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Assessment of micropollutants toxicity by using a modified *Saccharomyces cerevisiae* model

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Environment can be affected by a variety of micropollutants. In this paper, we develop a system to assess the toxicity on an environmental sample, based on the expression of a nanoluciferase under the control of the *STB5* promoter in a yeast. The *STB5* gene encodes for a transcription factor involved in a pleiotropic drug resistance and in the oxidative stress response. The response of the modified yeast was assessed using 42 micropollutants belonging to different families (antibiotics, pain killers, hormones, plasticizers, pesticides, etc.). Among them, 26 induced an increase of the bioluminescence for concentration ranges from pg.L^{-1} to ng.L^{-1} . Surprisingly, for concentrations higher than 100 ng.L^{-1} , no response can be observed, suggesting that other mechanisms are involved when the stress increases. Analyzing the different responses obtained, we highlighted six nonmonotonic types of responses. The type of response seems to be independent of the properties of the compounds (polarity, toxicology, molecular weight) and of their family.

In conclusion, we highlighted that a cellular response exists for very low exposition to environmental concentration of micropollutants and that it was necessary to explore the cellular mechanisms involved at very low concentration to provide a better risk assessment.

1. Introduction

A wide variety of micropollutants such as pharmaceuticals, agricultural and industrial chemicals, etc. are increasingly detected in environmental matrices (water, air, soil, etc.). The presence of these compounds in the various compartments of an ecosystem has become a worldwide issue of increasing concern. Indeed, although they are usually present in low concentrations in the environment of the order of ng.L^{-1} , it was demonstrated on many instances that a large number of these micropollutants were not degraded and were steadily found in unchanged forms in the environment (Fatta-Kassinos et al., 2011; Fernández et al., 2014; Gibs et al., 2007; Nam et al., 2014; Stackelberg et al., 2004). Persistent (Díaz-Cruz et al., 2003; Yamamoto et al., 2009), toxic to non-target organisms (Almeida et al., 2014; Barra Caracciolo et al., 2015) and raising unknown biological effects due to multiple exposures, these compounds are contributing to the overall toxicity of an ecosystem.

The identification of the toxicity of these compounds was for a long time based on bioassays carried out in animal models (mostly rats and

mice). However, in accordance with the three Rs (reduce, replace, refine) (Russel and Burch, 1959), the use of animal models should be avoided as much as possible. Moreover, these are very expensive models with which high throughput manipulations cannot be designed while the results are difficult to transpose to humans (Jové et al., 2013; Vilahur et al., 2011). To overcome these drawbacks, several *in vivo* assays and *in vitro* models were proposed. For example, *Daphnia*, that is a freshwater microcrustacean, is used to determine the toxicity of a liquid sample (ISO 6341, 2012). This test, based on the mortality of *daphnia* after 24–48 h of exposure, is restrictive in use, long and expensive. More recently, computational methods have been developed (Medema and Fischbach, 2015), but the quality of the results is highly dependent on the chemical descriptors and modelling approaches used. Another innovative solution consists in coupling computational methods with bioassays (Huang et al., 2016). All the data obtained from tests on 10,000 molecules allowed the construction of predictive models of the *in vivo* toxicity using both the chemical structure of these molecules and the responses to toxicological tests. However, this approach can be problematic to study cocktail effect and to predict the interaction

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between compounds, making the use of this strategy impossible for real environmental samples.

In this context, the development of cells able to give indications on the biological effects of contaminants alone or in mixture is a major concern to assess the environmental risk. This approach is a supplementary approach to the analytical methods (Chamas et al., 2017).

Among the cell biosensors developed, a standardized toxicity test called Microtox® was commercialized (Bulich and Isenberg, 1981). Based on luminescence variations of a bacterium, it can evaluate the toxicity of an environmental sample in 15 min. One of the major drawbacks of this test is the model used. Indeed, in addition to the constraints of use attributed to the lyophilization of these bacteria, bacterial cell systems do not respond to environmental factors such as eukaryotes. Therefore, it would be preferable to have a eukaryotic model to transpose the toxicity to humans.

All of these elements lead to the use of yeast as a model. Indeed, many studies have already used this microorganism to evaluate the toxicity linked to specific compounds. These studies have focused mainly on the determination of hormones such as estrogens, androgens and gestagen molecules. As examples, the E-Screen, A-YES (Körner et al., 1999), nAES (Kaiser et al., 2010), EstraMonitor apparatus (Pham et al., 2013, 2012), A-YAS (Gerlach et al., 2014) can be cited.

