

1 A 3D ecotoxi-topological profile: using concentration-time-response surfaces to
2 show peroxidase activity in *Zea mays* (L.) exposed to aluminium or arsenic in
3 hydroponic conditions

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ABSTRACT

This study sought to use concentration-time-response surfaces to show the effects of exposure to toxic (semi-)metals on peroxidase activity in higher plants as a function of exposure-concentration and exposure-time. Maize (*Zea mays* L.) seedlings (i.e., leaves and roots) were exposed to arsenic (as As^{3+}) or aluminium (as Al^{3+}) under hydroponic conditions, and their biomass and peroxidase enzyme responses were assessed at different concentration-time-exposures. The 3D ecotoxi-profile generated with these data showed two distinct regions: the first region is formed by exposures (i.e., points for time-concentration pairings) that were not statistically different from the results of the control points (i.e., zero toxicant concentration and all exposure-times), whereas the second region is formed by exposure pairings with results that were statistically different to those obtained from control pairings. Overall, the data show that enzyme activity increased over a shorter exposure-time when there was an increase in the exposure-concentration of the toxicant, which can be seen on a 3-D toxicity profile. We propose that quantitative relationship ratios from different assessed endpoints (e.g., biomass and enzyme activity) and enzymatic concentration-time-response surfaces could be helpful in the field of environmental-policy management.

Keywords: Ecotoxi-topology; Phytotoxicity; Maize; Arsenic; Aluminium; Peroxidase.

1. Introduction

Biological responses to toxins result from complex and dynamic cascades of events occurring between the initial exposure and the measured endpoints, and these responses may be reversible or irreversible, depending on the concentration-time-exposure (Byrne and Maher, 2019; Cho et al., 2016). Nevertheless, most experimental data generated in ecotoxicology studies assess biological responses over a specific exposure time under diverse toxicant concentrations, and the exposure time is represented only in the form of the measured endpoint (e.g., 72-h EC50) (Brooks et al., 2019; Mancini, 1983; OCDE, 1997; Van den Brink et al., 2019). It is possible that different conclusions on the effect of the same toxicant concentration could be derived for different exposure durations (Barata et al., 1999; Byrne and Maher, 2019; Hickey et al., 1991; Ragas, 2011). In this respect, concentration-time-response surfaces could provide a more complete presentation of the available data, and could reveal crucial information in environmental studies, including risk management, where the aim is to determine precise quantitative ecotoxicity values (Barata et al., 1999; Lee et al., 1995; Paumgarten, 1993; Sun et al., 1995).

Enzymatic activity responses can be easily measured over a short temporal scale, and peroxidase enzymatic activity in higher plants is a well-known experimental parameter for assessing the response of an organism exposed to a non-biodegradable toxicant (Jouili et al., 2011; Stegeman et al., 1992). Moreover, the study of biochemical parameters associated with more classical parameters (e.g., biomass yield) may help in organism quality assessment although it may be difficult to distinguish between adaptive responses from natural evolution and responses to temporary stress (Bijlsma and Loeschke, 2005; Calow and Berry, 1989; Forbes et al., 2006). Peroxidases are a group of oxidoreductase enzymes utilizing hydrogen peroxide as an electron acceptor

molecule. An example of their action in higher plants is the peroxidase transformation of phenolic compounds into phenolic radicals that are then polymerized to give rise to lignin (Polle et al., 1994; Tobimatsu and Schuetz, 2019). Peroxidase activity is very important for regulating plant growth, as this enzyme degrades, for example, the phytohormone indoleacetic acid (Rama-Rao et al., 1982). However, if this enzyme is used as a biomarker in environmental studies, it is important to remember that this class of enzymes is very susceptible to variations in environmental conditions, and is implicated in the oxidation of reactive oxygen species, and thereby help to avoid the damage resulting from oxidative stress (Bela et al., 2015; Gaspar et al., 1982; Gill and Tuteja, 2010; Sies, 1991).

