

## ESTIMATION OF THE ALLOWABLE CONCENTRATION OF CHLORIDES IN SOAKING EFFLUENTS FROM THE TANNING PROCESS USING ECOTOXICOLOGICAL TOOLS

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### ESTIMATION OF THE ALLOWABLE CONCENTRATION OF CHLORIDES IN SOAKING EFFLUENTS FROM THE TANNING PROCESS USING ECOTOXICOLOGICAL TOOLS

**ABSTRACT.** The use of sodium chloride for the preservation of pelts and skins destined for tanning is a very widespread technique in Peru, therefore the quantities used of this salt represent high concentrations of chlorides in the liquid effluents that are generated, which can affect the environmental quality of ecosystems and human health. The present study aimed to estimate an allowable concentration of chlorides in tanning effluents using ecotoxicological tools so that it would serve as a guide for the tanning industry and the authorities in their efforts to establish better conservation practices and effluent control parameters, which are currently absent from national environmental legislation. The permissible concentration of chlorides was determined using bioassays with seeds of *Lactuca sativa* species (lettuce), which was exposed to soaking effluents obtained from local tanneries. The studied species was determined based on sensitivity tests to zinc sulfate ( $ZnSO_4$ ) applied to three commercial lettuce species. The selected lettuce seed presented a germination rate higher than 90%, a CV lower than 30% and an  $IC_{50}$  of 38.97 mg/L of  $Zn^{+2}$  with a confidence interval between 19.30 mg/L and 58.65 mg/L of  $Zn^{+2}$ . The admissible guide concentration determined for chlorides in soaking effluents from  $IC_{50}$  was 582.30 mg/L with a minimum value of 258.45 mg/L and a maximum of 906.15 mg/L. Additionally, because of the presence of other pollutants in the soaking effluents, such as bactericides, organic matter, enzymes and salts added in soaking process, tests with synthetic solutions of sodium chloride at the same concentrations of the soaking effluents were conducted, to evaluate the effect produced by those substances.

**KEY WORDS:** waste valorization, chrome shavings, alkaline hydrolysis, tanning process

### ESTIMAREA CONCENTRAȚIEI ADMISIBILE DE CLORURI ÎN EFLUENȚII DE LA ÎNMUIERE DIN CADRUL PROCESULUI DE TĂBĂCIRE FOLOSIND INSTRUMENTE ECOTOXICOLOGICE

**REZUMAT.** Utilizarea clorurii de sodiu pentru conservarea blănurilor și a pieilor pentru tăbăcire este o tehnică foarte răspândită în Peru, prin urmare cantitățile de sare utilizate înseamnă concentrații mari de cloruri în efluenții generați, ceea ce poate afecta calitatea ecologică a ecosistemelor și sănătatea umană. Prezentul studiu are ca scop estimarea concentrației admisibile de cloruri în efluenții de la tăbăcire utilizând instrumente ecotoxicologice, astfel încât să servească drept ghid pentru industria de pielărie și pentru autoritățile în eforturile acestora de a stabili practici mai bune de conservare și parametrii de control al efluenților, care lipsesc în prezent din legislația națională de mediu. Concentrația admisibilă de cloruri a fost determinată folosind teste biologice cu semințe din specia *Lactuca sativa* (salată), care au fost expuse la efluenții de înmuiere obținuți din tăbăcăriile locale. Specia studiată a fost determinată pe baza testelor de sensibilitate la sulfatul de zinc ( $ZnSO_4$ ) aplicate la trei specii de salată comercială. Semințele de salată selectate au prezentat o rată de germinare mai mare de 90%, un coeficient de variație (CV) mai mic de 30% și o concentrație inhibitorie maximă 50% ( $IC_{50}$ ) de 38,97 mg/l de  $Zn^{+2}$  cu un interval de încredere între 19,30 mg/l și 58,65 mg/l de  $Zn^{+2}$ . Concentrația orientativă admisă determinată pentru cloruri în efluenții de înmuiere la  $IC_{50}$  a fost de 582,30 mg/l cu o valoare minimă de 258,45 mg/l și maxim de 906,15 mg/l. În plus, din cauza prezenței altor poluanți în efluenții de înmuiere, cum ar fi bactericidele, materia organică, enzimele și sărurile adăugate în procesul de înmuiere, s-au efectuat teste cu soluții de clorură de sodiu sintetice la aceleași concentrații ale efluenților de înmuiere, pentru a evalua efectul produs de aceste substanțe.