All of these biosensors are specifically constructed to detect a single class of compounds. It was therefore necessary to find another target that could provide a more global response to the presence of micropollutants. One of the effects that can be caused by this type of compound on the cell is oxidative stress, induced by numerous attack or defense mechanisms (Ayer et al., 2013; Okai et al., 2000). In yeast, the *STB5* gene codes for a transcription factor participating in the regulation of metabolic pathways involved in the response to oxidative stress. To this end, it induces the expression of genes of the phosphate pentose pathway to stimulate the production of NADPH (Cadière et al., 2010; Larochelle et al., 2006). This transcription factor is also involved in yeast drug resistance by activating the transcription of genes encoding efflux pumps (Akache et al., 2004).

In this work, we proposed to construct a genetically modified yeast expressing the nanoLuc gene reporter (gene coding for a luciferase) under the control of the *STB5* promoter. To assess the relevance of the system constructed, the response of the biosensors was assessed for 42 micropollutants at different concentrations.

2. Materials and methods

2.1. Strain

S. cerevisiae strain CBS 8340, CEN. PK 113-7D MATa, URA3, HIS3, LEU2, TRP1, MAL2-8c, SUC2 was obtained from the Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands, <http://www.westerdijkinstitute.nl/>).

2.2. Mutant construction

The aim was to obtain a strain expressing a nanoluciferase under *STB5* promoter control. To minimize impact on yeast metabolism and avoid the presence of antibiotic gene resistance in the final modified yeast that could be interfere with the cell response, it was chosen first to delete *URA3* gene and to reintroduce it in the native locus coupled with the expression cassette containing nanoluciferase.

2.2.1. Deletion of *URA3* gene

URA3 gene was deleted using the cassette Tefp-KanMX-Tefter amplified from pCM325 (Euroscarf, Germany) using a forward primer ACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAA-TAGCTTGCCTTGTCCCCGCCGG (underlined) complementary to the pCM325 plasmid and 39 complementary to the 116129–116165 position of the chromosome V region

and a reverse primer TTCGTCATTATAGAAATCATTACGACCGA-GATTCCCAGGGTTCGACACTGGATGGCGGCGT which included 20 nucleotides (underlined) complementary to the pCM325 plasmid and 40 complementary to the 117084-117046 position of the chromosome V region.

2.2.2. Expression of nanoluciferase under *STB5* promoter

A synthetic fragment containing *URA3-URA3*ter-*STB5*p-Nluc-*ADH1* term was obtained by fusion PCR of two fragments *URA3-URA3*-term and *STB5*p-Nluc-*ADH1* term.

The fragment containing *URA3-URA3*term was amplified from genomic DNA of *S. cerevisiae* strain CBS 8340, CEN. PK 113-7D using the primer the forward primer AACTGCACAGAA-CAAAAACCTGCAGGAAACGAAGATAAAAT which target is complementary to the 116126–116165 position of the chromosome V region and the reverse primer TCCTATACGCGAGAACCAGCGCCGCGAAGAA-GAAGCCACAATAACTGATATAATTAAATTG

Which included 22 nucleotides (underlined) complementary to the 117044 to 117023 position of the chromosome V region and 38 nucleotides complementary to the 38 first nucleotides of the second fragment *STB5*p-Nluc-*ADH1* term.

Fusion PCR was realized as previously described by Shevchuk et al. (2004). The two fragments were first amplified with overhang fragment. A first PCR was realized without primer and with the two fragments during 11 cycles. 5 µL of this product were used to a second PCR using the forward primer AACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAAAT and the reverse primer TTCGTCATTATAGAAATCATTACGACCGAGATTCGCCGGTTCGGTA-GAGGTGTGGTCAA. PCR were performed using Phusion DNA Polymerases (ThermoFisher, USA) according with the manufacturer instruction.

The fragment was purified on gel electrophoresis before being used to transform cells using the LiAC procedure (Schiestl and Gietz, 1989). The selection of *ura3* deletion mutant was realized on yeast extract peptone dextrose (YEPD) medium supplemented with G418 at 200 µg.mL⁻¹. The selection of a strain expressing the nanoluciferase was done on synthetic defined (SD) medium.

2.3. Culture conditions

Cells were cultivated at 28 °C with shaking at 210 rpm onto YEPD [composed of glucose (Fisher Scientific, Loughborough, UK), peptone (Sigma-Aldrich, St. Louis, MO, USA), yeast extract (Sigma-Aldrich, St. Louis, MO, USA)] or SD [0.67% yeast nitrogen base without amino acids (BD Difco)], containing 2% w/v glucose (Fisher Scientific, Loughborough, UK). Agar plates were prepared by the addition of 2% agar (w/v) to liquid media.