Anthropogenic emissions of (semi)metals into the natural environment from a variety of sources (e.g., metallurgy, agriculture, energy production, and waste disposal) can result in these elements being a stress-causing factor in plants and other living organisms, and they may disrupt entire ecosystems (Seregin and Ivanov, 2001; Verkleij and Schat, 1990). Arsenic is well-known to be one such plant-growth limiting contaminant, disrupting photosynthetic and respiratory systems and stimulating secondary metabolism (De Gara et al., 1993; Majumder et al., 2020; Panda et al., 2009). Aluminium phytotoxicity appears as a rapid inhibition of root elongation due to damage to root apex cells, with plants showing a reduced and atrophied root system as a consequence (Chandran et al., 2008; Awasthi et al., 2017). Cellular metabolic disturbance in roots reduces water and mineral uptake, affecting the physiology of the whole plant, which leads to an imbalance in ROS production entailing an oxidative stress process (Darkó et al., 2004; Sharma and Dubey, 2007).

In a general way, metal toxicity effects in plants include inhibition of the mitotic index (Gzyl et al., 2015), increased tissue permeability (Sen and Mondal, 1987), promotion of metal accumulation, increased energy expenditure to cope with high metal concentrations (Israr et al., 2006), reduced photosynthesis or chlorophyll-*a* synthesis (Dube et al., 2003), inhibition of root apex growth by inhibition of DNA synthesis (Liu and Jiang, 2001), disruption of enzymatic activity (Cakmak and Horst, 1991; Jaworska and Lemanowicz, 2019), and oxidative stress caused by reactive oxygen species (Jouili et al., 2011; AbdElgawad et al., 2020).

Within the ecological context of (semi-)metal phytotoxicity, the goal of this study was to use 3-D response surfaces to show peroxidase activity in the higher plant *Zea mays* (L.) as a function of time-concentration exposure to arsenic or aluminium ions while under hydroponic conditions. Using such 3D topological responses, a broader vision of the ecotoxicity profile can be obtained, which could be helpful in the field of environmental policy management. For example, sewage sludge can provide a large part of the nutrients required by plants, but aluminium coagulant-flocculant compounds used in wastewater treatment can be phytotoxic (Kluczka et al., 2017). Thus, it is necessary to understand the phytotoxic relationships between time and concentration responses of pollutants to evaluate the risks related to the application of sewage sludge to land used for non-human edible culture (e.g., maize used for animal nutrition).

2. Materials and methods

2.1. Contaminants

Sodium arsenite (NaAsO₂) and aluminium sulfate Al₂(SO₄)₃ (Anidrol, Diadema, SP, Brazil) were used as the experimental toxins in this study. In this study, the ranges

of concentrations used in the media solutions were based on Brazilian legislation (CONAMA resolution 357/2005). Preliminary assays were performed to determine the concentration ranges required to obtain concomitant biomass and biomarker responses. The specific tested concentrations were 0.0025, 0.005, 0.01, 0.02, and 0.04 mg $\text{As}^{3+}.\text{L}^{-1}$, and 0.025, 0.05, 0.1, 0.2, and 0.4 mg $\text{Al}^{3+}.\text{L}^{-1}$. The arsenic concentration of 0.04 mg $\text{As}^{3+}.\text{L}^{-1}$ and the aluminium concentration of 0.4 mg $\text{Al}^{3+}.\text{L}^{-1}$ were above the Brazilian legislated limits (0.033 mg $\text{As}^{3+}.\text{L}^{-1}$ and 0.2 mg $\text{Al}^{3+}.\text{L}^{-1}$) for Class 3 freshwater (supplied for human consumption, after conventional or advanced treatment) (CONAMA, 2005). Distilled water was used as a negative control in all exposure experiments.

2.2. *Plant species*

The seeds selected for the tests were obtained from genetically unmodified and pesticide-free plants of *Z. mays* purchased at the Organic Market in the city of Brumadinho (MG, Brazil). The tests were performed using only one ozone-sterilized seed batch, to eliminate possible problems with variations between different batches.

2.3. *Phytotoxicity tests in solution media*

Toxicant solution phytotoxicity tests were performed on 10-day-old *Z. mays* plantlets germinated in pots containing 1 kg of commercial autoclaved garden soil. Assays were performed according to the ISO (1995) guideline, with some slight modifications, i.e., fewer replicates per test series (three instead of four). The following is a summary of the adopted procedures.