**CUVINTE CHEIE:** valorificarea deșeurilor, răzătură de piele cromată, hidroliză alcalină, proces de tăbăcire

### ESTIMATION DE LA CONCENTRATION ADMISSIBLE DE CHLORURES DANS LES EFFLUENTS DE TREMPAGE DU PROCESSUS DE TANNAGE À L'AIDE D'OUTILS ÉCOTOXICOLOGIQUES

**RÉSUMÉ.** L'utilisation de chlorure de sodium pour la conservation des fourrures et des peaux destinées au tannage est une technique très répandue au Pérou, donc les quantités utilisées de ce sel représentent des concentrations élevées de chlorures dans les effluents liquides générés, ce qui peut affecter la qualité environnementale des écosystèmes et la santé humaine. La présente étude visait à estimer une concentration admissible de chlorures dans les effluents de tannage à l'aide d'outils écotoxicologiques afin qu'elle serve de guide à l'industrie du tannage et aux autorités dans leurs efforts pour établir de meilleures pratiques de conservation et des paramètres de contrôle des effluents, qui sont actuellement absents de législation environnementale nationale. La concentration admissible de chlorures a été déterminée à l'aide d'essais biologiques avec des graines d'espèces de *Lactuca sativa* (laitue), qui ont été exposées à des effluents de trempage provenant de tanneries locales. L'espèce étudiée a été déterminée sur la base de tests de sensibilité au sulfate de zinc ( $ZnSO_4$ ) appliqués à trois espèces de laitues commerciales. La graine de laitue sélectionnée présentait un taux de germination supérieur à 90%, un coefficient de variation (CV) inférieur à 30% et une concentration inhibitrice médiane ( $Cl_{50}$ ) de 38,97 mg/L de  $Zn^{+2}$  avec un intervalle de confiance entre 19,30 mg/L et 58,65 mg/L de  $Zn^{+2}$ . La concentration guide admissible déterminée pour les chlorures dans les effluents de trempage à partir de la  $Cl_{50}$  était de 582,30 mg/L avec une valeur minimale de 258,45 mg/L et un maximum de 906,15 mg/L. De plus, en raison de la présence d'autres polluants dans les effluents de trempage, tels que les bactéricides, la matière organique, les enzymes et les sels ajoutés dans le processus de trempage, des essais avec des solutions synthétiques de chlorure de sodium aux mêmes concentrations des effluents de trempage ont été effectués, pour évaluer l'effet produit par ces substances.

**MOTS CLÉS :** valorisation des déchets, copeaux de cuir chromé, hydrolyse alcaline, procédé de tannages

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## INTRODUCTION

The tanning industry generates pollutants with significant dangerous effects on the environment because traditional leather processing procedures involve large amounts of chemicals and water throughout their different stages [1, 2]. In the beamhouse operations, the initial stage, more water is needed than in any of the other stages because the skins that have previously gone through a conservation process have to be re-hydrated; this conservation is carried out with layers of salt (sodium chloride) to avoid its natural decomposition [3, 4] and consequently the effluents generated in this stage presents large amounts of sodium chloride (NaCl) which, if spilled into the environment, can cause damage to the soil and even impact waterbodies, altering the normal development of the living organisms that inhabit them [2].

Different studies had been carried out to evaluate the toxicity of tannery effluents; Hussain *et al.* [5] analyzed the effect of tannery effluents on seeds germination and growth of two sunflower cultivars in Pakistan, their results showed that the raw effluent caused the reduction in biomass accumulation and reproductive growth of sunflower cultivars, and that one of them was more resistant to lower concentrations, but due to the presence of chemicals, the effluents are not suitable for inclusion in irrigation system. Calheiros *et al.* [6] tested tannery effluents with high salinity treated by an activated sludge system on the germination and seedling growth of red clover (*Trifolium pretense*), where growth was inhibited by concentrations over 25% and undiluted effluent caused a complete germination inhibition. Kohli and Malaviya [7] evaluated the impact of tannery effluents with high salinity (45.97 ppm) at different concentrations on the germination pattern of ten varieties of wheat (*Triticum aestivum*), where some varieties exhibited more tolerance at lower concentrations of tannery effluents, showing a potential to grow in tannery effluents contaminated soils.

