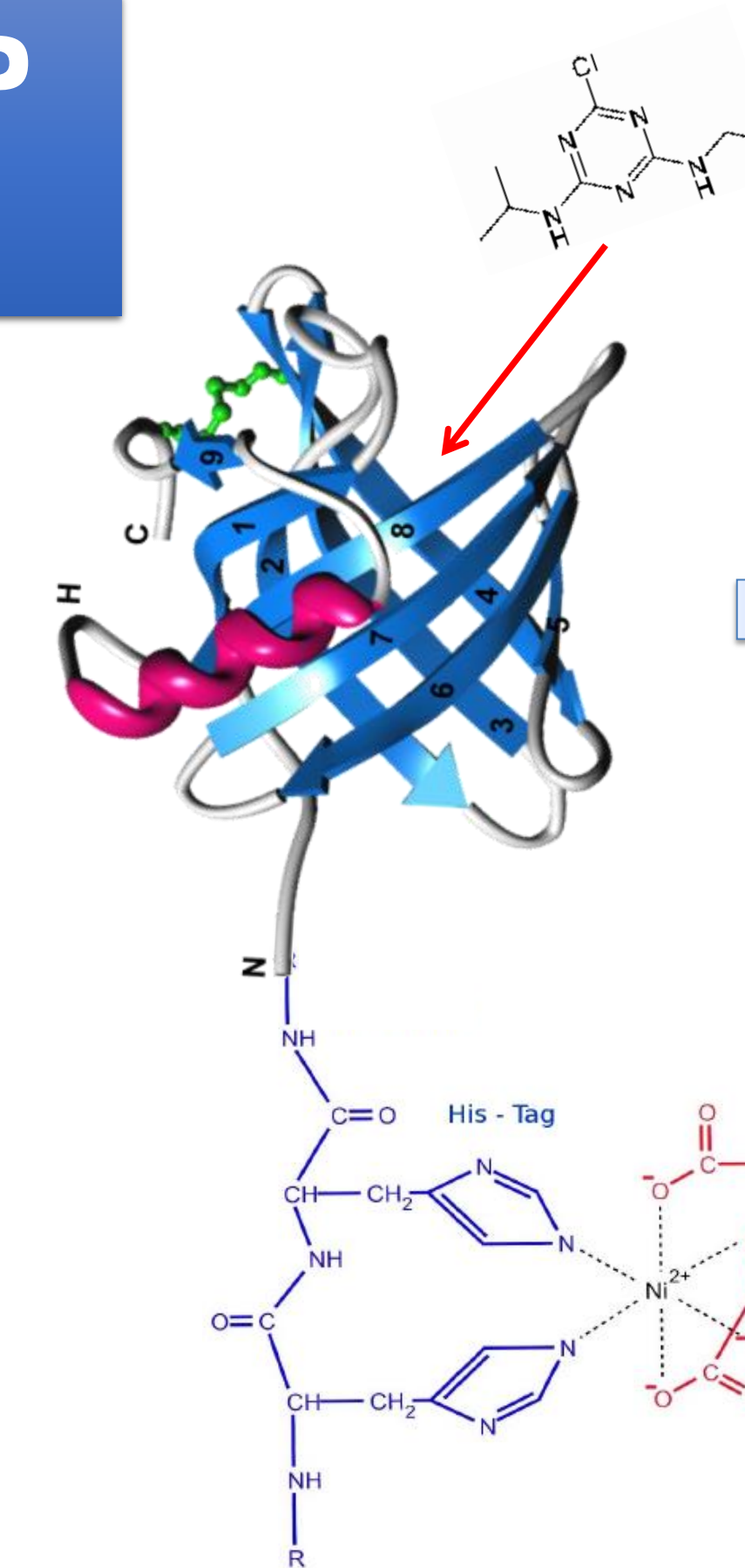
Endocrine disruptor contaminated water



Ni-NTA- Agarose



Decontaminated water



atrazine (simazine)

PORCINE OBP BINDS AND REMOVES THE TRIAZINE POLLUTANTS FROM WATER.