Three series of tests were performed at different exposure times of 0, 20, 45, 90, 180, 360, 720, and 2880 minutes. For each series of test plants, three pots (disposable

hydrophobic plastic, 120 mL) were used for each test concentration and the controls. Immediately after preparing the test solutions, 38 *Z. mays* plantlets were added to each pot. Plants were placed in a culture room with a $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ daytime temperature, $17^{\circ}\text{C} \pm 2^{\circ}\text{C}$ night-time temperature, and 16:8 hour light/dark photoperiods with $82 \text{ mE m}^{-2} \text{ s}^{-1}$ lighting from fluorescent tubes. Biological parameters were analysed for all exposure times. For each time-analysis, four plants from each pot were harvested and separated into two parts, and the wet weights of the roots and aerial parts were immediately measured on an analytical balance. The total weight of the harvested plant parts (expressed in grams wet weight) was divided by the number of plants harvested from each pot. Half of the biomass was then used to measure the plant dry weight in a stove, while the other half was used to measure enzyme activity and protein.

2.4. Measured endpoints

2.4.1. Biomass

Biomass was calculated from the dry mass (as described above) in relation to the number of plants (g plant^{-1}).

2.4.2. Quantification of total soluble proteins

The protein content in the *Z. mays* leaf and root samples was determined using bovine serum albumin (BSA) as a standard (Bradford, 1976). The protocol was validated according to the linearity of the responses obtained from the samples on a calibration curve obtained using commercial BSA. Thus, a stock solution of the commercial protein (SIGMA, P-8000) was prepared by dissolving 100 mg of the protein in 100 mL of phosphate buffer (50 mM; pH 7.6). A dilution range of 6.25, 12.5, 25.0,

50.0, and 100.0 $\mu\text{g}\cdot 100\text{ }\mu\text{L}^{-1}$ was used to prepare the calibration curve used to express the results obtained from the samples. The mean linear regression equation for the three curves was: $Y = 0.0038 X + 0.0315$ ($R^2 = 0.9938$).

2.4.3. Peroxidase enzyme activity (EC 1.11.1.7)

Enzyme activity was measured using a slightly modified version of the published method (Byl et al., 1994). Briefly, 0.5 g of the fresh aerial and root parts were weighed, homogenized separately using a mortar and pestle, and maintained at 4°C in 4 mL of 50 mM potassium phosphate buffer (pH 7.6). The samples were centrifuged (3000 g, 10 minutes, 4°C) and the supernatants were stored at -20°C awaiting subsequent analysis. After defrosting, the supernatant (0.1 mL) was placed in a 1.5-mL glass cuvette, and 0.7 mL of solution A (prepared by adding 810 mg phenol to 25 mg 4-aminoantipyrine in 50 mL of distilled water) was added just before the assay. Then, 0.75 mL of solution B (prepared by adding 0.01% of hydrogen peroxide in 0.01 M Phosphate buffer, pH 7.1) was added to start the reaction, and the absorbance was monitored for 5 minutes at 510 nm. The measured variation in absorbance was reported according to the dry weight of the sample, and was compared with the protein content of each sample. The enzymatic unit of the peroxidase forms 1.0 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 and 20°C (equivalent to ~18 μM units per minute at 25 °C), and was defined by a calibration curve using horseradish POD (SIGMA, 10670U). The commercial enzyme stock solution (1067 EU mg^{-1}) was prepared by dissolving 10 mg of the enzyme in 10 mL of 0.01 M phosphate buffer (pH 7.1). A dilution range was used to prepare the calibration curve utilised to express the results obtained from the samples. The dilutions used to create the calibration curve were: 1/4, 1/6, 1/8, 1/10, 1/12, 1/14, and 1/16. The mean linear regression equation for the three curves was: $Y = -49.92 X + 13871$ ($R^2 =$

0.9992). The values obtained from the calibration curve were divided by the protein content of the sample (dry weight basis).

2.5. Graphical

The graphic drawings shown in this study were made with Origin software (OriginLab Corporation, Washington, USA).

2.6. Statistical analysis

Three test series were performed at three different periods (20-day intervals between the series). For each test series, data on enzyme activity, biomass, and protein content were generated in triplicate (three pots) for each treatment (or control; $n = 9$). After checking the data for normality (Shapiro–Wilk’s test) and homogeneity of variances (Hartley’s test), analysis of variance (ANOVA) was performed, followed by generation of the lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) values using Williams’ test ($p \leq 0.05$) (Williams, 1971). TOXSTAT 3.0 software (University of Wyoming, Laramie, WY, USA) was used for these calculations.

