LE POSSIBILI APPLICAZIONI DEI BIOSENSORI IN CAMPO AMBIENTALE E CLINICO

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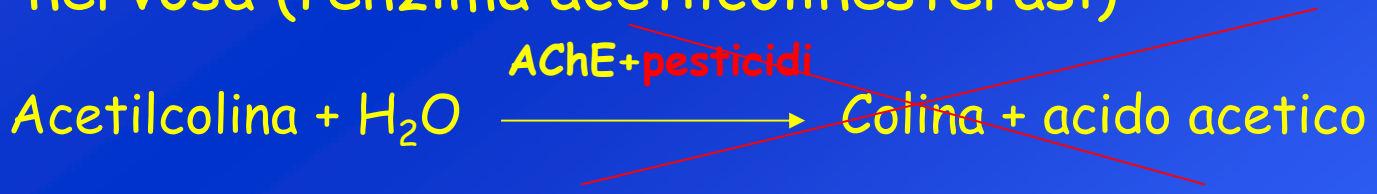
BIOSENSORI PER LA DETERMINAZIONE DI PESTICIDI ORGANOFOSFORICI E CARBAMMICI

SVILUPPO DI UN BIOSENSORE PER LA MISURA DI PESTICIDI

I pesticidi organofosforici e carbammici sono ampiamente utilizzati in agricoltura in quanto caratterizzati da una bassa persistenza nell'ambiente e da una comunque alta tossicità

Tali pesticidi hanno la capacità di inibire irreversibilmente un enzima chiave della trasmissione nervosa (l'enzima acetilcolinesterasi)



SVILUPPO DI UN BIOSENSORE PER LA MISURA DI PESTICIDI

.. Misurando l'attività enzimatica (AChE) prima (P_0) e dopo (P_i) l'esposizione del biosensore al campione è possibile determinare la concentrazione dei pesticidi organofosforici e carbammici presenti nel campione reale

$$I\% = \left(\frac{P_0 - P_i}{P_0} \right) x100$$

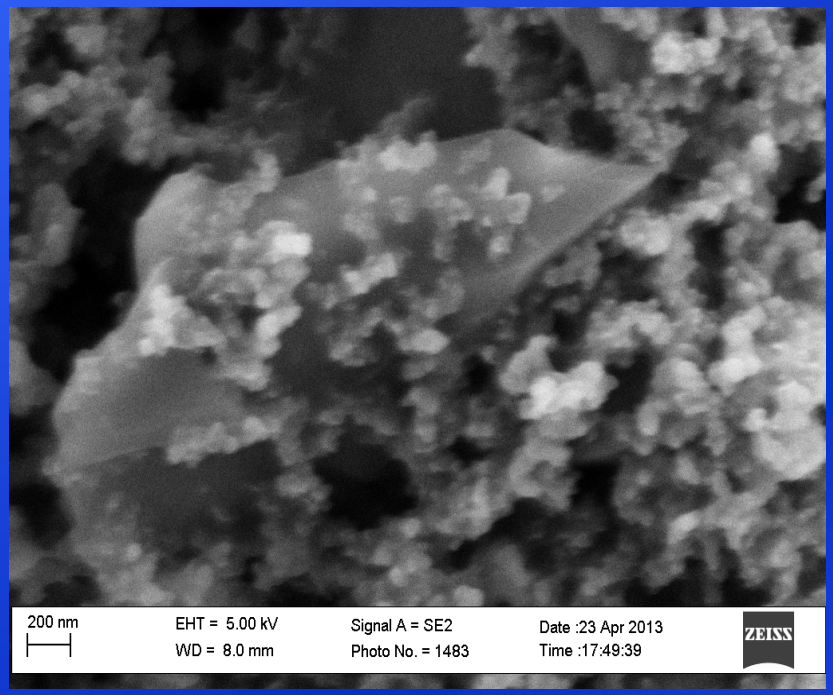
SVILUPPO DI UN BIOSENSORE ELETTROCHIMICO MONOENZIMATICO PER LA MISURA DI PESTICIDI

BIOSENSORE MONOENZIMATICO

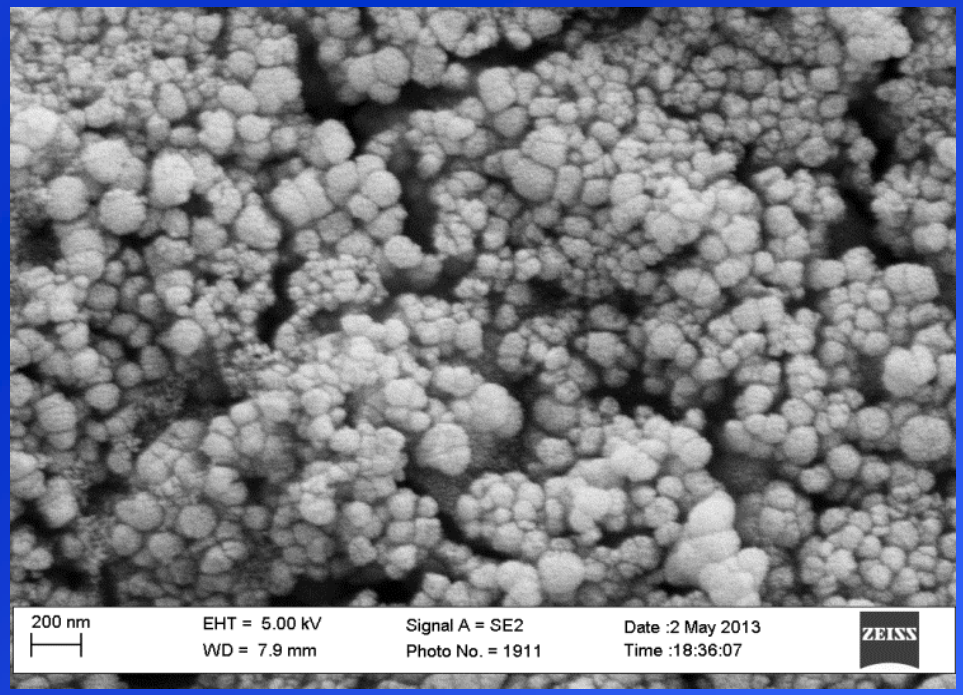
Acetiltiocolina + H_2O $\xrightarrow{\text{AChE}}$ ***tiocolina*** **+ acido acetico**