Evaluation of toxicity was realized using the minimum medium (MM) previously described by Routledge and Sumpter (1996).

2.4. Micropollutants

All micropollutant standards used, classified into several groups according to their representativeness in the environment (Table 1), were of high purity grade (>90%). Individual stock standard solutions were prepared on a weight basis in methanol for almost all compounds, except for aminomethylphosphonic acid (AMPA), anatoxin A, cephalixin, cycloheximide, cylindrospermopsin, diamide, glyphosate and β-N-methylamino-L-alanine (BMAA) which were prepared in ultrapure water; for ciprofloxacin, that was prepared in ultrapure water with 0.1 N hydrochloric acid; and for ofloxacin prepared in methanol with 0.2% sodium hydroxide at 1 M. All the stock solutions are to an initial concentration of 5 mg.L⁻¹. These standard solutions were stored at –20 °C until their use. Cycloheximide and diamide were used as positive control (Akache et al., 2004; Larochelle et al., 2006). To obtain the desired concentration in the wells, dilutions of the stock solutions will be made

Table 1

List of the analyzed micropollutants classified according to group classes.

Antibiotics	Antidepressants/ Narcotics	Antidiabetic	Anti-inflammatory/ Painkillers	Hormones and plasticizers	Pesticides	Toxins	β -blockers	Controls
Azithromycin ^a	Carbamazepine- 10,11-epoxide ^a	Metformin ^a	Acetaminophen ^c	Bisphenol A ^a	AMPA ^b	Anatoxin A ^d	Atenolol ^a	Cycloheximide ^a
Cephalexin ^a	Carbamazepine ^a		Diclofenac ^a	4-nonylphenol ^a	Atrazine ^a	Cylindro- spermopsin ^d	Metoprolol ^c	Diamide ^a
Ciprofloxacin ^a	Oxazepam ^a		Ibuprofen ^a	Ethinyl estradiol ^c	Chlorpyrifos ^a	BMAA ^d	Propranolol ^a	
Clarithromycin ^a			Ketoprofen ^a		Diuron ^a		Sotalol ^a	
Erythromycin ^a			Salicylic acid ^a		Glyphosate ^c			
Flumequine ^a			Xylazine ^a		Metolachlor ^a			
Ofloxacin ^a					Simazine ^c			
Roxithromycin ^a					Terbutylazine ^a			
Spiramycin ^a					Warfarin ^c			
Sulfamethoxazole ^a								
Trimethoprim ^c								

AMPA: Aminomethylphosphonic acid.

BMAA: β -N-methylamino-L-alanine.^a Sigma-Aldrich (St-Louis, MO, USA).^b Supelco (Bellefonte, PA, USA).^c Fluka (Steinheim, Germany).^d Novakits (Nantes, France).

to obtain daughter solutions corresponding to a concentration 20-fold higher than the desired concentration in the well. All dilutions of these standard compounds were performed in ultrapure water. The concentration of the different solutions were checked by LC-MS/MS (3200 Qtrap).

2.5. Microtiter plate assay

For micropollutant screening, the strain was cultivated overnight onto MM at 28 °C with shaking at 210 rpm. From this first preculture, a second one was performed during eight to 10 h in the same conditions, before performing a culture of *S. cerevisiae*, inoculated at 0.1 optical density unit per milliliter. After about 15 h of incubation (in order to reach the midpoint of the exponential phase), 95 μ L of this culture were directly added to each well of a 96-well microplate (Dutscher, Brumath, France). Previously, 5 μ L of the micropollutant to be tested (in a final concentration range of 1 μ g. L⁻¹ to 1000 ng.L⁻¹) or sterile ultrapure water for controls were added to each well. The cellular concentration was measured with a Tecan Sunrise™ reader at 610 nm before and after micropollutant exposition. Based on the instructions of the Nano-Glo® Luciferase Assay System, the reconstituted reagent is composed of 20 μ L of substrate mixed with 1 mL of lysis buffer. After 3 h of exposition at 28 °C with shaking at 180 rpm, the bioluminescence of the cell suspension was measured by GloMax® Navigator GM2000 (Promega, Charbonnières-les-Bains, France) in white polystyrene microplates (VWR, Fontenay-sous-Bois, France). Each well contained 50 μ L of reconstituted reagent and 50 μ L of exposed culture. The microplates were placed on a shaking tray for 30 min at room temperature before performing the measurement. All experiments were performed in triplicate.