3. Results and discussion

It is difficult to predict how the toxicity of a specific concentration of a toxin varies as a function of the exposure duration. A possible method to alleviate this problem is to use a 3D topological profile of the measured endpoint response, such as enzyme activity. In this type of data representation, the relationships between time and concentration-exposure can be seen from different perspectives, as the representation

system allows for 360° rotation. Thus, in this phytotoxicity study, discussions of peroxidase activity are based on NOEC and LOEC values, which are useful for exploring more generic ecotoxicological situations (e.g., in a hypothesis test). In many environmental issues, the acquisition of accurate data (i.e., point estimation approach) can be problematic, as is the case where toxicity effects must be associated with different concentrations of toxins and different exposure durations. The main value of a hypothesis testing approach occurs in the initial stages of investigations, and it may not result in the most insightful metric for judging toxicity effects (OECD, 2006). Therefore, we created 3D ecotoxicological profiles showing the LOEC values for peroxidase activity and dry biomass measured in the leaves of maize plantlets exposed to different concentrations of arsenic over different durations (Table S1 – Supplementary information). Fig. 1 (panels A-D) shows different 3D profiles of peroxidase activity as a function of concentration-time-exposure. We found a $LOEC_{2880\text{ min}}$ of 0.04 mg.L^{-1} for dry biomass of *Z. mays* leaves, whereas for the peroxidase activity endpoint, the $LOEC_{720\text{ min}}$ value was 0.005 mg.L^{-1} . The shortest exposure time over which peroxidase showed increased activity was 90 minutes, with a 0.04 mg L^{-1} exposure concentration.

Figure 1

Two different regions are of particular interest in Fig. 1. The first region (the white region or NOEC region) is formed by exposures (i.e., points for time-concentration pairings) that were not statistically different from the results of the control points (i.e., zero toxicant concentration and all exposure-times), whereas the second region (the grey

region or LOEC region) is formed by exposure pairings with results that were statistically different to those obtained from control pairings.

The two profiles shown at the bottom of Fig. 1 represent diametrically opposed views of the exposure conditions. Thus, it is possible to obtain an ecotoxi-topological view of the time when a given concentration of the toxicant begins to cause significant adverse effects on the exposed maize plantlets. A more detailed analysis of Fig. 1 shows that at the point of the greatest exposure values (i.e., the longest exposure duration time and greatest toxicant concentration), peroxidase activity showed a decrease, just at the point where a significant decrease in leaf biomass began.

Quantitative sensitivity relationships (QSR) between biomarkers and classical endpoints have received little attention, despite their importance. These responses try to numerically link lower and higher levels of biological responses, for example, the LOEC value for enzyme activity variation and “catastrophic” EC50 or LC50 values. Thus, QSRs could be useful for regulatory purposes in the environmental management domain, because there are a lack of studies that could facilitate establishment of a numerical relationship range for chemical exposure among the responses observed at different levels of biological organization in the same or different species (Corrêa et al., 2006; Silva et al., 2013).

It is important to note that in our experiments, the quantitative relationships among the different assessed endpoints are not subject to medium interference, because distilled water will not generate different LOEC values; this is not the case with data obtained for soil exposure (as an example), where different soil properties will generate different ecotoxicity values (Martins et al., 2019). In our experiment, where *Z. mays*

leaves were exposed in solution to different arsenic concentrations and exposure-times, the LOEC dry biomass/enzyme activity ratio was 8.

The same endpoints (i.e., LOEC values for peroxidase activity and dry biomass) measured in the roots of maize plantlets exposed to arsenic are presented in Fig. 2 that shows the 3D profile of peroxidase activity as a function of concentration-time-exposure in four angles (panels A-D) (Table S2 – Supplementary information).

Figure 2

The data presented in Table S2 indicate that the $\text{LOEC}_{2880 \text{ min}}$ for the dry biomass of *Z. mays* roots exposed to As^{3+} was 0.04 mg L^{-1} . A $\text{LOEC}_{720 \text{ min}}$ of 0.0025 mg.L^{-1} was found for peroxidase activity in roots, while a LOEC of 0.04 mg.L^{-1} exposure-concentration was found after an exposure time of 45 minutes. In the case of *Z. mays* roots, the LOEC dry biomass/enzyme activity ratio was 16.

Again, it is possible to observe two different regions in Fig. 2, with these showing similar profiles to those observed in Fig. 1, including the profile of the point of greatest exposure values (i.e., the longest exposure duration and the greatest concentration), where peroxidase shows a tendency to decrease its activity, just at the point where a significant decrease in root biomass begins.