CARATTERIZZAZIONE DELLA SUPERFICIE ELETTRODICA MEDIANTE SEM

PRUSSIAN BLUE (PB) $KFe^{III}[Fe^{II}(CN)_6]$



bare-SPE

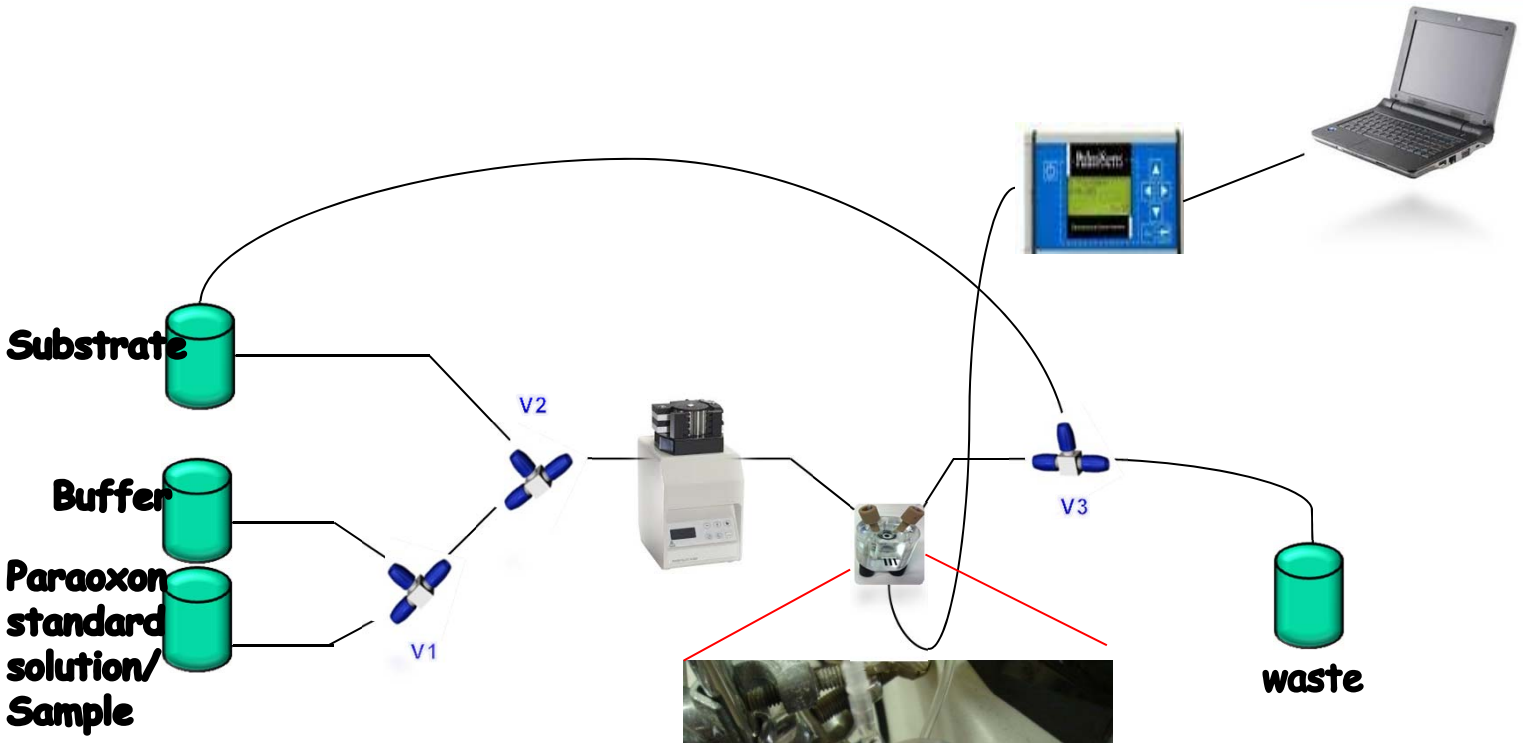


PBNPs-SPE

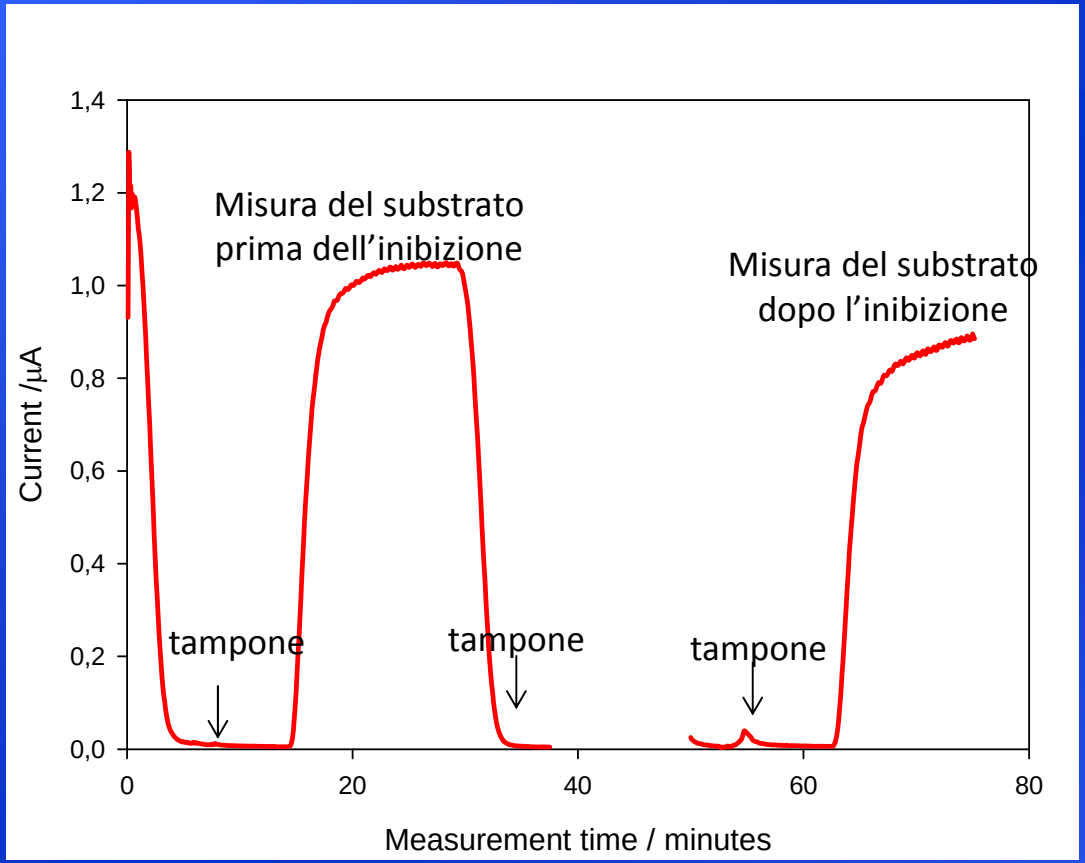
d=(95±15) nm

S. Cinti, F. Arduini, G. Vellucci, I. Cacciotti, F. Nanni, D. Moscone, Electrochemistry Communications (2014), 47, 63-66

INTEGRAZIONE DEL BIOSENSORE IN UN SISTEMA IN FLUSSO



CARATTERIZZAZIONE ANALITICA DEL BIOSENSORE

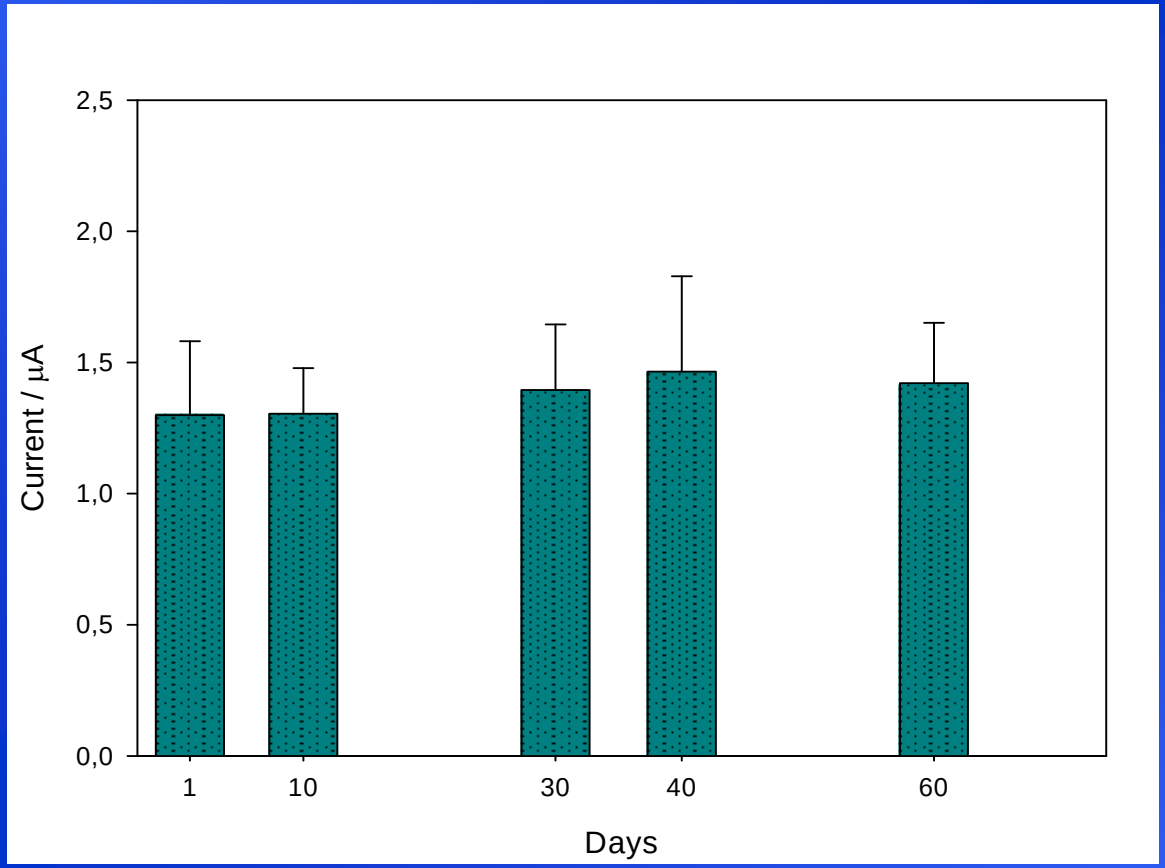


Intervallo di linearità : 2 - 10 ppb

$y = 5.17x + 20.7$,
$R^2 = 0.952$

LOD 1 ppb (10% di inibizione)

STUDIO DELLA STABILITA' IN CONDIZIONI NON OPERATIVE



Potenziale applicato:+200 mV vs Ag/AgCl, tampone fosfato 0.05 M + KCl 0.1 M, pH 7.4, butirriltiocolica 5 mM, storage conditions: dry a RT

APPLICAZIONE IN DIVERSI CAMPIONI DI ACQUA

Sample	*Campione diluito 1:4 (v/v) con tampone*	*Campione fortificato con 25 ppb paraoxon*	*Recupero (%)*
	Concentrazione di paraoxon trovata (ppb)		
Acqua potabile	-	22.5±1.2	90±5
Lago Albano	-	28.8±3.1	115±12
Tevere	-	29.8±2.3	119±9

ACQUASENSE

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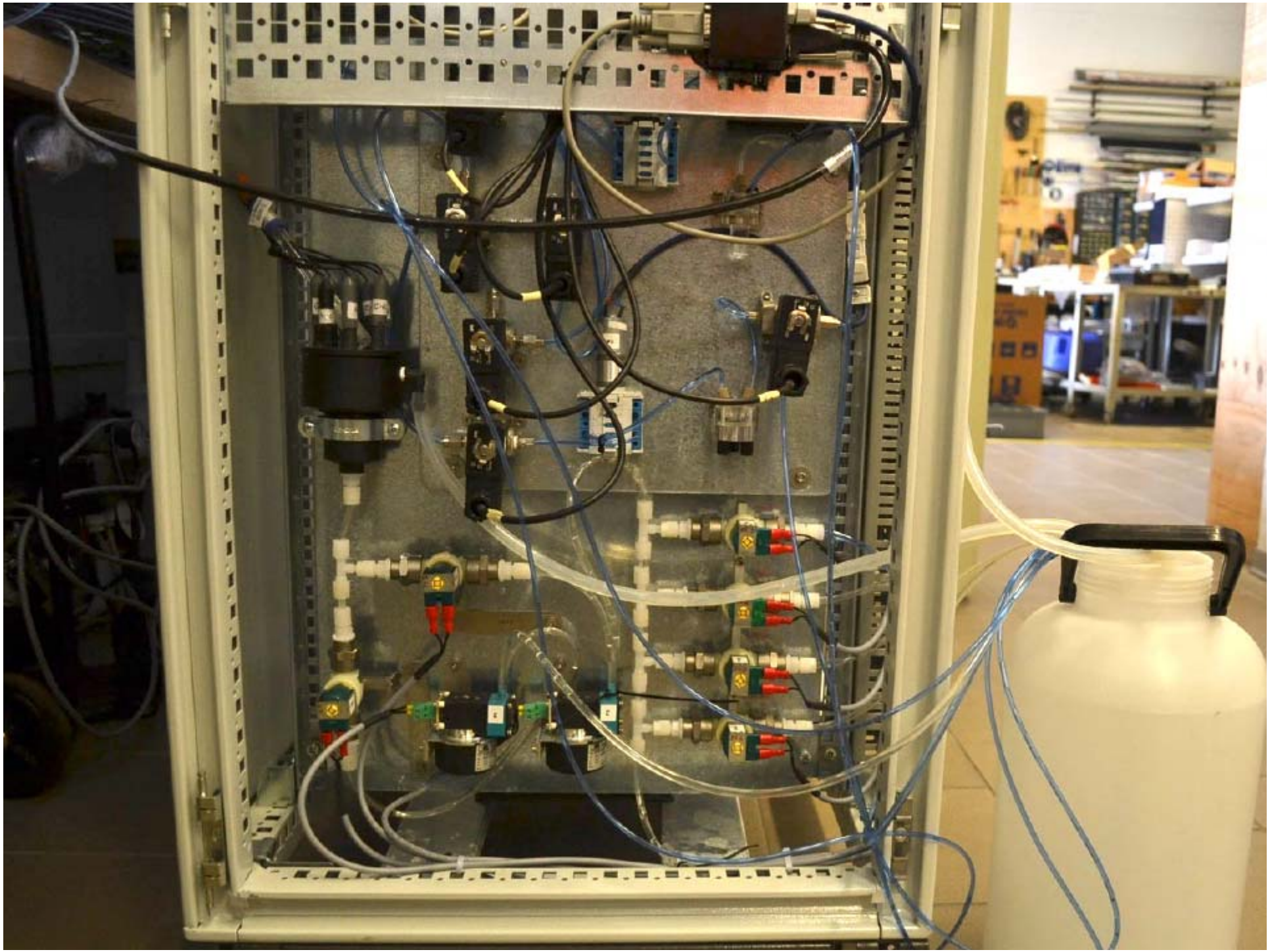
Progetto
Industria 2015