Currently, Peru has environmental legislation for the regulation of physical and chemical parameters in the effluents generated by the tanning industry. However, despite the harmful consequences that high concentrations of chlorides on waterbodies can generate

[8], this parameter is not being regulated, and when a tannery treats its effluents, they don't know how much this parameter must be reduced to establish its treatment level. One of the methods used for establishing maximum allowable concentrations is the use of bioassays [9, 10], where the toxicity of an effluent sample is tested by measuring its effect on the growth of bioindicator organisms; in view of this, the present study aimed at proposing a maximum allowable concentration of chlorides, supported on the basis of toxicity bioassays with seeds of *Lactuca sativa* species exposed to local tannery effluents to determine the half maximal inhibitory concentration (IC50) [11, 12]. Likewise, the toxicity of synthetic samples of sodium chloride was evaluated to determine the effect of other substances from the soaking process. The analysis of the bioassay results was done by the statistical complement REAL STATISTICS, ANOVA tests and multiple comparisons.

## EXPERIMENTAL

### Materials and Methods

#### Sample

The tanning effluents used in this research corresponded to those generated in the soaking phase of the tanning processes of local tanneries in the city of Lima. The samples were kept at a temperature below 4°C, immediately after being taken.

#### Determination of Chloride Concentration in Effluents

The determination of the concentration of chlorides in mg/L in the soaking effluent was carried out in a laboratory accredited by INACAL using the EPA 300.0 method. Rev. 2.1:1993. Determination of Inorganic Anions by Ion Chromatography.

#### Toxicity Test

The toxicity tests were carried out in the R&D Laboratory of the Center for Productive Innovation and Technology Transfer of the Leather, Footwear and Related Industries (CITEccal Lima) and included the application of sensitivity tests for the selection of the test

species from commercial *Lactuca sativa* seeds [11, 13, 14], the toxicity analysis of soaking effluents and synthetic samples of sodium chloride with the selected seed.

The chemical reagents used were sodium bicarbonate for analysis ACS, Reag. Ph Eur MERCK, magnesium sulfate heptahydrate for analysis EMSURE® ACS, Reag. Ph Eur MERCK, potassium chloride for analysis Reag. Ph Eur SCHARLAU for the preparation of the reconstituted water. Anhydrous zinc sulfate, brand name, for the sensitivity test. As for the equipment, an analytical balance AND, model GF-1000 was used; an oven JP SELECTA, model DRY - BIG and an incubator VELP, model FTC 90I; all calibrated. In all procedures, reconstituted water was used and the materials used were washed with neutral detergent, EXTRAN, rinsed with tap water and distilled water. The volumetric glass materials were dried in an oven at a temperature not exceeding 50°C and the petri dishes were dried at a temperature of 100°C to sterilize them.

The reconstituted water was prepared with distilled water and had a composition of 192 mg/L of sodium bicarbonate, 120 mg/L of anhydrous magnesium sulfate and 8 mg/L of potassium chloride. The stock solution of zinc sulfate had a concentration of 500 mg/L from which the following concentrations were obtained by dilution with reconstituted water: 8.15 mg/L, 16.30 mg/L, 32.40 mg/L, 48.89 mg/L and 65.19 mg/L. The synthetic samples of sodium chloride were prepared from a 28.57 g/L chloride stock solution using reconstituted water as the solvent.

The selection of the species for the toxicity tests was made from the study of red lettuce seeds from the Hydroponics Unit of the National Agrarian University La Molina (UNALM), romaine lettuce seeds from the bio garden of the UNALM and long light green romaine lettuce seeds, butter variety, from BATLLE S.A. 08750 Molins de Rei, Barcelona, Spain. Seeds were exposed to reconstituted water and to the diluted concentrations of the ZnSO<sub>4</sub> stock solution, being determined for each of them the germination

percentage, the coefficient of variation between the replicates and the IC<sub>50</sub>.