These similarities were expected, as both figures represent the same plant species exposed to the same conditions, despite the sensitivities of the two plant parts (i.e., leaves and roots) being different. Previous studies have also shown alterations in the activity of peroxidase enzyme due to the influence of different concentrations of arsenic in different plant species (Armendariz et al., 2016; Khan et al., 2009). A significant synergistic effect on antioxidant enzyme activity was noted in *Lemna minor*

(L.) when these macrophytes were exposed to different time-concentrations of As(III) and As(V) ions under hydroponic conditions (Duman et al., 2010).

Fig. 3 shows the results from the same experimental design described previously, but with the leaves of *Z. mays* exposed to different concentrations of aluminium solutions. Numerical data are presented in Table S3 (Supplementary information). The phytotoxicity data indicate a LOEC_{2880 min} for the dry biomass of *Z. mays* leaves exposed to an Al³⁺ concentration of 0.4 mg.L⁻¹. A LOEC_{720 min} of 0.025 mg.L⁻¹ was found for peroxidase activity in *Z. mays* leaves, while a LOEC of 0.4 mg.L⁻¹ was found after an exposure-time of 90 min. In the case of *Z. mays* leaves, the LOEC dry biomass/enzyme activity ratio was 8.

Fig. 3 shows a similar profile to the previous figures, despite it being for a different toxic element. The same observations made for maize leaves exposed to As³⁺ are valid for maize leaves exposed to Al³⁺.

Figure 3

Fig. 4 shows the results measured in roots of *Z. mays* exposed to aluminium under hydroponic conditions and numerical data are shown in Table S4 (Supplementary information). For biomass, the LOEC value increased significantly at an exposure-concentration of 0.4 mg L⁻¹ after an exposure-time of 2880 min. For peroxidase activity in *Z. mays* roots, a LOEC_{720 min} was found at a 0.02 mg.L⁻¹ concentration of aluminium, while the shortest duration over which peroxidase showed increased activity in roots

was 90 min at a 0.4 mg.L⁻¹ exposure-concentration. For *Z. mays* roots, the LOEC dry biomass/enzyme activity ratio was 16.

Figure 4

Fig. 4 profile is quite similar to Figs 1 – 3 and previous comments made also apply here. Comparison with Fig. 3 reveals that the significant responses observed in the roots occurred at lower aluminium concentrations than those observed in maize leaves.

In relation to the variation in peroxidase activity response, it is well-known that (semi-)metals generally promote the formation of reactive oxygen species, oxidative stress (AbdElgawad et al., 2020; Ahmed et al., 2018;; Tang et al., 2016), and consequently oxidative damage (Gill and Tuteja, 2010; Hassan et al., 2005; Tiryakioğlu et al., 2006), before they affect the biomass of plant species. However, oxidative damage could be counterbalanced by the anti-oxidative defence system (Foyer, 1993; Sies, 1991), and the equilibrium point between these processes can be seen at the limit points of Figs. 1–4 (i.e., near the longest duration and greatest concentration exposures), precisely where biomass begins to decrease significantly. An overview of the data pool measured in this study is shown in Table 1.

Table 1

If we compare sensitivity between the different parts of the plantlets for the same endpoints, the roots of *Z. mays* can be seen to be more sensitive to hydroponic solution

exposure than the leaves, with this being the case for both toxicants. In a similar manner, it was reported that shoots of Indian mustard (*Brassica juncea* L. Czern.) seemed to be less efficient than roots at scavenging reactive oxygen species, as indicated by different responses to Cd stress shown by peroxidase and catalase activities (Mohamed et al., 2012). A similar observation was made on the peroxidase response of roots and primary leaves of bean seedlings as a part of the defence mechanism against copper- and zinc-imposed oxidative stress (Cuypers et al., 2002).

The two regions of the 3D ecotoxi-topology plots shown in all figures (i.e., with and without statistical significance or NOEC and LOEC) are dependent on the level of significance used. In this sense, environmental toxicological studies relate statistical significance with biological relevance (or significance) of a toxic effect, but this does not always represent the real ecological consequence (e.g., in the case of reversible toxic effects). Therefore, in the 3D graphical interpretation shown above, we must bear in mind that the statistical NOEC/LOEC values are very dependent on the range of tested concentrations, generated data variability, and level of statistical significance applied in the analysis (Newman and Clements 2008). However, from a practical point of view, the management of pollution issues can be greatly enriched when 3D ecotoxi-topology profiles are used as a complementary ecotoxicity approach.