2.6. Statistical analysis

To identify the compounds that induced a response from the strain, a Student's test was performed with RStudio software (<https://rstudio.com/>). Luminescence results for each replicate were compared with those obtained for the corresponding controls using the dplyr package. The results of luminescence variations obtained for each micropollutant at each concentrations tested were modelled with the shinyHeatmaply package (Galili et al., 2018), using the Euclidean distance method and Ward clustering linkage. Correlation matrix with hierarchical cluster analysis was performed with ade4, factoextra and FactoMineR packages. Patterns within the different datasets were modelled by Principal

Component Analysis (PCA) to identify relationships between variables characterizing the molecules that responded to the system with the specific packages ade4, factoextra and FactoMineR.

3. Results and discussion

3.1. Exposure time to micropollutants

The first step was to optimize the exposure time to the micropollutants, which allowed the highest luminescence to be obtained, while at the same time providing good reproducibility between replicates. After preliminary experiments on several media (supplementary figure S1) and different exposure times (supplementary figure S2), the results obtained show a variation of luminescence at 4 h and not at 2 h of exposition on SD medium. In the remainder study, we chose to replace the SD medium by another minimal medium because in MM, all the compounds are controlled.

Thus, the 3 h, 4 h, 5 h and 6 h exposure times were tested in triplicate using the control molecule diamide on minimum medium. Indeed, diamide was described to induced *STB5* expression (Larochelle et al., 2006). Six concentrations between 1 μ g. L⁻¹ and 1000 ng.L⁻¹ were used and luminescence was read after each time (Fig. 1).

Significant differences (p-value < 0.05) were evaluated using a Student's test between control and each concentration.

The highest luminescence was observed at the 3-h time for all concentrations. Then, a decrease of about 10% in luminescence was noted at

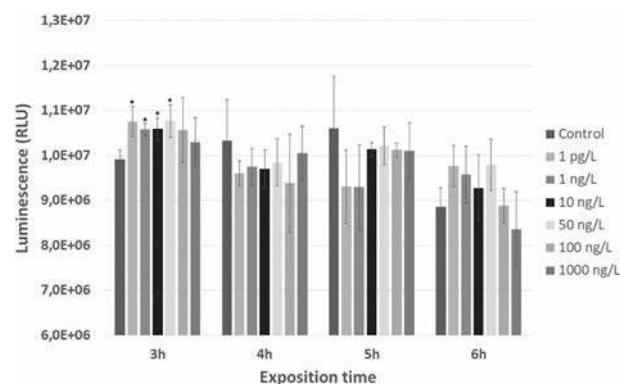


Fig. 1. Evaluation of the luminescence emitted after different exposure times to diamide. Significant differences (p-value < 0.05) were indicated by a star.

the other three times, 4 h, 5 h and 6 h, although it was not significant. It is therefore likely that nanoluciferase is continuously produced by the yeasts and destroyed as it is produced. However, significant variation from the control was demonstrated only for the lowest time. It is therefore not necessary to wait too long, as this will not improve the amount of nanoluciferase produced. Thus, a duration of 3 h of exposition to micropollutants was chosen for the remaining experiments.

Finally, to validate this time, this test was carried out with some representative molecules of the different families allowing to confirm that 3 h seems to be the best time to detect the induction of *STB5* gene after exposition to micropollutants (supplementary figure S3).

3.2. Screening of each micropollutant

The level of luminescence produced by *S. cerevisiae* is dependent on cell concentration. In order to assess a potential increase in cell concentration that would lead to erroneous results, cell density measurements were taken before and after the 3-h exposure to the various micropollutants. The results of these measurements showed an average cell density variation of $1.63\% \pm 2.94\%$ (data not shown). This slight variation did not affect the level of luminescence emitted by the cells and is therefore not taken into account in the data processing.

A total of 252 conditions were evaluated (42 molecules at six different concentrations), each time with measurements performed in triplicate. Since the cell concentration does not vary, only the luminescence variation from the control was used (Fig. 2).

Of the 42 molecules tested, 26 provide a statistically significant system response for at least one of the concentrations tested. For each of these molecules, response ranges were established and are variable with increases in induction ranging from 106.2% for exposure to ketoprofen at 1000 ng.L⁻¹ to 131.3% for exposure to propranolol at 50 ng.L⁻¹.