After the selection of the test organism, the IC<sub>50</sub> of the tanning effluents was determined by exposing them to tanning effluent dilutions corresponding to 1%, 3%, 10%, 30% and 100% prepared with reconstituted water. The exposure of the seed to the synthetic sodium chloride solution was made from dilutions of the stock solution corresponding to 285.7 mg/L, 857.1 mg/L, 2857.1 mg/L, 8571.4 mg/L, 28 571, 4 mg/L.

The procedure of exposing the seeds to reconstituted water, stock solution dilutions and effluent dilutions was initiated by placing filter paper discs on the plates, then using a pipette to saturate each of the plates with 2 mL of the solutions to prevent air bubbles. Later, using a tweezer, 20 seeds were carefully placed in each plate, leaving enough space between the seeds to allow the elongation of the roots. The plates were then covered and placed in plastic bags to prevent them from losing humidity, and finally they were placed in the incubator for 120 hours (5 days) at a temperature of 22 ± 2°C. Each concentration or dilution was worked in triplicate [10, 15, 16].

For all cases the final evaluation consisted of comparing the effects generated in the test organisms exposed to the sample (zinc sulphate, soaking effluent and synthetic sodium chloride sample) with the response of the exposed organisms to the negative control (reconstituted water) under the same test conditions. Once the exposure period was completed (120 hours), the effect on germination and elongation of the radicle was quantified. Samples with necrosis, fungi or other aspects that may have interfered with germination and growth were observed. Seed elongation was performed by measuring root growth.

The germination percentage (GP) was determined by comparing the sown seeds with the germinated ones according to the following equation:

$$GP (\%) = \frac{\text{Number of total germinated seeds}}{\text{Total number of seeds tested}} \times 100 \quad (1)$$

The radicle growth inhibition percentage (RIGP) was estimated with the average elongation for each dilution with respect to the average elongation of the negative control.

$$RIGP (\%) = \left( \frac{\bar{x} \text{ elongation at dilution } \tau - \bar{x} \text{ control elongation}}{\bar{x} \text{ control elongation}} \right) \times 100 \quad (2)$$

The half maximal inhibitory concentration (IC<sub>50</sub>) is the concentration where the 50% of the test organisms are inhibited, in this study, where the elongation of the radicle is inhibited at 50%. The IC<sub>50</sub> was determined on the basis of

a fitting equation with a regression coefficient greater than 80% which is derived from the graph of the percentage inhibition vs. the diluted concentrations of the samples (dose-response curve). From the fitting equation:

$$y = ax + b, \text{ with } R^2 > 0.8$$

$$x = CI50 \text{ when } y = 50\%, \text{ then } CI50 = \frac{50 - b}{a} \quad (3)$$

Where:

x: Dilution

y: Radicle growth inhibition percentage (RIGP)

The coefficient of variation (CV) was

determined by determining the elongation standard deviation of a series of measurements between the average elongations.

$$CV (\%) = \left( \frac{\text{standard deviation of elongation data}}{\text{Average elongation}} \right) \times 100 \quad (4)$$

For the statistical analysis of the results, the analysis of the normality of the data for each dilution with the Shapiro Wilk test was considered. Likewise, by means of an ANOVA analysis and multiple comparison tests through Tukey Test and Dunnett Test, it was evaluated if there were significant differences between the sample dilutions of each toxicity test with the negative control and between the dilutions. This analysis was performed with the supplement Real Statistics for MS. Excel.

## RESULTS AND DISCUSSIONS

### *Evaluation of the Seeds*

Table 1 shows the germination results for the three species evaluated. The highest average percentage of germination in reconstituted water corresponds to light green romaine lettuce with a value of 93.89%. On the other hand the lowest average value for the coefficient of variation, also corresponds to this species and is equal to 24.53% according to the methodology of Acute Toxicity Test with seeds of *Lactuca sativa* L. [10, 17] it is considered that there is a good percentage of germination if this is greater or equal to 90% and an adequate CV if this is less or equal to 30%.