4. Conclusions

In this study, measured toxicity endpoints (i.e., dry biomass and peroxidase enzyme activity) in *Z. mays* showed different sensitivities, with biomass showing a response only at the highest exposure-concentration and highest exposure-duration, whereas peroxidase activity responses generally occurred at lower exposure-

concentrations and highest exposure-durations for both the toxicants As^{3+} and Al^{3+} . However, peroxidase also responded to lower exposure-durations at higher exposure-concentrations. The highest (semi-)metal exposure-concentration and highest exposure-duration produced a less intense increase in enzymatic activity, just at the point where a significant decrease in biomass was beginning. For peroxidase activity, a 3D ecotoxi-topological view can be obtained to indicate the time at which a given concentration of the toxicant begins to cause significant adverse effects on the exposed organism. These ecotoxi-topological views based on concentration-time-response surfaces can be used to predict ecotoxic concentration levels at different exposure durations, which can be very useful in setting regulatory goals to enhance, protect, and preserve ecosystems. However, this ecotoxi-topology approach must be considered as an approximate approach for now, because additional studies are necessary to improve and validate the methodology.

CRedit authorship contribution statement

Fernanda Engel: Investigation, Methodology; **Sylvie Cotel:** Formal analysis, Writing - original draft; **Cleder A. Somensi:** Investigation, Writing - review and editing; **Renan C. Testolin:** Methodology; Editing; **Rogério Corrêa:** Resources, Formal analysis; **Hela Toumi:** Instrumental analysis, Methodology; **Jean-François Férard:** Writing - review and editing, Supervision; **Claudemir M. Radetski:** Writing - review and editing, Supervision.

Declaration of competing interest

The authors have no conflicts of interest to declare in relation to the work described in this study.

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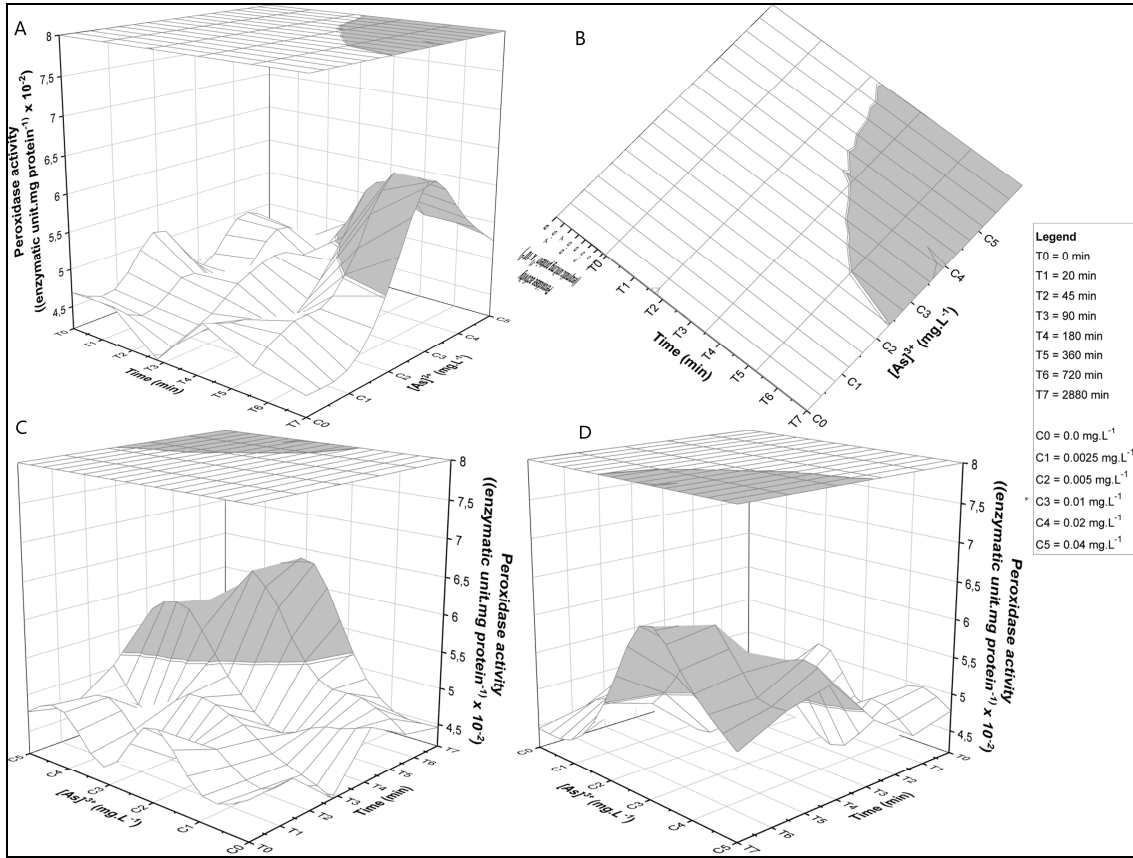