SENSORI PER LA DETERMINAZIONE DI PIOMBO, CADMIO, ZINCO ARSENICO, MERCURIO

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The Effects of Metals as Endocrine Disruptors

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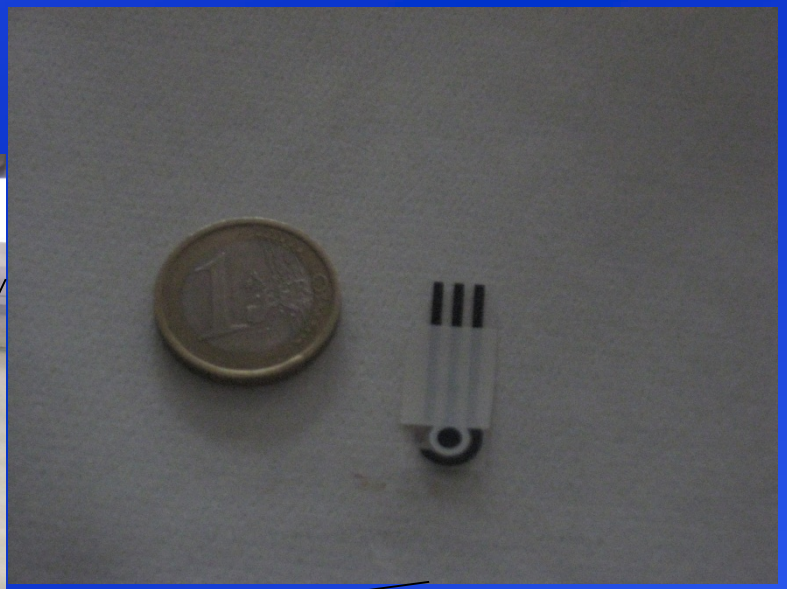
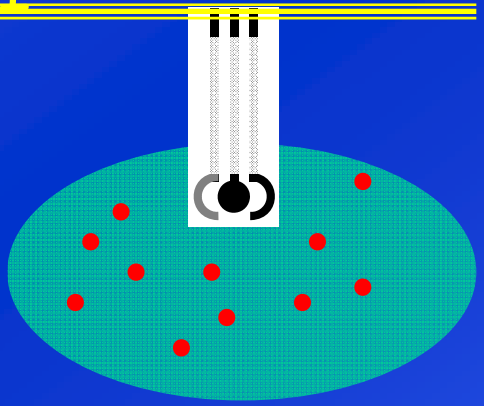
TABLE 1. Effects of Metals on Endocrine System

Metals	Effects	References
Cadmium	Alterations of the secretory patterns of pituitary hormones	Lafuente et al., 2003
	Stimulation of progesterone synthesis (low doses)	Powlin et al., 1997; Massanyi et al., 2000
	Inhibition of progesterone synthesis (high doses)	Paksy et al., 1997; Piasek and Laskey, 1999; Jolibois et al., 1999a; 1999b; Kawai et al., 2002
	Estrogenic effect	Garcia-Morales et al., 1994
	Increase in early delivery	Nishijo et al., 2002
	Lower birth weight	Frery et al., 1993; Nishijo et al., 2002
	Early onset of puberty	Johnson et al., 2003
Mercury	Stimulation of progesterone synthesis	Mondal et al., 1997
	Reduction in plasma levels of testosterone and 17-beta-estradiol	Drevnick and Sandheinrich, 2003; Vachhrajani and Chowdhury, 1990; Ng and Liu, 1990
	Reduction in sperm motility and sperm count	Chowdhury et al., 1989
	Increase in plasma levels of T4, TSH, estrone, and estradiol	Barregård et al., 1994; Ellingsen et al., 2000; Agusa et al., 2007; Abdelouahab et al., 2008
Arsenic	Increase in GR-, MR-, PR-, AR-, RAR-, and TR-mediated transcription (low doses)	Bodwell et al., 2004; Bodwell et al., 2006; Davey et al., 2008
	Inhibition of GR-, MR-, PR-, AR-, RAR-, and TR-mediated transcription (high doses)	Kaltreider et al., 2001; Bodwell et al., 2004; Davey et al., 2008
	Inhibition of ER-mediated transcription	Davey et al., 2007
	Estrogenic effect	Jana et al., 2006
	Inhibition of spermatogenesis	Sarkar et al., 2003; Pant et al., 2004; Jana et al., 2006
Lead	Alterations of affinity of estrogen and luteinizing hormone receptors	Wide, 1980; Wiebe and Barr, 1988; Wiebe et al., 1988
	Action at multiple sites on the hypothalamus–pituitary–gonadal axis	Ronis et al., 1996; Srivastava et al., 2004
	Reduction in serum levels of IGF-1, LH, testosterone and estradiol	Ronis et al., 1996; Dearth et al., 2002; Srivastava et al., 2004;
	Alterations of onset of puberty	Iavicoli et al., 2004; 2006
	Morphological and functional alterations of sperm	Sokol and Berman, 1991; Kempinas et al., 1994; Wadi and Ahmad, 1999; Gennart et al. 1999; Bonde et al., 2002; Kasperczyk et al., 2008
	Inhibition of GH synthesis	Huseman et al., 1992; Ronis et al., 1996
Manganese	Increase in serum levels of LH, FSH and testosterone	Pine et al., 2005; Lee et al., 2006
	Stimulation of spermatogenesis	Lee et al., 2006
	Stimulation in the secretion of LH and LHRH	Prestifilippo et al., 2007; Lee et al., 2007
	Early onset of puberty	Pine et al., 2005
Zinc	Stimulation of spermatogenesis	Fuse et al., 1999; Chia et al., 2000; Ali et al., 2005; Yuyan et al., 2007

SENSORI ELETTROCHIMICI BASATI SU ELETTRODI STAMPATI



PRINCIPIO PER LA MISURA DEI METALLI PESANTI (e.g. piombo) UTILIZZANDO ELETTRODI STAMPATI

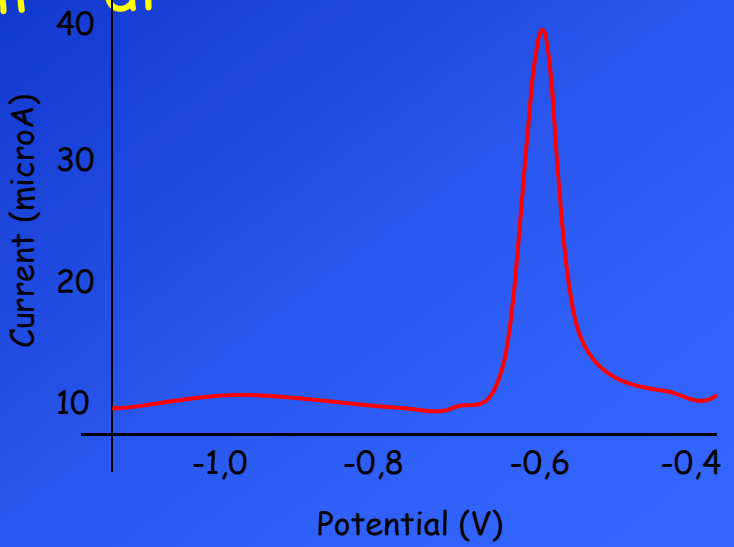


1 – PRE-CONCENTRATION STEP

Il Pb^{2+} viene ridotto e accumulato sulla superficie dell'elettrodo di lavoro del sensore sul quale viene depositato anche un film di bismuto