Table 1: Comparison between the results of percentage of germination, coefficient of variation and CI50 for the species of lettuce studied

Day	Red lettuce			Romaine lettuce			Light green romaine lettuce		
	% of germination	Root CV	IC <sub>50</sub> (mg/L)	% of germination	Root CV	IC <sub>50</sub> (mg/L)	% of germination	Root CV	IC <sub>50</sub> (mg/L)
01	70.00	33.38	95.87	55.00	48,35	82.79	91.67	29.07	121.77
02	85.00	44.33	80.13	66.67	63.88	88.94	98.33	22.40	71.83
02	65.00	34.24	45.63	88.33	38.95	42.57	91.67	22.12	56.03

In terms of species sensitivity analysis, long light green romaine lettuce has the highest sensitivity expressed as an average IC<sub>50</sub> of 48.07 mg/L. It should be noted that the correlation coefficients of the dose response curves were greater than 0.80 for all the trials, and in their determination the statistical conditions were met that there is normality in the data and significant differences between the concentrations or dilutions with the negative control and among themselves.

*Sensitivity Test - Positive Control*

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To establish the reproducibility of the applied methodology, sensitivity tests were performed with the selected species from the following Zn (II) concentrations: 8.15 mg/L, 16.30 mg/L, 32.40 mg/L, 48.89 mg/L and 65.19 mg/L. The IC<sub>50</sub> of Zn (II) determined from root growth assessment was 38.97 mg/L with a 95% confidence interval between 19.30 mg/L and 58.65 mg/L. Figure 1 shows the established control chart for this test.

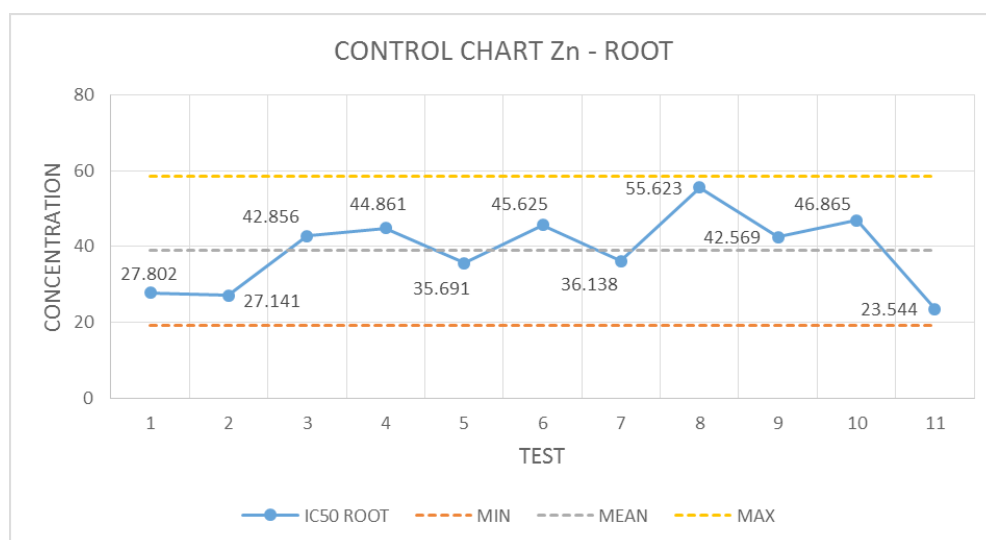


Figure 1. Control Chart Zn-Root

Bohórquez P. and Campos C. [16] determined an IC<sub>50</sub> for *L. sativa* of 24.48 mg/L of Zn<sup>+2</sup> with 95% confidence interval between 14.60 mg/L and 34.36 mg/L.

*Toxicity Tests on Soaking Effluents and Proposal of a Permissible Value for Chlorides in Tanning Effluents*

Table 2 presents the results of the chloride concentration in soaking effluents from tanneries in Metropolitan Lima as well as the respective IC<sub>50</sub> obtained in the toxicity tests. On average, an IC<sub>50</sub> of 582.30 mg/L and a range with a minimum value of 258.45 mg/L and a maximum value of 906.15 mg/L are determined. In order to determine the admissible concentration to be proposed, the safety factor or assessment factor approach was considered. This approach is applied to ensure the protection of the

most sensitive organisms and is inversely proportional to the quantity and quality of the toxicity data available. However, when comparing the IC<sub>50</sub> value obtained with the value of the Environmental Quality Standards for water in national legislation, which considers a value of 500 mg/L of chlorides for irrigation of vegetables and animal beverages (category 3) and with the value considered by international legislation for the case of Bogotá, which establishes a maximum value of chlorides in tanning effluents of 3,000 mg/L, the conclusion was reached that the value of 582.30 mg/L of chloride is sufficiently restrictive to ensure that tanning effluents that come into contact with a water body do not affect the normal development of the species, therefore the safety factor applied was 1.