Fig. 1. Different 3D profiles of peroxidase activity as a function of concentration-time-exposures measured in the leaves of maize grown in arsenic (As^{3+}) solution. Coordinate references were: A (Azimuth= 310; Inclination = 15.8; Roll = 0.2); B (Azimuth= 319; Inclination = 81.0; Roll = 0.8); C (Azimuth= 220; Inclination = 15.8; Roll = 0.2); D (Azimuth= 40; Inclination = 15.8; Roll = 0.2).

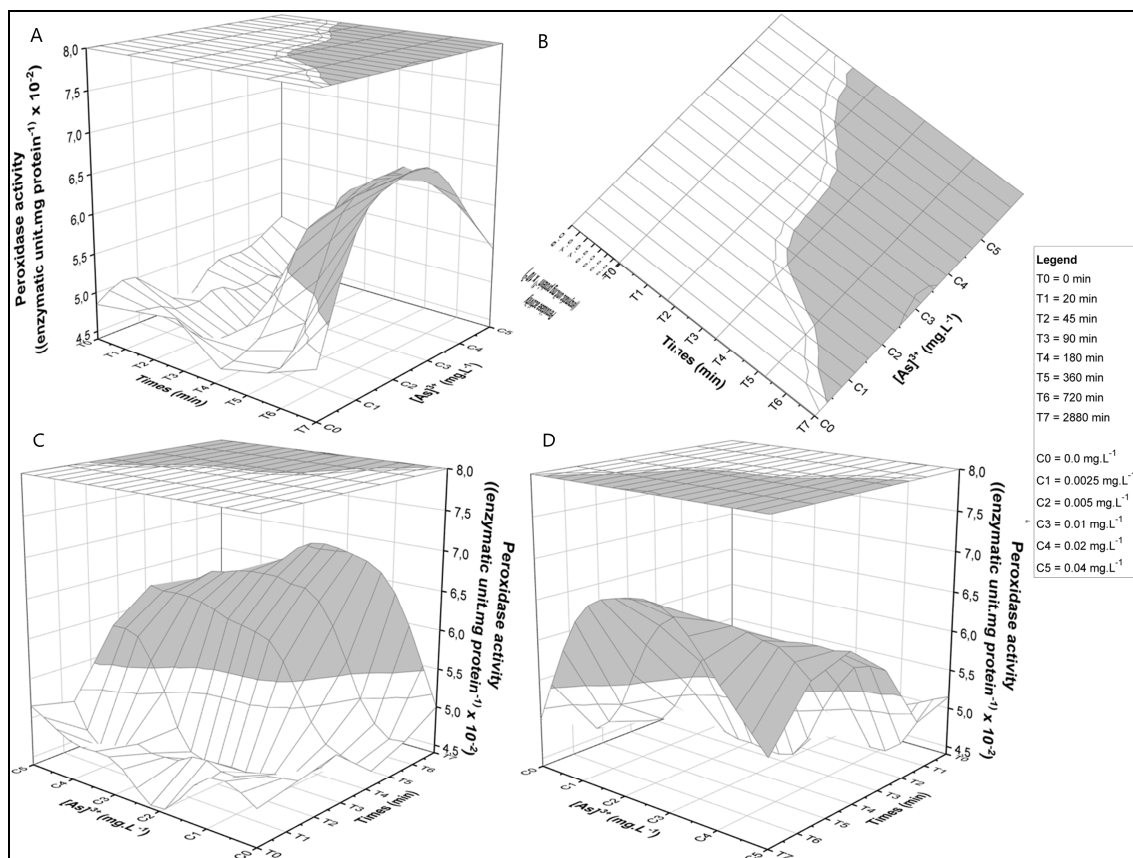


Fig. 2. Different 3D profiles of peroxidase activity as a function of concentration-time-exposures measured in roots of maize grown in arsenic (As^{3+}) solution. Coordinate references were: A (Azimuth= 310; Inclination = 15.8; Roll = 0.2); B (Azimuth= 319; Inclination = 81.0; Roll = 0.8); C (Azimuth= 220; Inclination = 15.8; Roll = 0.2); D (Azimuth= 40; Inclination = 15.8; Roll = 0.2).