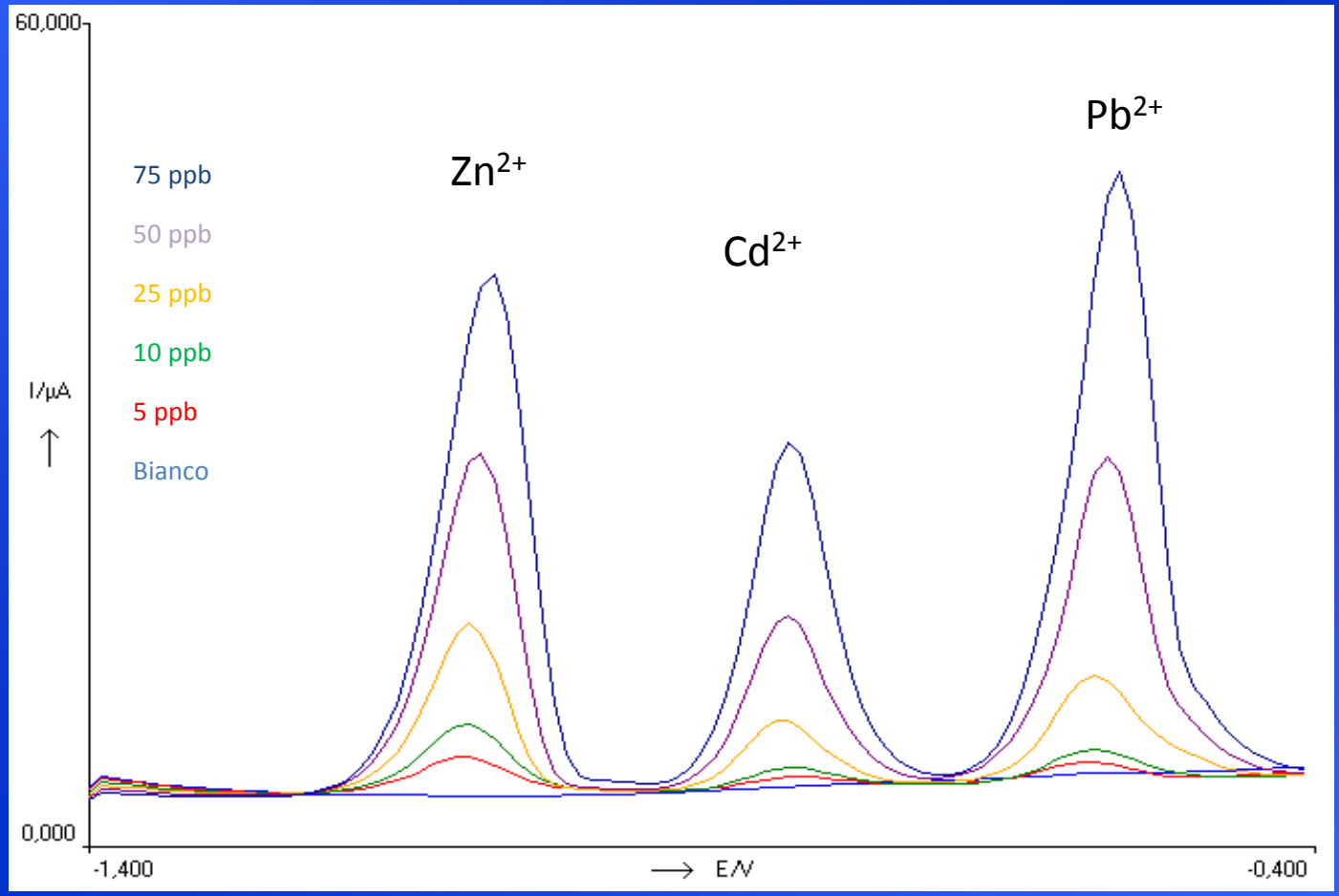
2 – STRIPPING STEP

Il Pb^{2+} viene ridissolto e il picco osservato



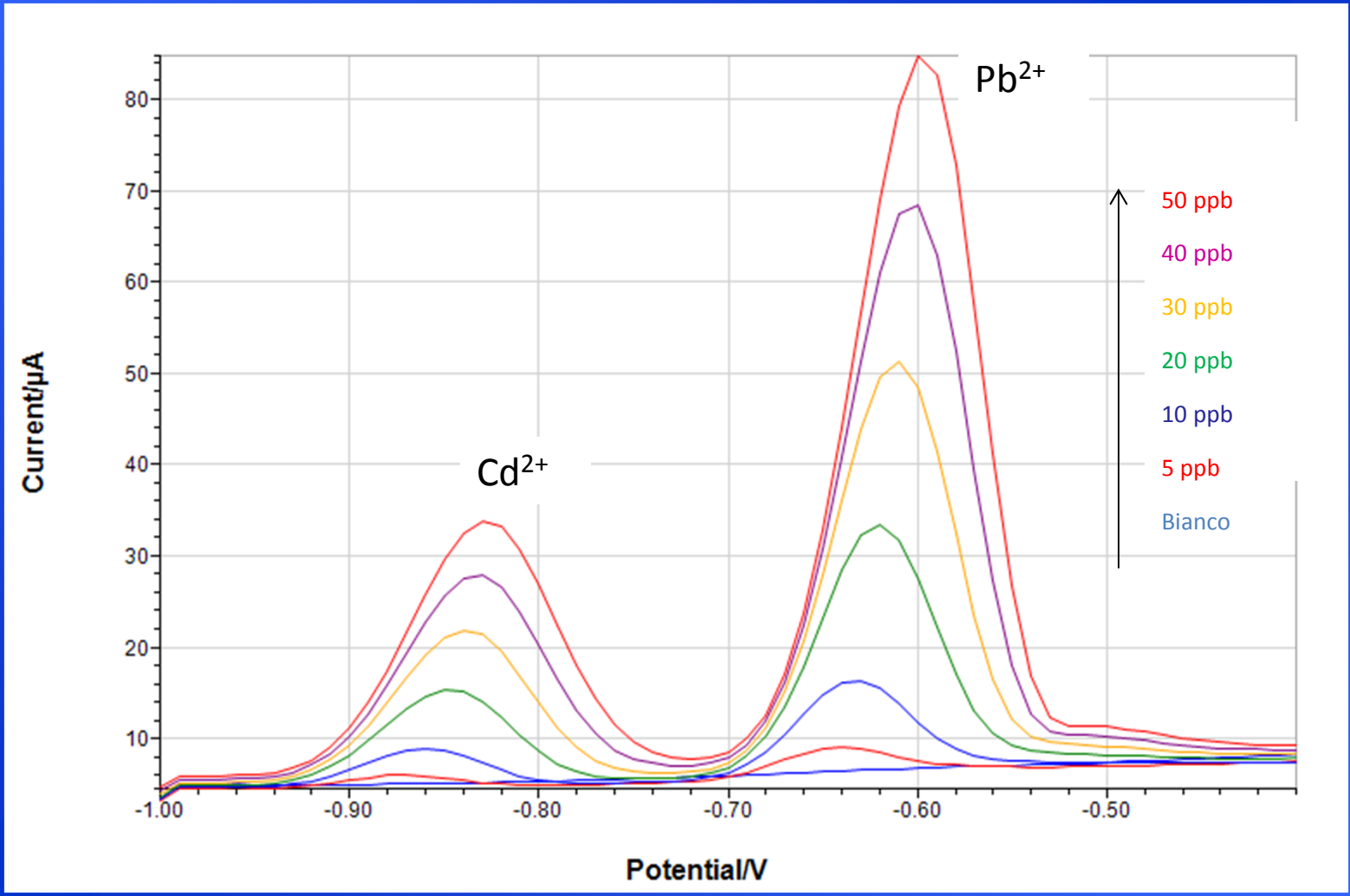
F. Arduini, J. Calvo Quintana, A. Amine, G. Palleschi, D. Moscone,Trends in Analytical Chemistry, 2010, 29, 1295

Misura di Zn^{2+}, Cd^{2+}e Pb^{2+} e utilizzando SPE modificato con film di bismuto



Anodic stripping voltammograms using SPE modified with Bi film without and with Zn^{2+}, Cd^{2+} and Pb^{2+} at 5, 10, 25, 50 and 75 ppb in acetate buffer pH=4.6.

Misura di Cd^{2+} e Pb^{2+} utilizzando SPE modificato con film di bismuto



Anodic stripping voltammograms using SPE modified with Bi film without and with Cd^{2+} and Pb^{2+} at 5, 10, 20, 30, 40 and 50 ppb in acetate buffer pH=4.6.

ENVIRONMENTAL Science & Technology

Environ. Sci. Technol. 2014, 48, 7477−7485

Article

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Disposable Electrochemical Sensor to Evaluate the Phytoremediation of the Aquatic Plant *Lemna minor* L. toward Pb^{2+} and/or Cd^{2+}

