Estimating the xenobiotics mixtures toxicity on aquatic organisms: the use of α-level of the fuzzy number

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Abstract. Agricultural practices that use various xenobiotics can contaminate surface water and groundwater with xenobiotics mixtures concentrations which cause serious risks to water quality and to the health of aquatic organisms that inhabit them. Xenobiotics in water when present as mixtures can exacerbate or reduce the toxic effects in aquatic organisms, when compared to the toxic effects of each individual component concentrations of the xenobiotics mixture. The objective of this study is to develop a mathematical method using α-level of the fuzzy numbers with less accounts and simpler calculations to sort ecotoxicological effects in aquatic organisms of xenobiotics mixtures concentrations occurring in water, classifying them into antagonistic, additive or synergistic and also establishing the magnitude of the effects of concentrations of mixtures. The proposed method in this paper using fuzzy numbers can be suggested in protocols established by regulatory agencies to classify ecotoxicological effects of xenobiotics mixtures in water.

Keywords: mixtures, fuzzy numbers, α-level, ecotoxicological.

1 Introduction

Agricultural practices that use various xenobiotics can contaminate surface water ([3] [22]) and groundwater ([14] [6] [5]) with xenobiotics mixtures concentrations that can cause serious risks to water quality and to the health of aquatic organisms that inhabit them ([18] [1] [9]). Xenobiotics when present in water as

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mixtures can exacerbate or reduce the toxic effects on aquatic organisms, when compared to the toxic effects of each individual component concentrations of the xenobiotics mixture. For instance, the combination of the insecticides quinalphos and phenthoate showed synergistic toxicity to tilapia *Oreochromis mossambicus* [7]. The association of the fungicides piraclostrobin and epiconazole increased the toxicity to microalgae in 13.6 times when compared to the toxicity of individuals compounds [20]. According to Nair et al. [16], the insecticide combination malathion-endosulfan showed a "more than additive" effect to juveniles of rohu fishes (*Labeo rohita*). Qu et al. [21] proposed an ecological risk assessment of pesticide residues for wetland ecosystems and the risks of eight pesticides in Taihu Lake wetland were assessed, as single substances and in mixtures.

The simultaneous presence of substances in the aquatic compartment can also be derived from the commercial formulations that contain more than one active ingredient, or from the mixture of products in the spray tanks ([19] [23]). In this context, the use of more than one active ingredient is seen as an advantage due to the reduced cost and the reduced spraying of the recommended dose. Also because the increase of the number of pest species to be chemically controlled.

When two xenobiotics enter concurrently in a biological system there is a need to characterize the toxic effect of the combination in relation to the toxic effect of each compound individually [12]. Some methods allow the classification of such chemical interactions. In this classification, additivity can be generalized for two compounds that act independently on the same target and their effects are additive. Synergism is defined as an interaction among compounds producing a higher effect (more than additive effect) when compared with the individual effect of each compound. Conversely, antagonist compounds would reduce the effect [8].

The toxicity of a compound can be expressed by the value of the median effective concentration (LC_{50}), or concentration that affects 50% of individuals in a population in a given time interval. Therefore, the smaller this value, the more toxic the compound [15]. Thus, by knowing the LC_{50} values for the individual compounds and LC_{50} values for the compounds in the mixture (with their confidence intervals), one can classify the magnitude of the mixtures effect when compared to the individual component concentrations of mixture. Also it is possible to establish confidence intervals for the magnitude of the effect [12].

A fuzzy set has been defined as a collection of objects with membership values between 0 (complete exclusion) and 1 (complete membership). The membership values express the degrees to each object with respect to the properties or distinctive features to the collection. Recently, a fuzzy model have been applied in the field of mixture toxicity prediction to solve the limitations of existing prediction models of mixture toxicity [24].

Then, the objective of this study was to develop a mathematical method using α-level of the fuzzy numbers with less accounts and simpler calculations to sort ecotoxicological effects in aquatic organisms of xenobiotics mixtures concentrations occurring in water. The method allows classify the mixture into antagonistic, additive or synergistic and also establish the magnitude of the effects of concentrations of mixtures. The legislation establishing limits of chemicals in water bodies in Brazil [4] does not report such limits for chemicals mixtures. Thus, the importance in detecting a synergistic action, that results in a potentiation of the effect, contributes to the establishment of public policies in order to improve the water quality standards.

2 Additive toxicity

Toxicity was defined by the median effective concentration LC_{50}, that is, the concentration calculated to produce 50% of effect and 95% confidence intervals according to the procedures of ([11] [12]) . The procedures for determining the additive index is based on the toxic unit concept in which each component in the mixture contributes to toxicity.