Table 2: Chloride and IC<sub>50</sub> concentration of soaking effluents from tanneries in Metropolitan Lima

Sample code	Chloride concentration (mg/L)	IC <sub>50</sub> (mg/L)
PC-11	9 428.86	551.76
PC-12	16 578.55	477.08
PC-13	9 626.20	393.87
PC-15	4 082.95	695.29
PC-16	16 418.50	793.49

Table 3: IC<sub>50</sub> in synthetic samples of sodium chloride

Sample code	IC <sub>50</sub> (mg/L) for sodium chloride in synthetic samples	IC <sub>50</sub> (mg/L) for chloride in synthetic samples
1	1 040.59	631.23
2	1 688.22	1 024.08
3	1 016.98	616.91

Table 3 shows the IC<sub>50</sub> values determined for synthetic sodium chloride samples. It can be seen that these values are higher than those obtained with the soaking effluent samples, this is because the synthetic sample does not contain other substances that normally result from a soaking effluent. The chemicals used in the soaking process are: sodium hydroxide, sodium hypochlorite, surfactants, bactericides and enzymatic preparations, these by their nature add to the final effluent a higher toxicity, on the

other hand, besides salt, blood and dirt adhered to the skin are dragged away. This difference in toxicity would evidence the effect of salt in its pure and mixed state, which was also observed by Lyu *et al.* [18] who performed toxicity tests on *Lactuca sativa L.* with pure compounds and industrial effluent from Soyo and Daejeon in Korea, determining that the Zn concentration required to inhibit root elongation in *L. sativa* by 50% (IC<sub>50</sub>) was higher in Daejeon effluent than pure Zn, concluding that Zn mixed with the

effluent is less toxic than in pure form. Therefore, for the acceptable concentration to be proposed to be valid for the tanning industry, the joint effect of the chloride immersed in the soaking effluent should be considered. On the other hand, Campagna-Fernandes, Marin, & Penha [12], determined that the effects of sodium chloride on root growth are detected from a concentration of 1,000 mg/L of NaCl, that is, at 606,655 mg/L of Cl<sup>-</sup>, values that coincide with those established in the present investigation.

## CONCLUSIONS

The species of *Lactuca sativa L.* that validates the toxicity test is the long light green romaine lettuce, because it has a high percentage of germination (more than 90 %) that is preserved in time, a low variability of root elongation and hypocotyl (CV < 30 %) and presents the lowest values of IC<sub>50</sub>, that is to say, it is the most sensitive among those evaluated. The IC<sub>50</sub> calculated by sensitivity tests with ZnSO<sub>4</sub> for *Lactuca sativa L.* is 38.97 mg/L of Zn<sup>+2</sup> with confidence interval between 19.30 mg/L and 58.65 mg/L of Zn<sup>+2</sup>. Analysis with synthetic samples of industrial sodium chloride led to the conclusion that chlorides have a more toxic effect if they are discharged to the environment as part of the steeping effluent than in their pure state, since the IC<sub>50</sub> values of chlorides in synthetic samples were higher than the IC<sub>50</sub> values of chlorides in steeping effluents.

The maximum allowable concentration of chlorides for soaking effluent determined in this work is 582.30 mg/L with a minimum value of 258.45 mg/L and a maximum of 906.15 mg/L. This value is the starting point for establishing a limit and minimizing the effects of chlorides when discharged into the environment. The reduction of the chloride levels in the soaking effluents, implies for the tanneries to implement source reduction measures, applying the Cleaner Production process focused on minimization and reuse, with the last alternative being to treat the effluent at the end of the process.

It is important to strengthen the present study with a greater number of samples, representative of the national tanneries, to validate the findings of the present investigation. For this purpose, a more in-depth study should be carried out to analyze samples of tanning effluents in the main areas where this industry is developed in Peru, which is not only Lima, but also Trujillo and Arequipa, and thus have a national scope. It is suggested that at the legislative level, the maximum permissible limit values for tanning effluents be updated to include a limit value for chlorides based on studies that consider the toxicity of the element, as is the case with the present research, and even promote research that analyzes toxicity with species of different trophic levels.

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