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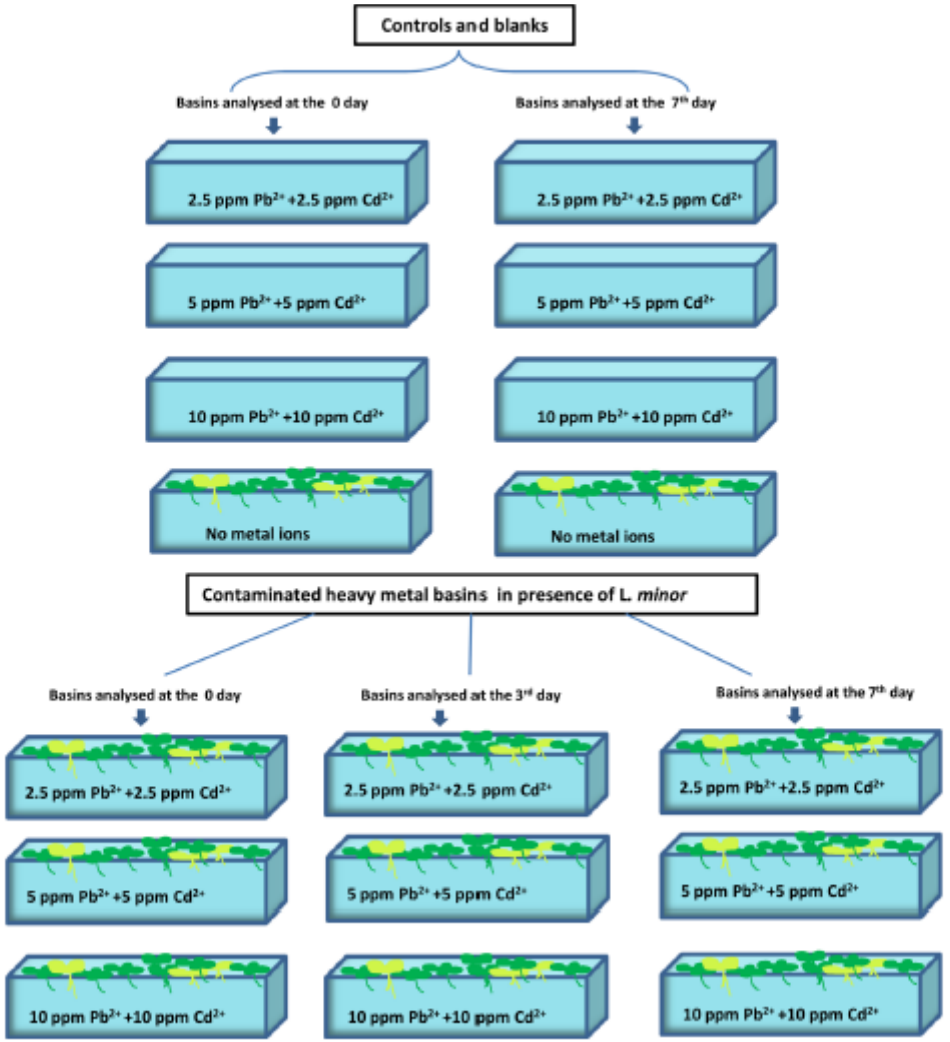
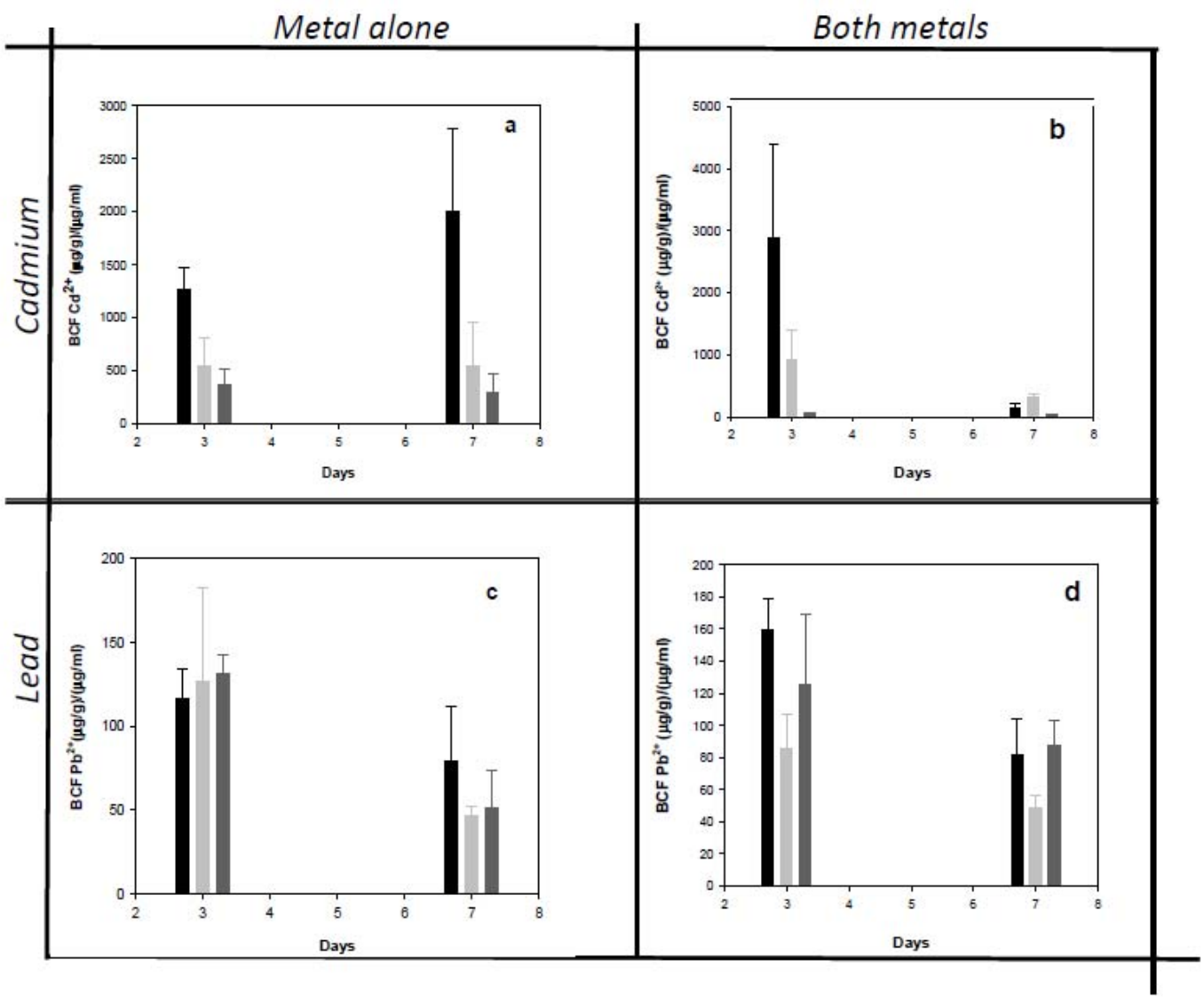


Figure 3. Experimental setup used to evaluate the phytoremediation of *Lemna minor* in the case of medium polluted with lead and cadmium ions.

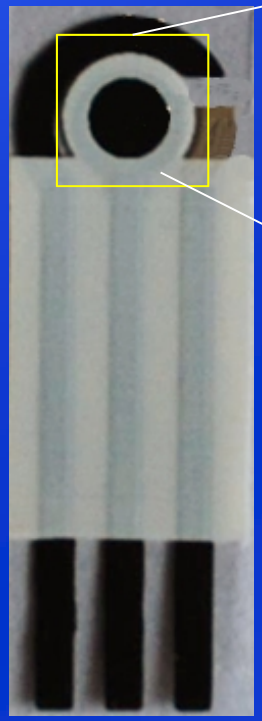
Table 1. Analytical Performances of the Sensor in Standard Solutions (STD Solutions), IRRI Medium and *L. minor* Extract

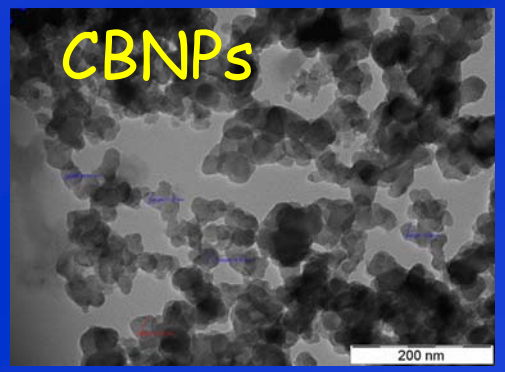
heavy metal calibration type		working solution	linear range (ppb)	equation	LOD (ppb)	RSD% (n = 3)
Cd^{2+}		STD solution	5−80	$y = -1.97 + 0.78x$	2	6.0
		IRRI medium	5−50	$y = -1.04 + 0.35x$	2	7.0
		L. minor extract	5−50	$y = -1.83 + 0.63x$	2	10
Pb^{2+}		STD solution	5−80	$y = -4.07 + 1.35x$	0.3	9.5
		IRRI medium	5−50	$y = -3.59 + 1.06x$	0.3	11
		L. minor extract	5−50	$y = -4.68 + 1.75x$	0.3	15
Cd^{2+}+Pb^{2+}	Cd^{2+}	STD solution	5−50	$y = -1.27 + 0.48x$	2	6.3
		IRRI medium	5−50	$y = -1.04 + 0.44x$	2	11.5
		L. minor extract	5−50	$y = -1.59 + 0.67x$	2	11
	Pb^{2+}	STD solution	5−50	$y = -4.57 + 1.59x$	0.3	4.7
		IRRI medium	5−50	$y = -5.26 + 1.66x$	0.3	17
		L. minor extract	5−50	$y = -5.38 + 1.84x$	0.3	14


Metal alone
Both metals
Cadmium
Lead
a
b
c
d
BCF Cd2+ (µg/g)/(µg/ml)
BCF Pb2+ (µg/g)/(µg/ml)
Days

SENSORE PER LA DETERMINAZIONE DELL'ARSENICO

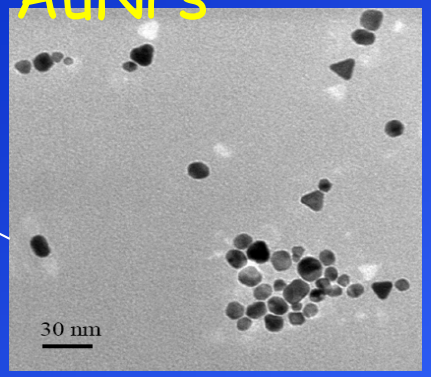
SENSORE PER LA DETERMINAZIONE DELL' As(III)





+

AuNPs



Metrohm

Method 2: Determination of As(III)

Reagents

All reagents used should have the highest possible degree of purity and be as arsenic-free as possible (p.a. or «suprapur»). Only ultrapure water is to be used.

- Hydrochloric acid, w(HCl) = 30%, suprapur
- Ascorbic acid, puriss. p.a., CAS 50-81-7
- As(III) stcok solution, β[As(III)] = 1 g/L (commercially available)

Ready-to-use solutions

As(III) standard solution	**β[As(III)] = 1 mg/L** This solution is prepared from the As(III) stock solution by dilution with c(HCl) = 0.1 mol/L (oxygen-free).
Ascorbic acid solution	**w(ascorbic acid) = 1%** 100 mg ascorbic acid is dissolved in 10 mL oxygen-free ultrapure water.