Definition 1. *The contributions of two components of chemical mixtures are summed accordingly*

$$(A_m/A_i) + (B_m/B_i) = S,$$

where A *and* B *are chemicals,* A_i *and* B_i *are toxicities (*LC_{50}*) of the individual chemicals,* A_m *and* B_m *are toxicities (*LC_{50}*) of the mixtured chemicals and* S *is the sum of biological activity [12].*

Definition 2. *The additive index is defined by*

$$AI = \begin{cases} (1/S) - 1.0 & if \;\; S \leq 1.0 \\ (-S) + 1.0 & if \;\; S > 1.0 \end{cases} \tag{1}$$

The range for additive index is derived by selecting values of 95% confidence interval yielding the greatest derivation from the additive index. The lower limits of the individual toxicants – A_i and B_i – and the upper limits of the mixtures – A_m and B_m – are substituted for LC_{50} to determine the lower limit of the index. Analogously, the upper limits of the mixture – A_m and B_m – are substituted into the formula to determine the upper limit of the index.

If the range overlaped zero, then toxicity of the chemicals in combination is considered additive [12].

We suggest [12] and [11] for a detailed study of the procedures for classifying the mixture into antagonistic, additive or synergistic.

Remark: The additive toxicity of n chemicals in a mixture is assessed by adding the contributions of additional chemicals according to the formula

$$(A^1_m/A^1_i) + (A^2_m/A^2_i) + (A^3_m/A^3_i) + \ldots + (A^n_m/A^n_i) = S$$

3 Fuzzy Numbers

Next, we develop brief reviews of the concept of fuzzy numbers, and we detail the method suggested in this paper.

Fuzzy Sets and Fuzzy Logic have become one of the emerging areas in contemporary technologies of information processing. Fuzzy Sets Theory was first developed by [25] in the mid-1960s to represent uncertain and imprecise knowledge. It provides an approximate but effective means of describing the behavior of the system that is too complex, ill defined, or not easily analyzed mathematically.

Definition 3. *Let U be a classical non-empty set. A fuzzy subset F of U is described by a function,*

$$F : U \to [0, 1],$$

called membership function of fuzzy set F [25].

The value $F(x) \in [0, 1]$ indicates the membership degree of the element x of U in fuzzy set F, with $F(x) = 1$ and $F(x) = 0$ designating, the belonginness and not-belongingness of x in F, respectively. Note that the membership function of empty, $\emptyset$, and universe, U, sets are, respectively, $\emptyset(x) = 0$ and $U(x) = 1$ for all $X \in U$ [13].

Linguistic variables (or fuzzy) are variables whose values are fuzzy sets [17].

The set of all elements that belong to a fuzzy set A with at least α degree is called α-level of A and denoted by $[A]^\alpha$.

Definition 4. *Let A be a fuzzy subset of X and $\alpha \in [0, 1]$. The α-level of A is the subset of X defined by*

$$[A]^\alpha = \{x \in X/A(x) \geq \alpha\}$$

for $0 < \alpha \leq 1$.

So, the set $[A]^\alpha$ consists of those elements of the universe X whose membership degree is larger than α [10]

A very special class of fuzzy sets is the so-called "fuzzy numbers". This is due to the fundamental role that they play in fuzzy modeling. In this sense, the majority of the fuzzy sets belongs to the fuzzy numbers class [13].

Definition 5. *A fuzzy subset A in $\mathbb{R}$ is called fuzzy number when:*

1. *all α-levels of A are non-empty with $0 \leq \alpha \leq 1$, that is, A must be normal;*
2. *all α-levels of A are closed intervals of $\mathbb{R}$;*
3. *the support of A, that is, $suppA = \{x \in \mathbb{R}/A(x) > 0\}$.*

Definition 6. *Let us represent the α-levels of the fuzzy numbers A by*

$$[A]^\alpha = [a_1^\alpha, a_2^\alpha].$$

A fuzzy subset F of real numbers is called triangular if its membership function is a triangular function. This function is specified by three parameters,

$F(x : a, b, c)$, such as:

$$F(x:a,b,c)=\begin{cases} 0 & if\ \ x<a \\ \frac{x-a}{b-a} & if\ \ a\leq x<b \\ \frac{c-x}{c-b} & if\ \ b\leq x<c \\ 0 & if\ \ x\geq c \end{cases}$$

where a, b, c are given numbers.

The α-levels of triangular fuzzy numbers have the following simplified from:

$$[a_1^\alpha, a_2^\alpha] = [(b-a)\alpha + a, (b-c)\alpha + c], \tag{2}$$

fo ll $\alpha \in [0, 1]$.