Interestingly, 1000 ng.L⁻¹, the highest concentration tested, had the

lowest response rate. Indeed, only six molecules responded at this concentration (propranolol, acetaminophen, erythromycin, bisphenol A, cycloheximide and ketoprofen). Cytotoxicity of compounds at this high concentration was considered, but yeast growth was observed even after several hours of exposure to these different micropollutants. One hypothesis is that for higher concentrations, the cell needs to implement more important and specific detoxification systems that do not go through *STB5* induction. Indeed, *STB5* is a pleiotropic factor and a too strong induction of this transcription factor leads to deleterious effects on the growth, in particular by the inhibition of glycolysis (Cadière et al., 2010). Based on the hierarchical classification, 100 ng.L⁻¹ and 1000 ng.L⁻¹ showed close responses, with little relationship to other concentrations. Concentrations 1 ng.L⁻¹ and 10 ng.L⁻¹ had the most similar responses. Very close, the response obtained at 1 pg.L⁻¹ highlighted the low concentration of micropollutant required to activate a response by the biosensor.

3.3. Micropollutants classification

Eight classes of micropollutants were tested (Table 1). Of these, most can be grouped under the term pharmaceuticals (antibiotics, antidepressants/narcotics, antidiabetic, anti-inflammatory/painkillers and β -blockers). These selected pharmaceuticals have the ability to activate different metabolic signaling pathways and different cellular response mechanisms that may be interesting to evaluate with this tool.

The objective was to determine whether our toxicity assessment tool presents one or more response specificities based on this categorization. This is why a correlation matrix with a hierarchical classification was carried out in order to highlight clusters of molecules with the same characteristics and therefore able to react in the same way to the biosensor (Fig. 3).

A hierarchical cluster highlighted six groups with different response profiles. Each profile was illustrated with model curves showing the average percentage of variation in luminescence as a function of the concentration tested (Fig. 4).

In contrast to other models, model A, comprising only acetaminophen, showed a response that increases slightly and proportionally with increasing concentration. On the other hand, models B to F showed nonmonotonic responses. This type of response is characterized by a slope of the curve whose sign changes somewhere within the range of doses examined (Vandenberg et al., 2012). Indeed, nonmonotonic dose-response curves are often U-shaped, inverted U- or J-shape (Ortiz de García et al., 2016; Vandenberg et al., 2012), as shown in the models B and F, containing respectively bisphenol A, carbamazepine, cephalexin, cycloheximide, erythromycin, ibuprofen, ketoprofen, simazine and atenolol, chlorpyrifos, cylindrospermopsin, glyphosate, metformin, oxazepam, propranolol, that represent 57% of the compounds tested. These two groups also showed the highest correlations between the molecules (Fig. 3).

Model C (diamide only) showed an inconsistent decrease in response when the dose was increased. For Model D (azithromycin, flumequine, terbuthylazine and trimethoprim), a concentration-proportional increase in response is observed in a first phase of the curve, then a sharp drop is noted at high concentrations. A similar pattern is observed with model E containing carbamazepine-10,11-epoxide, 4-nonylphenol, anatoxin A, atrazine and diuron, with two response peaks followed by a sharp decrease in response at high concentrations. Except model A, all others showed a decrease in response at high concentration. These non-linear variations may demonstrate the possibility that micropollutant responses to toxicity tests may not be correlated with concentration, especially at low doses.

By looking at the different classes of micropollutants, it appeared that all classes were represented, i.e. at least one micropollutant from each of the classes evaluated responded positively to the yeast system. Nevertheless, none of the groups of molecules defined by the models are specific to a class of micropollutants. As a result, some classes of

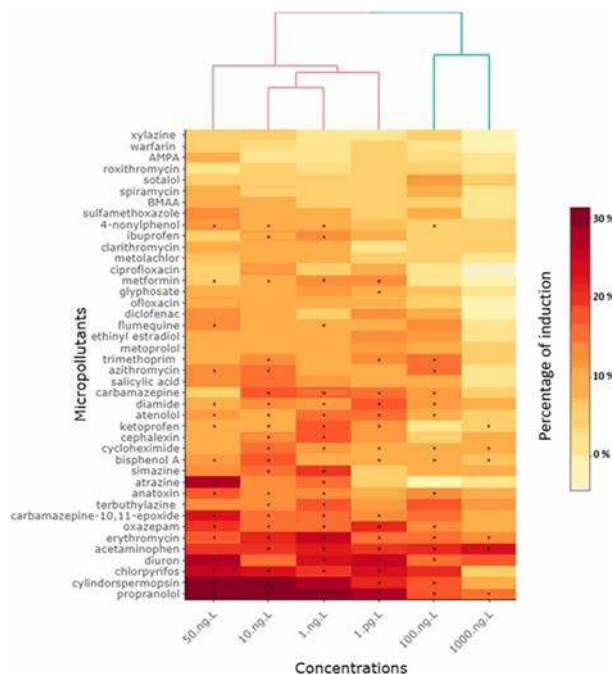


Fig. 2. Heatmap representing the luminescence variation between each micropollutant and the negative control based on the concentration tested. The gradation scale from yellow to red gives the percentage of induction, with luminescence variations of up to 30%. Clustering link was plotted using Euclidean distance method and Ward clustering linkage. Significant luminescence variations are indicated by a star. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