Analysis

Sample solution:

10 mL w(HCl) = 30%

is first degassed for 5 min. Then

100 µL w(ascorbic acid) = 1% and
10 mL (diluted) sample

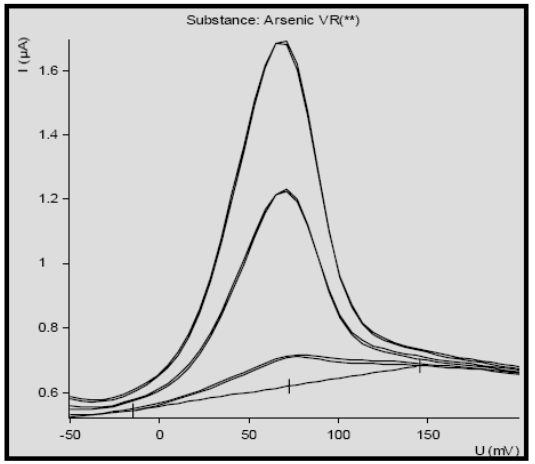
are added and the voltammogram is recorded with the following parameters:

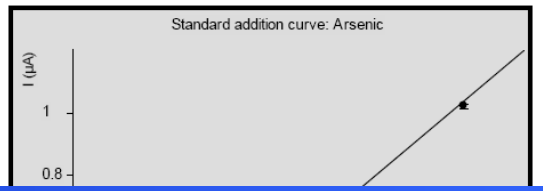
Working electrode	RDE
Stirrer speed	2000 rpm
Mode	DP
Purge time	10 s
Voltage step	6 mV
Voltage step time	0.3 s
Sweep rate	20 mV/s
Peak potential As	50 mV

The concentration is determined by standard addition.

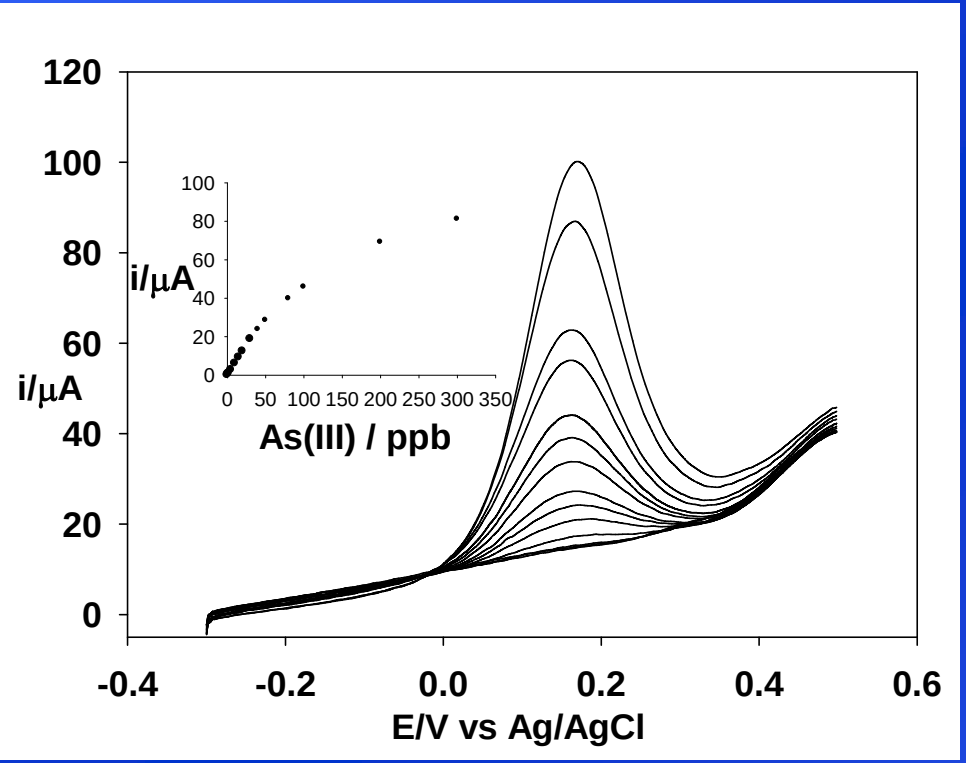
Example

Determination of As(III) in mineral water (without carbon dioxide) with the 746 VA Trace Analyzer





Caratterizzazione analitica



LS-ASV in 0.1 M HCl +0.01% w/v acido ascorbico, scan rate = 0.8 V/s, t deposizione = 300 s, E cleaning=0.2 V, t cleaning= 10 s.

Intervallo di linearità: 2-30 ppb
$Y=0.629x-0.25$, $R^2=0.999$

LOD = 0.4 ppb

Limite di legge= 10 ppb

S. Cinti, S. Politi, D. Moscone, G. Palleschi, F. Arduini, Electroanalysis (2014), 26, 931-939

Applicazione in campioni reali

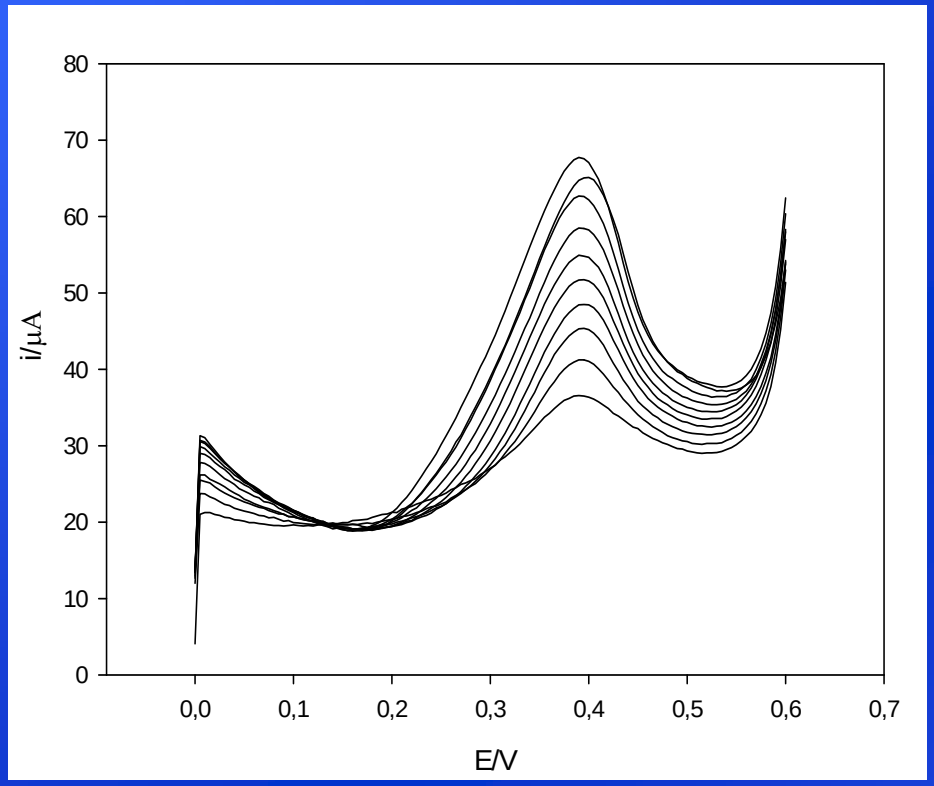
Studi di recupero

BASF
The Chemical Company

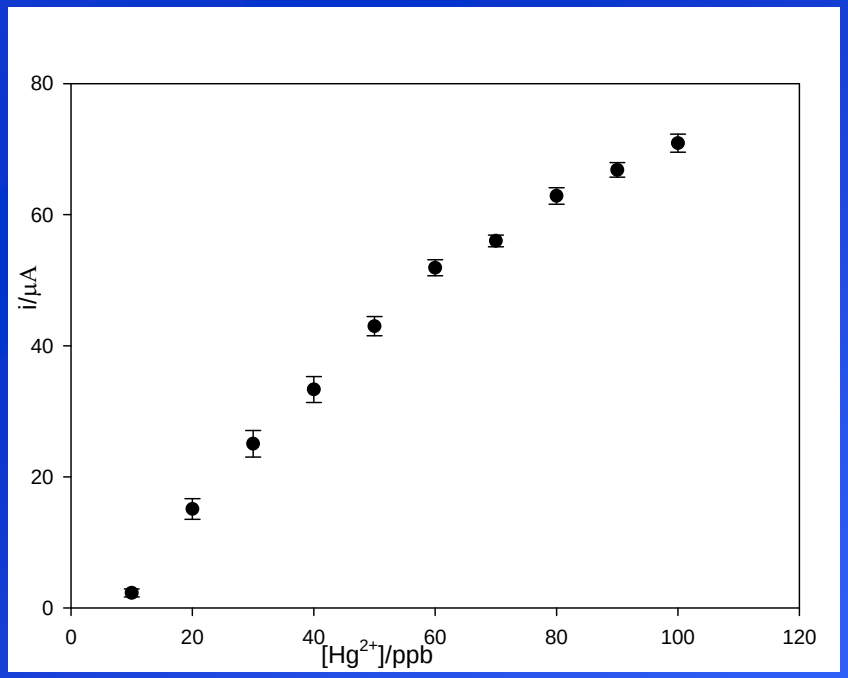
As(III)	Campione di acqua potabile	Campione di acqua potabile + 10 ppb	Campione di acqua potabile + 20 ppb
Recupero%± RSD% (n=3)	-	99±9	108±4