The great advantage of fuzzy numbers is that it is possible to compute with them. Thus, we can define arithmetic operations on fuzzy numbers.

Definition 7. *Let A and B be fuzzy numbers and λ a real number.*

1. *the addition of A and B produces a third fuzzy number $A+B$, whose membership function is given by:*

$$\psi_{A+B}(x)=\begin{cases} sup_{\phi(z)}min[\psi_A(x),\psi_B(x)] & if\ \ \phi(z)\neq 0 \\ 0 & if\ \ \phi(z)=0 \end{cases}$$

   where $\phi(z) = \{(x, y) : x + y = z\}$.
2. *the subtraction of two fuzzy numbers A and B produces a third fuzzy number $A - B$, whose membership function is given by:*

$$\psi_{A-B}(x)=\begin{cases} sup_{\phi(z)}min[\psi_A(x),\psi_B(x)] & if\ \ \phi(z)\neq 0 \\ 0 & if\ \ \phi(z)=0 \end{cases}$$

   where $\phi(z) = \{(x, y) : x - y = z\}$.
3. *the multiplication of λ by fuzzy number A produces a third fuzzy number λA, whose membership function is given by:*

$$\psi_{\lambda A}(x)=\begin{cases} sup_{\{x:\lambda x=z\}}min[\psi_A(x)] & if\ \ \lambda\neq 0 \\ \chi_{\{0\}}(x) & if\ \ \lambda=0 \end{cases}$$

   where $\chi_{\{0\}}$ is the characteristic function of $\{0\}$.
4. *the multiplication of A and B produces a third fuzzy number $A.B$, whose membership function is given by:*

$$\psi_{A.B}(x)=\begin{cases} sup_{\phi(z)}min[\psi_A(x),\psi_B(x)] & if\ \ \phi(z)\neq 0 \\ 0 & if\ \ \phi(z)=0 \end{cases}$$

   where $\phi(z) = \{(x, y) : x.y = z\}$.

5. *the division of* A *and* B*, if* $0 \notin supp(B)$*, produces a third fuzzy number* A/B*, whose membership function is given by:*

$$\psi_{A/B}(x) = \begin{cases} sup_{\phi(z)} min[\psi_A(x), \psi_B(x)] & if \;\; \phi(z) \neq 0 \\ 0 & if \;\; \phi(z) = 0 \end{cases}$$

where $\phi(z) = \{(x, y) : x/y = z\}$*.*

From concept of α-level we have a "practical method" to obtain the results of each arithmetic operation between fuzzy numbers, because the arithmetic operations with fuzzy numbers are closely linked to the interval mathematics [2].

Theorem 1. *Let* A *and* B *be fuzzy numbers with* α*-levels* $[A]^\alpha = [a_1^\alpha, a_2^\alpha]$ *and* $[B]^\alpha = [b_1^\alpha, b_2^\alpha]$*, respectively; and*$\lambda$ *a real number. Then, we have the following properties:*

1. *the addition between* A *and* B *is a fuzzy number* $A + B$*, whose* α*-levels are given by*
$$[A + B]^\alpha = [A]^\alpha + [B]^\alpha = [a_1^\alpha + b_1^\alpha, a_2^\alpha + b_2^\alpha].$$
2. *the subtraction between* A *and* B *is a fuzzy number* $A - B$ *whose* α*-levels are given by*
$$[A - B]^\alpha = [A]^\alpha - [B]^\alpha = [a_1^\alpha - b_2^\alpha, a_2^\alpha - b_1^\alpha].$$
3. *the multiplication of a real number* λ *by the fuzzy number* A *produces is a fuzzy number* λA*, whose* α*-levels are given by*
$$[\lambda A]^\alpha = \lambda[A]^\alpha = [\lambda a_1^\alpha, \lambda a_2^\alpha] \qquad if \;\; \lambda \geq 0$$
or
$$[\lambda A]^\alpha = \lambda[A]^\alpha = [\lambda a_2^\alpha, \lambda a_1^\alpha] \qquad if \;\; \lambda < 0.$$
4. *the multiplication of a fuzzy number* A *by a fuzzy number* B *is a fuzzy number* $A.B$*, whose* α*-levels are*
$$[A.B]^\alpha = [A]^\alpha.[B]^\alpha = [\min P^\alpha, \max P^\alpha],$$
where $P^\alpha = \{a_1^\alpha b_1^\alpha, a_1^\alpha b_2^\alpha, a_2^\alpha b_1^\alpha, a_2^\alpha b_2^\alpha\}$*.*
5. *the division of a fuzzy number* A *by a fuzzy number* B*, if* $0 \notin suppB$*, is a fuzzy number* A/B*, whose* α*-levels are given by*
$$\left[\frac{A}{B}\right]^\alpha = \frac{[A]^\alpha}{[B]^\alpha} = [a_1^\alpha, a_2^\alpha].\left[\frac{1}{b_2^\alpha}, \frac{1}{b_1^\alpha}\right].$$

Proof: See [2] .