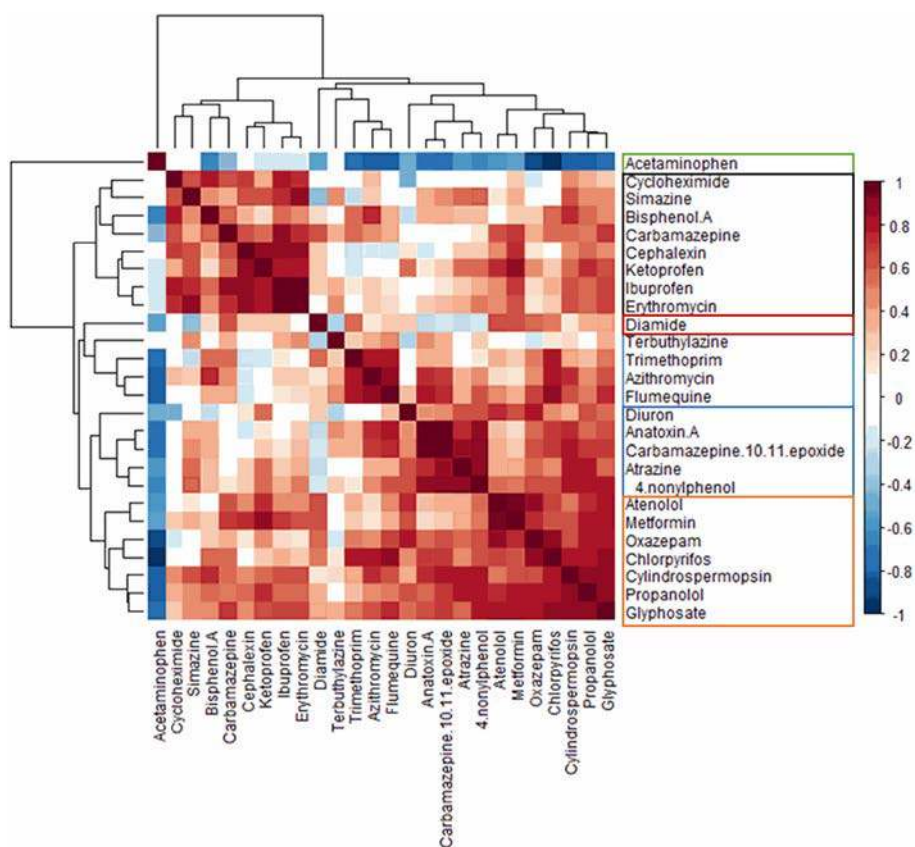


Fig. 3. Correlation matrix of micropollutants activating the yeast system. The compounds were classified according to a hierarchical cluster. The six different groups of molecules, identified using hierarchical clusters, were distinguished by color frames. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