Misura di Hg^{2+} utilizzando SPE modificato con CBNPs e AuNPs

Voltammogrammi ottenuti nell'intervallo 10-100 ppb Hg^{2+}



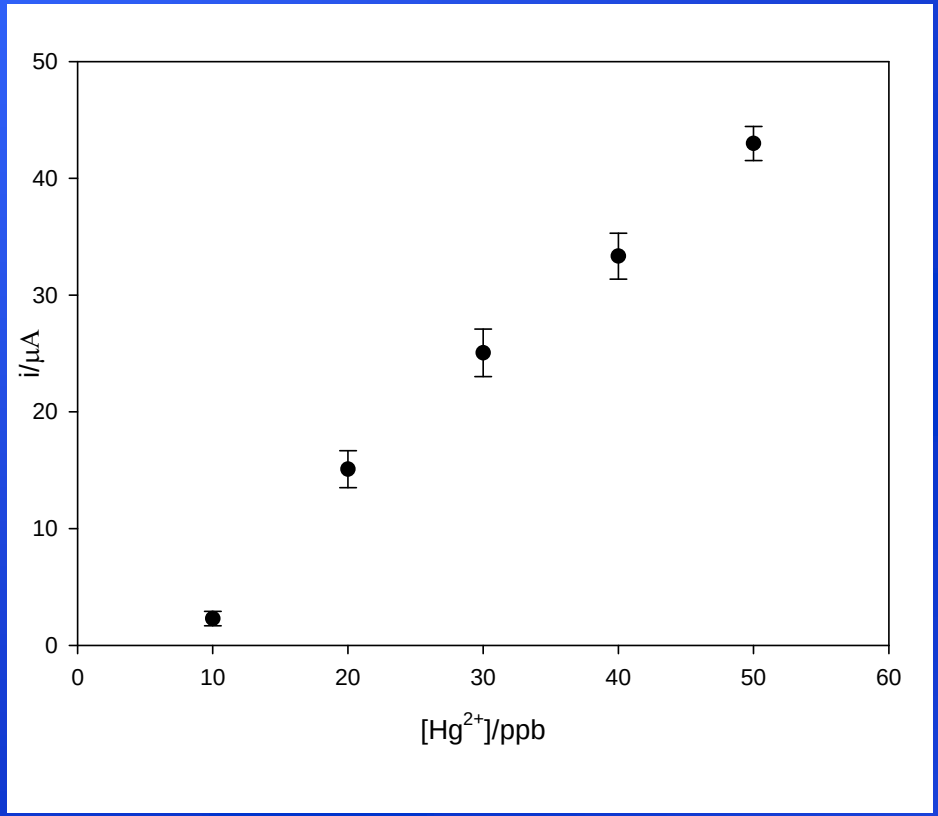
Curva di calibrazione 10-100 ppb Hg^{2+}



Voltammetria di stripping ad onda quadra,
E dep=0.2 V, t dep=400 s, E cond=0.6 V, Freq=60 Hz,
E ampl= 0.1 V, t cond=10 s, t eq =10 s ,Estep= 0.01 V

CARATTERIZZAZIONE ANALITICA DEL SENSORE

Curva di calibrazione 10-50 ppb di Hg^{2+}



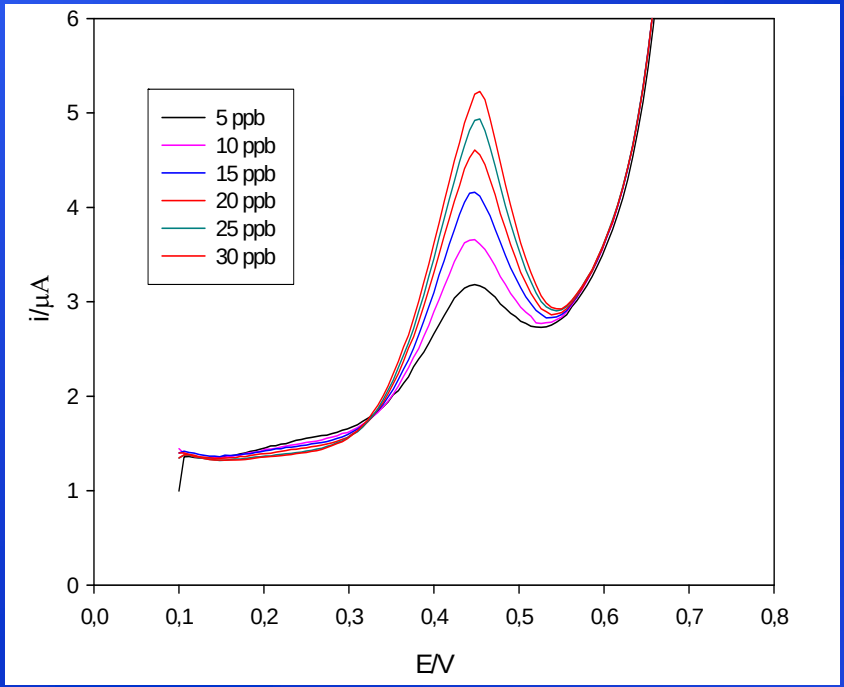
$y = -8{,}77 + 1{,}09x$ $R^2 = 0{,}90$

LOD = 3.4 ppb

Suolo	Siti ad uso verde pubblico, privato e residenziale	$mg \cdot kg^{-1}$	1
	Siti ad uso commerciale e industriale	$mg \cdot kg^{-1}$	5
Acqua	Acque sotterranee	$\mu g \cdot l^{-1}$	1
	Acque superficiali	$\mu g \cdot l^{-1}$	1

Standard di qualità e CSC per il mercurio totale (Dlgs 152/06, Reg. CE n. 1881/2006, ACGIH 2001)

Misura di Hg^{2+} utilizzando SPE d'oro modificato con AuNPs



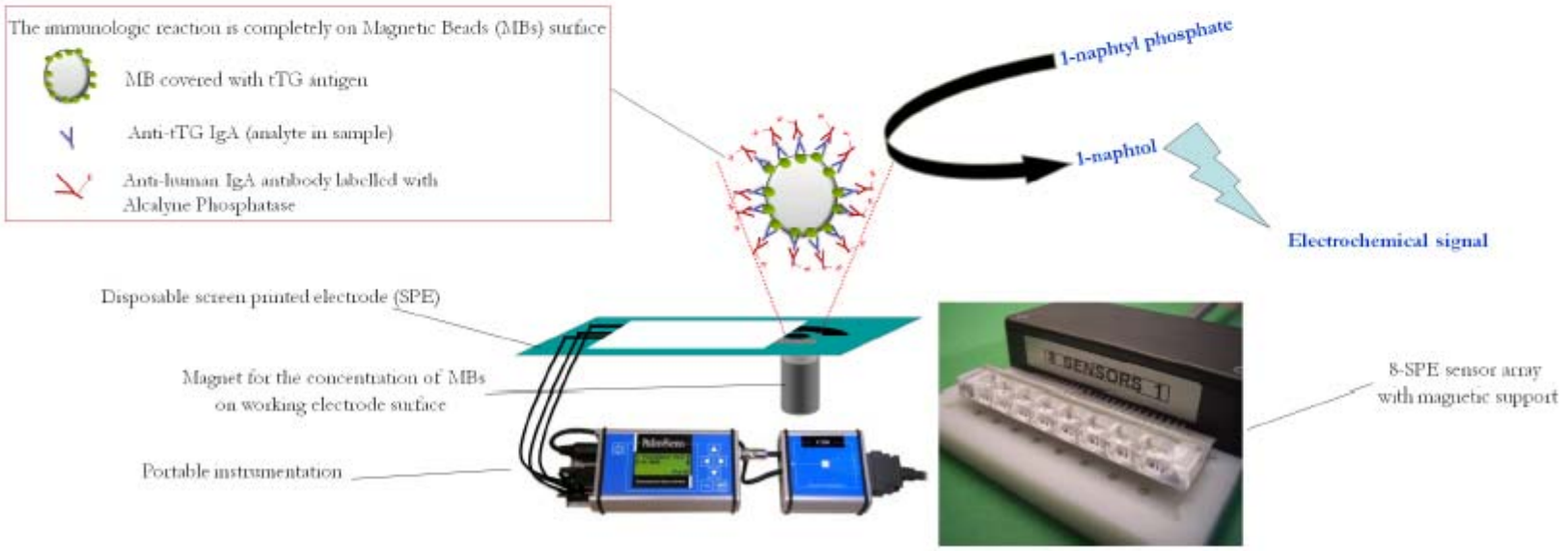
SWV E dep=0,2 V, t dep=400 s, E cond=0,6 V,
Freq=60 Hz, E ampl= 0,1 V, t cond=10 s, t eq =10s ,
Estep= 0,01 V

$Y = 0.352 + 0.0978x$
$R^2 = 0.999$

LOD= 0.16 ppb

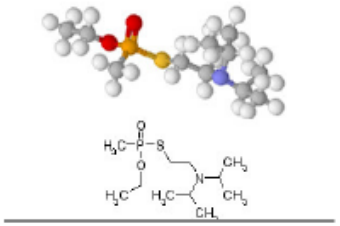
Immunosensori elettrochimici per la determinazione di:

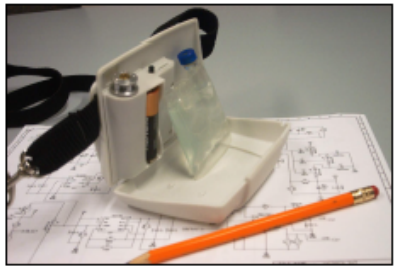
- **Tossine algali**: acido domoico, saxitossina→ *ELISA competitivo, SPE*
- **Micotossine**: AFs, OTA, tricoteceni (T2+HT-2, DON)→ *ELISA competitivo, array 8-SPEs, IMBs*
- **Anabolizzanti** (estradiolo) e **farmaci veterinari** (eritromicina e tilosina) → *ELISA compet, SPE*
- **Proteine in OGM** → *ELISA sandwich, IMBs,SPE*
- **Microrganismi patogeni** (Salmonella, Listeria, S. aureus) → *ELISA sandwich, IMBs, array 8-SPEs*
- **Ab anti-transglutaminasi** (celiachia) → *ELISA diretto, IMBs, array 8-SPEs*
- **Allergeni** → *ELISA competitivo, IMBs, array 8-SPEs*