Thus, it is enough to consider the interval arithmetic operations.

4 Additive toxicity using α-level of the fuzzy numbers

In this section, we have developed a mathematical method using α-level of the fuzzy numbers to sort ecotoxicological effects in aquatic organisms of xenobiotics mixtures concentrations occurring in water, classifying them into antagonistic, additive or synergistic and also establishing the magnitude of the effects of concentrations of mixtures.

We have proposed to use α-level of the fuzzy numbers to classify the mixture prepared by the adaptation of classic method (1). By these means, we intend to simplify the calculus of the additive index.

For this method, we consider the values of the LC_{50} individually and in combination of the chemicals A and B and the $100(1-\alpha)\%$ confidence interval individually and in combination of each chemicals as being the α-level.

Definition 8. *Let $[A]^\alpha$ be the α-level of the fuzzy number (LC_{50}) the xenobiotic A individually, $[A_m]^\alpha$ the α-level of the fuzzy number (LC_{50}) the xenobiotic A in combination, $[B]^\alpha$ the α-level of the fuzzy number (LC_{50}) the xenobiotic B individually, $[B_m]^\alpha$ the α-level of the fuzzy number (LC_{50}) the xenobiotic B in combination, then we define the interval sum of biological activity, denoted IS, by*

$$\frac{[A_m]^\alpha}{[A]^\alpha} + \frac{[B_m]^\alpha}{[B]^\alpha} = IS. \tag{3}$$

And considering $1 = [1,1]$, we have

Definition 9. *The fuzzy additive index,FAI, is defined by*

$$FAI = \begin{cases} ([1,1]/IS) - [1,1] & if \;\; SM \leq 1.0 \\ IS(-[1,1]) + [1,1] & if \;\; SM > 1.0 \end{cases} \tag{4}$$

where SM is the arithmetic mean between the lower limit and the upper one of IS.

We can see (4) as the adaptation of classic method (1).

5 Results

In this section we use the method of the Section 4 to classify the toxicity of mixtures. Fish are exposed simultaneously to more than one contaminant because some chemicals are applied as combinations to increase efficacy or reduce costs [12].

In Table 1 the columns 2, 3 and 4 are available in [12]. Note that the column 4 is the values obtained by classic model (Section 2). In column 5, we have the corresponding fuzzy number obtained through the mathematical model proposed in Section 4 with $\alpha = 0.05$.

It has been highlighted that is possible to choose any α, but it was chosen $\alpha = 0.05$ in order to compare the results by the classic method [11] and the results

Table 1. Toxicity and additive indices for xenobiotics, pairs of xenobiotics combinations against rainbow trout in soft water at 12^{o}C.

Xenobiotics	LC_{50} and interval 95% confidence interval individually	LC_{50} and interval 95% confidence interval in combination	additive index and range	fuzzy additive index
Antimycin	0.0312	0.03		
$\mu g/L$	[0.0266, 0.0366]	[0.0272, 0.0331]	-0.574	[-1.4307, -0.1729]
Dibrom	0.049	0.03	[-1.43, -0.173]	Antagonism
mg/L	[0.0279, 0.0633]	[0.0272, 0.0331]	Antagonism	
TFM	1.81	1.16		
lampricide, mg/L	[1.53, 2.14]	[0.998, 1.35]	-0.326	[-0.8083, 0.0287]
Bayer 73	0.0346	0.0237	[-0.808, 0.0295]	Additive
lampricide, mg/L	[0.0204, 0.0275]	[0.0204, 0.0275]	Additive	
Malathion	70	3.44		
$\mu g/L$	[59.2, 82.7]	[2.92, 4.06]	7.20	[5.0851, 10.0106]
Delnav	47.2	3.44	[5.09, 10.0]	Synergism
$\mu g/L$	[42.4, 52.6]	[2.92, 4.06]	Synergism	

obtained by our method because the classical method considers 95% confidence intervals.