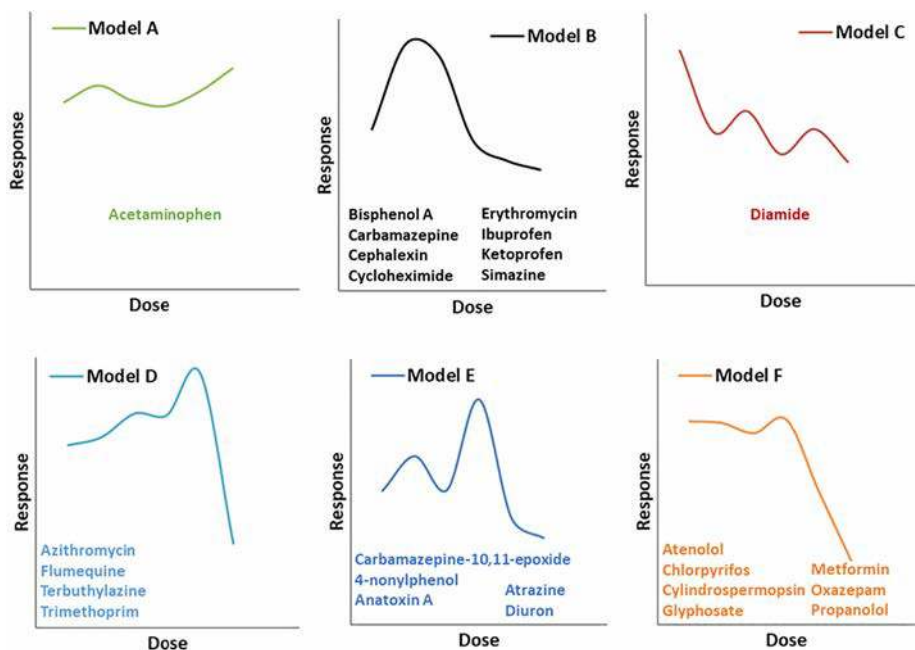


Fig. 4. Models of dose-response curves. These models represent nonmonotonic response following yeast exposure to micropollutants.

micropollutants exhibited similar response patterns, although not all molecules in these classes responded positively. We demonstrate here that the concentration required to activate a response does not require a high dose. On the contrary, it seems that a high dose can inhibit the

system.

These results are of interest from an environmental point of view since the concentrations of micropollutants found in water are usually in the nanogram range.

3.4. Relationship between parameters characterizing micropollutants and their responses to the biosensor

To identify the relationships between some physico-chemical, ecotoxicological and experimental variables characterizing the molecules that responded positively to the biosensor, a PCA was carried out. Among the physico-chemical factors, molar mass, octanol/water partition coefficient and topological polar surface area (TPSA) were taken into account. This last factor was chosen for its ability to represent the penetration of a molecule through the cell membrane (Pajouhesh and Lenz, 2005). In addition, this variable was used as a descriptor in quantitative structure-activity relationship (QSAR) models to assess the pharmacological activity of various chemical compounds (Prasanna and Doerksen, 2009). Three ecotoxicological factors were also selected, namely bioaccumulation, persistence and lethal dose 50. Finally, the last two factors were experimental with the response rate, which corresponds to the average variation percentage of luminescence of the compounds that showed a significant positive response at the concentrations tested and the minimum concentration leading to a response. Six variables representing the different models defined in Fig. 4 were added to the PCA. These parameters are additional, i.e., they do not affect the PCA result. In the resulting PCA plot, the data points were separated along a curve running across the first two principal components, which account for 56.7% of the variance (Fig. 5A).

It was possible to notice a strong correlation between molar mass and persistence ($r = 0.72$). Interestingly, the minimum concentration

leading to a response also shown a correlation with persistence ($r = 0.55$) and molecular weight ($r = 0.58$) which could demonstrate that the minimum concentration inducing a response from the system is particularly influenced by these two variables. Indeed, the higher the molecular weight and persistence, the higher the concentrations of micropollutants would be required to induce a response.

On the other hand, on this plot, additional variables representing the different response patterns were indicated by blue dotted arrows. They can indicate, among the different parameters characterizing the molecule, those that would influence the response according to the model. However, none of these factors correlated with the defined models, which would suggest that other parameters influence the response.

The projection of the micropollutants having responded positively to the system in the two-dimensional space defined by axes Dim 1 and Dim 2 was shown in Fig. 5B. Although erythromycin and azithromycin on the one hand, and chlorpyrifos on the other hand were outside the group formed by the other molecules, because of their high molecular weights or their high bioaccumulation and log Kow respectively, it appeared that the majority of the compounds were not separated according to patterns based on the different variables studied. Consequently, the way in which micropollutants respond seems independent of their physico-chemical or ecotoxicological properties.