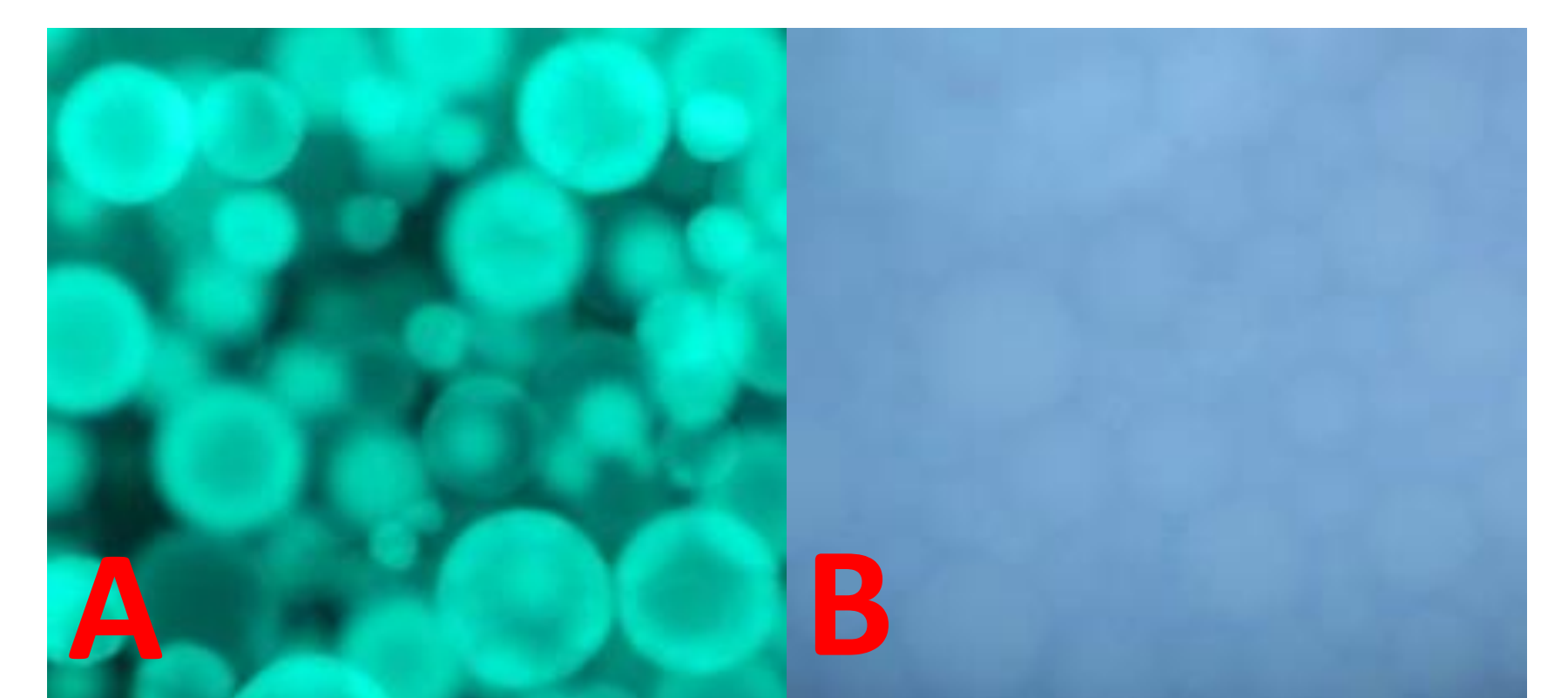
Agarose bead

His-tagged porcine OBP coupled to Ni-NTA- Agarose

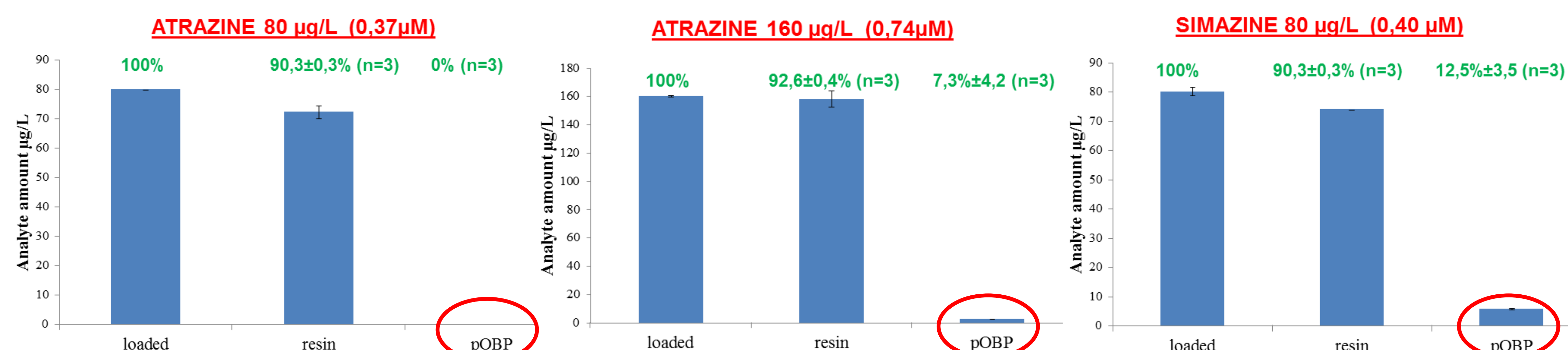
RESULTS AND CONCLUSIONS

1 - Visualization of the functionality of the porcine OBP-based filtering matrix:

A - Fluorescence microscopy photography of Ni-NTA agarose beads functionalized with porcine OBP after incubation with 50 μ M of AMA solution followed by extensive washing.
B - As in A, but with the uncoupled Ni-NTA agarose resin.



2 - Removal capability of the 6XHis-Porcine OBP filtering system (pOBP) and of the Ni-NTA agarose resin (RESIN) used as control, after loading aqueous solutions (LOADED) of Atrazine and Simazine in the 0,1–1,0 μ M range.



These data, that are comparable to those previously obtained with bovine OBP (1), confirm that protein based filters, employing OBPs as retention elements, are suitable for the removal of traces of triazine derivatives from water, that are not retained by the normally employed filtration matrixes (surface-modified activated carbons, polymeric phases, etc.), which are effective for higher concentration values of these compounds.

1) Bianchi et al. Anal Bioanal Chem. 2013 (2-3):1067-75.