Considering case 1 of the Table 1:

Let A and B be two xenobiotics such that:

- LC_{50} of xenobiotic A individually is equal to 0.0312 $\mu g/L$;
- LC_{50} of xenobiotic B individually is equal to 0.049 mg/L;
- LC_{50} of xenobiotic A in combination is equal to 0.03 $\mu g/L$;
- LC_{50} of xenobiotic B in combination is equal to 0.03 mg/L;
- 95% confidence interval of xenobiotic A individually is equal to $[0.0266, 0.0366]$;
- 95% confidence interval of xenobiotic B individually is equal to $[0.0279, 0.0633]$;
- 95% confidence interval of xenobiotic A in combination is equal to $[0.0272, 0.0331]$;
- 95% confidence interval of xenobiotic B in combination is equal to $[0.0272, 0.0331]$.

According to Section 2, the classic method to calculate the additive index and the range is

$$S = \frac{0.03}{0.0312} + \frac{0.03}{0.049} = 1.574.$$

Since $S > 1$, then

$$-1.574 + 1.0 = -0.574.$$

Thus, the additive index for A and B is equal to -0.574.

Next, we calculate the range for A and B:

– the lower limit of range is equal to $(-S) + 1.0 = -2.431 + 1.0 = -1.43$, since $S = \frac{0.0331}{0.0266} + \frac{0.0331}{0.0279} = 2.43 > 1$.
  And
– the upper limit of range is equal to $(-S) + 1.0 = -1.173 + 1.0 = -0.173$, since $S = \frac{0.0272}{0.0366} + \frac{0.0272}{0.0633} = 1.173 > 1$.

Hence, the range of mixture is equal to $[-1.43, -0.173]$, i.e., $0 \notin [-1.43, -0.173]$ and $[-1.43, -0.173] \subset \mathbb{R}^*_-$. Therefore, the toxicity of A mixed with B is antagonistic because the fuzzy additive index belongs to negative values.

Now, the fuzzy method to calculate the fuzzy additive index is

– for the xenobiotic A, we defined the fuzzy number LC_{50} of A individually is equal to $A = (x : 0.0264, 0.0312, 0.0369)$, that is, $[A]^{0.05} = [0.0266, 0.0366]$;
– for the xenobiotic B, we defined the fuzzy number LC_{50} of B individually is equal to $B = (x : 0.0268, 0.048, 0.0641)$, that is, $[B]^{0.05} = [0.0279, 0.0633]$;
– for the xenobiotic A, we defined the fuzzy number LC_{50} of A_m in combination is equal to $A_m = (x : 0.0271, 0.03, 0.0333)$, that is, $[A_m]^{0.05} = [0.0272, 0.0331]$;
– for the xenobiotic B, we defined the fuzzy number LC_{50} of B_m in combination is equal to $B_m = (x : 0.0271, 0.03, 0.0333)$, that is, $[B_m]^{0.05} = [0.0272, 0.0331]$;

that is,

$$IS = \frac{[0.0272, 0.0331]}{[0.0266, 0.0366]} + \frac{[0.0272, 0.0331]}{[0.0279, 0.0633]} = [1.1729, 2.4307].$$

Since $SM > 1$, then

$$FAI = [1.1729, 2.4307](-[1, 1]) + [1, 1] = [-1.4307, -0.1729].$$

Therefore, the fuzzy additive index is equal to $[-1.4307, -0.1729]$. Observe that, we get the same range of the combination between A and B of the classic method (1). In this way, the toxicity of A mixed with B is antagonism.

Nevertheless, the quantity of the mathematical calculations is smaller than the classic method and the formula (4) is easiest solution than the classic formula (1).

Analogously, we determine the values of the Table 1 for cases 2 and 3.

6 Conclusions

In this study we develop a method using fuzzy numbers to sort ecotoxicological effects in aquatic organisms of xenobiotics mixtures concentrations occurring in water, classifying them into antagonistic, additive or synergistic and also establishing the magnitude of the effects of concentrations of mixtures.

It can be observed that the values obtained by the fuzzy model are very close to the values found in the literature but there are less accounts and simpler calculations. It has been used the formulas (1) of Section 2 three times to determine the values of the additive index, the lower limit and the upper one of the confidence interval. And it has been used the formulas (4) just once to determine the fuzzy additive index of Section 4.

Furthermore, it can be applied our method for any confidence interval. Simply take the α-level as desired. It has been highlighted that is possible to choose any α, but it was chosen $\alpha = 0.05$ in order to compare the results by the classic method [11] and the results obtained by our method.

The method developed using fuzzy numbers can be suggested in protocols established by regulatory agencies to classify ecotoxicological effects of xenobiotics mixtures in water. In this way, public policies would be implemented to ensure the health of the aquatic environment.

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