4. Conclusion

For twenty-six of chemical compounds tested, positive responses

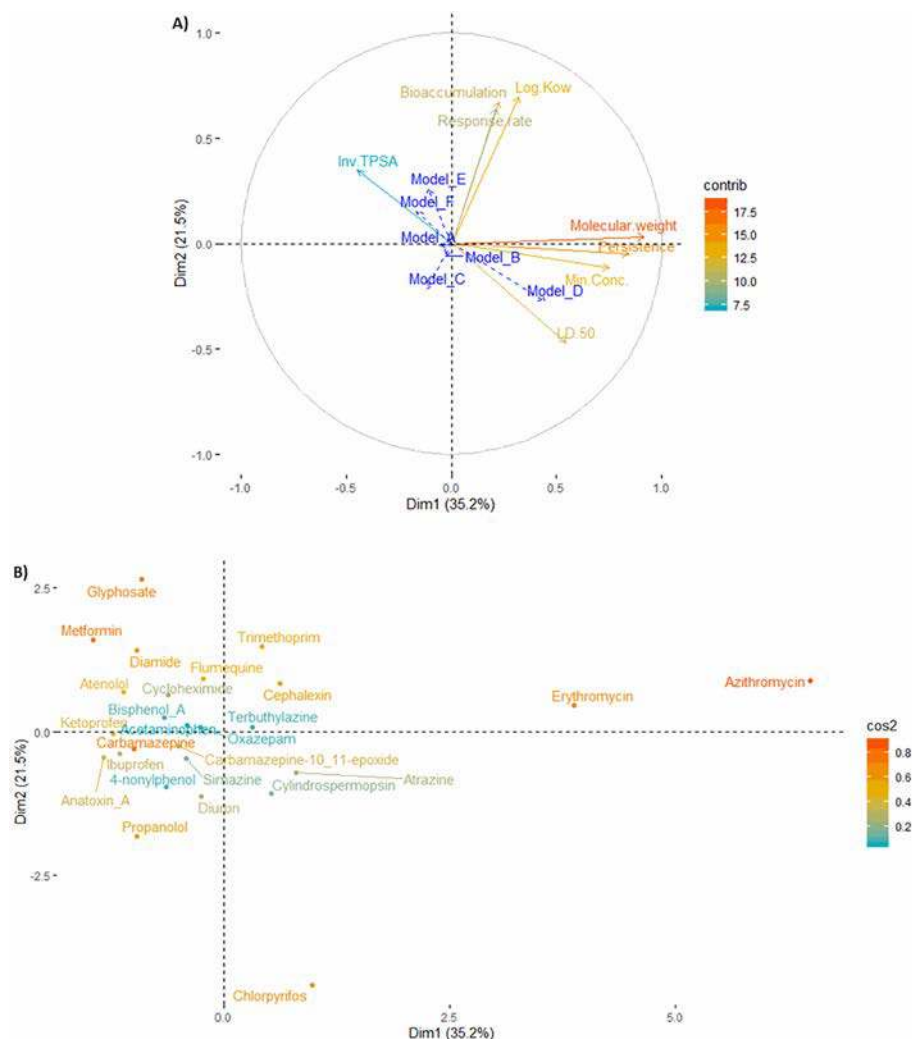


Fig. 5. Principal component analysis. A) Map of variable factors. The variables used for PCA analysis were the octanol/water partition coefficient (Log. Kow), the molecular weight (Molecular.weight), the average response rate of each compound (Response.rate), the minimum concentration leading to a response (Min.Conc.), the capacity of an organism to absorb contaminants (Bioaccumulation), the capacity to resist degradation (Persistence), the lethal dose causing 50% death in a population (LD50), the inverse of the topological polar surface area (Inv.TPSA). The various previously defined models to which the compounds belong were added as additional variables, indicated by dotted blue arrows. B) Distribution of micropollutants on PCA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

induced a cellular response for range concentrations between pg. L^{-1} and ng.L^{-1} suggesting that the expression of *STB5* is very sensitive to the external stress variation. Moreover, in these concentration ranges, it appeared that the yeast response to these micropollutants is non-monotonic, i.e. it does not evolve in a linear way as a function of concentration.

This article report for the first time that an indicator was able to response to very low concentration, compared to existing system ($\mu\text{g.L}^{-1}$ for antibiotic in *Vibrio fischeri* for example). In addition, we observed that for a higher concentration, no response was observed, suggesting the establishment of other cellular mechanisms. The recent work of Pandey et al. (2020) on the analysis of the yeast proteome in response to different oxidative stresses highlighted the existence of a specific signaling pathway by oxidant. Indeed, for each compound, a specific signaling pathway is induced to allow to the cell to survive, to repair damage etc. Interestingly, the concentration of diamide used to perform the proteomic analysis is 250 mg.L^{-1} which is 250,000 fold higher than our highest concentration tested. The analysis of the data provided in this publication, did not reveal any change in the level of expression of the *Stb5p*, which is consistent with our results.

For further studies, we will evaluate the transcriptomic responses of the cells for each model obtained and at different concentrations of responses, to try to evaluate the signaling pathways involved. The use of such low concentrations of chemical compounds has never been used to date and to our knowledge to evaluate the cellular mechanisms of responses. Moreover, we have initiated work on this biosensor on mixtures of the molecules. Finally, to assess the effect of a long-term exposition, the fermentation in continuous culture will be explored.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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