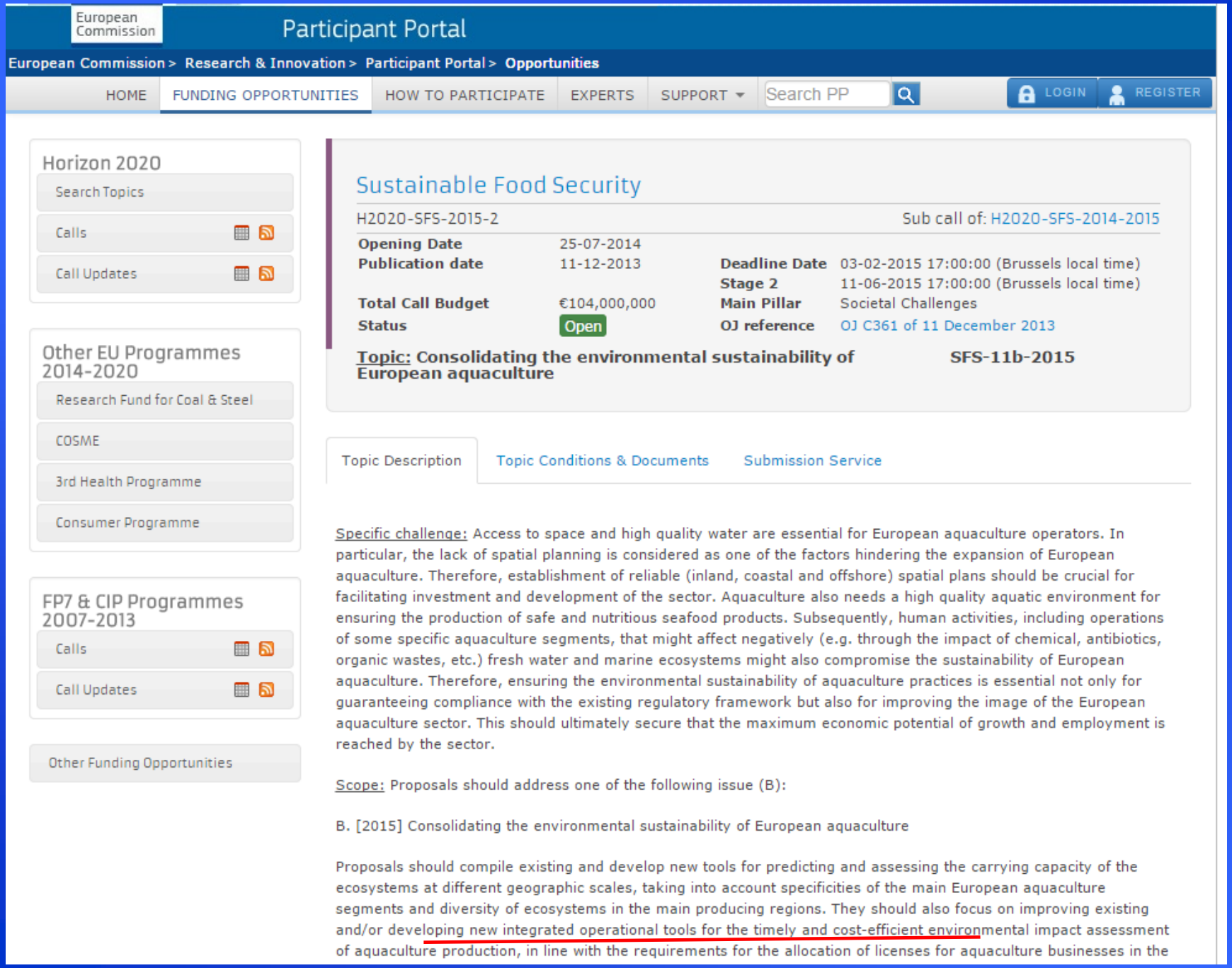


Biosensori elettrochimici enzimatici per:

- **monitoraggio della fermentazione alcolica e malo-lattica**→ *GOD, FDH, AO, SPE, mediatori elettr.*
- **determinazione ammine biogene in campioni alimentari e biologici**→ *DAO, SPE*
- **determinazione dell'acido okadaico e dei suoi derivati**→ *GOD-AP/sensore H_2O_2, FIA, FosfA+PP2A*
- **amilasi in saliva**→ *SPE, PB, FIA, glucosio ossidasi, α–glucosidasi*
- **determinazione dell'AFB_1** (biosensore elettrochimico/kit rapido spettrofotometrico) →*AChE*
- **determinazione di polifenoli** →*tirosinasi*
- **determinazione dei trigliceridi** → *lipasi, glicerolo deidrogenasi, SPE*
- **determinazione di pesticidi organofosforici e carbammici** →*ChE, SPE*
- **determinazione di gas nervini**→*BChE, SPE, mediatori*
- **monitoraggio in continuo di glucosio in pazienti diabetici** → *SPE, PB, GOD*
- **acido lattico in saliva** → *SPE, PB, lattato ossidasi*
- **studio e ripulitura di manufatti cartacei** → *SPE, GOD, gel, amilasi*



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Sustainable Food Security
H2020-SFS-2015-2
Sub call of: H2020-SFS-2014-2015
Opening Date 25-07-2014
Publication date 11-12-2013
Deadline Date 03-02-2015 17:00:00 (Brussels local time)
Stage 2 11-06-2015 17:00:00 (Brussels local time)
Total Call Budget €104,000,000
Main Pillar Societal Challenges
Status Open
OJ reference OJ C361 of 11 December 2013
Topic: Consolidating the environmental sustainability of European aquaculture SFS-11b-2015
Topic Description
Topic Conditions & Documents
Submission Service
Specific challenge: Access to space and high quality water are essential for European aquaculture operators. In particular, the lack of spatial planning is considered as one of the factors hindering the expansion of European aquaculture. Therefore, establishment of reliable (inland, coastal and offshore) spatial plans should be crucial for facilitating investment and development of the sector. Aquaculture also needs a high quality aquatic environment for ensuring the production of safe and nutritious seafood products. Subsequently, human activities, including operations of some specific aquaculture segments, that might affect negatively (e.g. through the impact of chemical, antibiotics, organic wastes, etc.) fresh water and marine ecosystems might also compromise the sustainability of European aquaculture. Therefore, ensuring the environmental sustainability of aquaculture practices is essential not only for guaranteeing compliance with the existing regulatory framework but also for improving the image of the European aquaculture sector. This should ultimately secure that the maximum economic potential of growth and employment is reached by the sector.
Scope: Proposals should address one of the following issue (B):
B. [2015] Consolidating the environmental sustainability of European aquaculture
Proposals should compile existing and develop new tools for predicting and assessing the carrying capacity of the ecosystems at different geographic scales, taking into account specificities of the main European aquaculture segments and diversity of ecosystems in the main producing regions. They should also focus on improving existing and/or developing new integrated operational tools for the timely and cost-efficient environmental impact assessment of aquaculture production, in line with the requirements for the allocation of licenses for aquaculture businesses in the

Sustainable Food Security

H2020-SFS-2015-2 — Sub call of: H2020-SFS-2014-2015

Opening Date	25-07-2014		
Publication date	11-12-2013	**Deadline Date**	03-02-2015 17:00:00 (Brussels local time)
		Stage 2	11-06-2015 17:00:00 (Brussels local time)
Total Call Budget	€104,000,000	**Main Pillar**	Societal Challenges
Status	Open	**OJ reference**	OJ C361 of 11 December 2013

Topic: Biological contamination of crops and the food chain **SFS-13-2015**

Specific challenge: The occurrence of biological contamination in various crops is of major concern as it has major implications for food and feed safety, food security and international trade. Worldwide, it is estimated that mycotoxins are responsible for losses of up to 5-10% of crop production. Contaminations are due to a series of events including weather conditions, possible climate change effects, land use, crop management and varieties as well as harvest and post-harvest techniques. Integrated approaches rather than isolated solutions are required to effectively control the incidence of mycotoxins in crops and reduce contamination throughout the feed and food chains.

Scope: Proposals should aim at reducing the risk of mycotoxin contamination in crops and all along the feed and food chains. They should bring about technical, management and organisational solutions (including HACCP techniques) that are effective at the various stages of production as well as at pre- and post-harvest levels and also deal with the safe use of contaminated batches. Proposals should take into account the development of ICT solutions as well as reliable and cost effective control tools and support policies in the area. Proposals should benefit conventional and organic supply chains and fall under the concept of 'multi-actor approach'[1] and allow for adequate involvement of the farming sector in proposed activities. In line with the objectives of the EU strategy for international cooperation in research and innovation and in particular with the implementation of the EU-China dialogue, proposals are encouraged to include third country participants, especially those established in China[2].

The Commission considers that proposals requesting a contribution from the EU in the range of EUR 3–5 million would allow this specific challenge to be addressed appropriately. Nonetheless, this does not preclude submission and selection of proposals requesting other amounts.

Expected impact:

· a toolkit (plant resources, management tools, technologies) to effectively monitor and reduce the incidence of mycotoxin contaminants in crop production as well as in the food and feed supply chains.

Personalising health and care

H2020-PHC-2015-single-stage — Sub call of: H2020-PHC-2014-2015

Opening Date	30-07-2014		
Publication date	11-12-2013	**Deadline Date**	21-04-2015 17:00:00 (Brussels local time)
Total Call Budget	€104,500,000	**Main Pillar**	Societal Challenges
Status	Open	**OJ reference**	OJ C 361 of 11 December 2013

Topic: Advanced ICT systems and services for integrated care **PHC-25-2015**

Specific challenge: Research on new models of care organisation demonstrates that advanced ICT systems and services may have the potential to respond to, amongst others, the increasing burden of chronic disease and the complexity of co-morbidities and in doing so contribute to the sustainability of health and care systems.

One challenge in re-designing health and care systems is to develop integrated care models that are more closely oriented to the needs of patients and older persons: multidisciplinary, well-coordinated, anchored in community and home care settings, and shifting from a reactive approach to proactive and patient-centred care.

Scope: Proposals should go beyond the current state of art in tele-health and tele-care systems by developing new approaches for integrated care supported by ICT systems and services. Proposals should address barriers from technological, social and organisational points of view in the following domains:

- Development of robust, privacy compliant, accurate and cost-effective systems that facilitate monitoring of patient status, patient activity and compliance with therapy;

- Fusion, analysis and interpretation of patient and care provider data, to improve decision making among formal and informal care givers and patients;

- Multi-channel and multi-actor interaction and exchange of knowledge in integrated care settings, across digital collaborative platforms;

- Development of patient-oriented services to support patient empowerment, self-care, adherence to care plans and treatment at the point of need;

- Development of new patient pathways, new training programmes for the care workforce and new organisational models to improve the coordination of care services as well as the skills and collaboration of health professionals, social carers and informal care givers;

B.E.A.T.

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Grazie per la gentile attenzione

SCELTA DEL TIPO DI IMMOBILIZZAZIONE