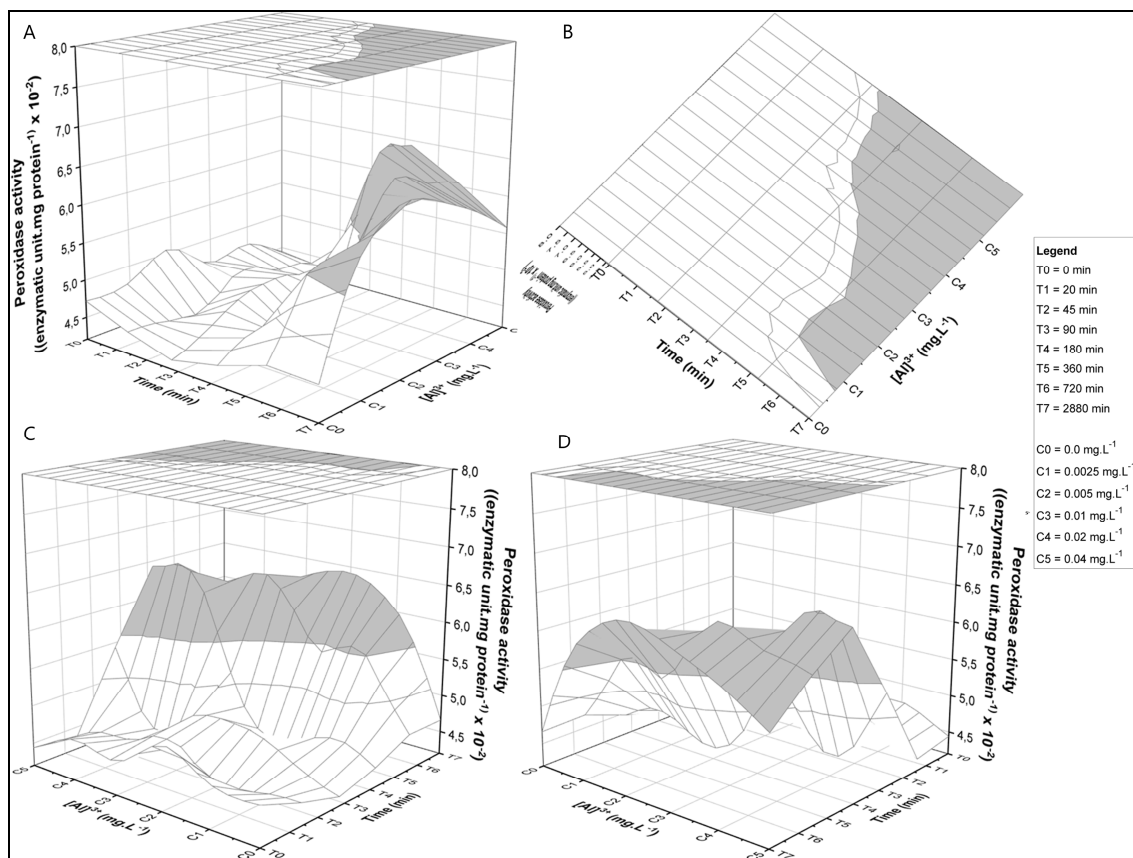


Fig. 3. Different 3D profiles of peroxidase activity measured in the leaves of *Zea mays* as a function of concentration-time-exposures to aluminium (Al³⁺) in solution. Coordinate references were: A (Azimuth= 310; Inclination = 15.8; Roll = 0.2); B (Azimuth= 319; Inclination = 81.0; Roll = 0.8); C (Azimuth= 220; Inclination = 15.8; Roll = 0.2); D (Azimuth= 40; Inclination = 15.8; Roll = 0.2).

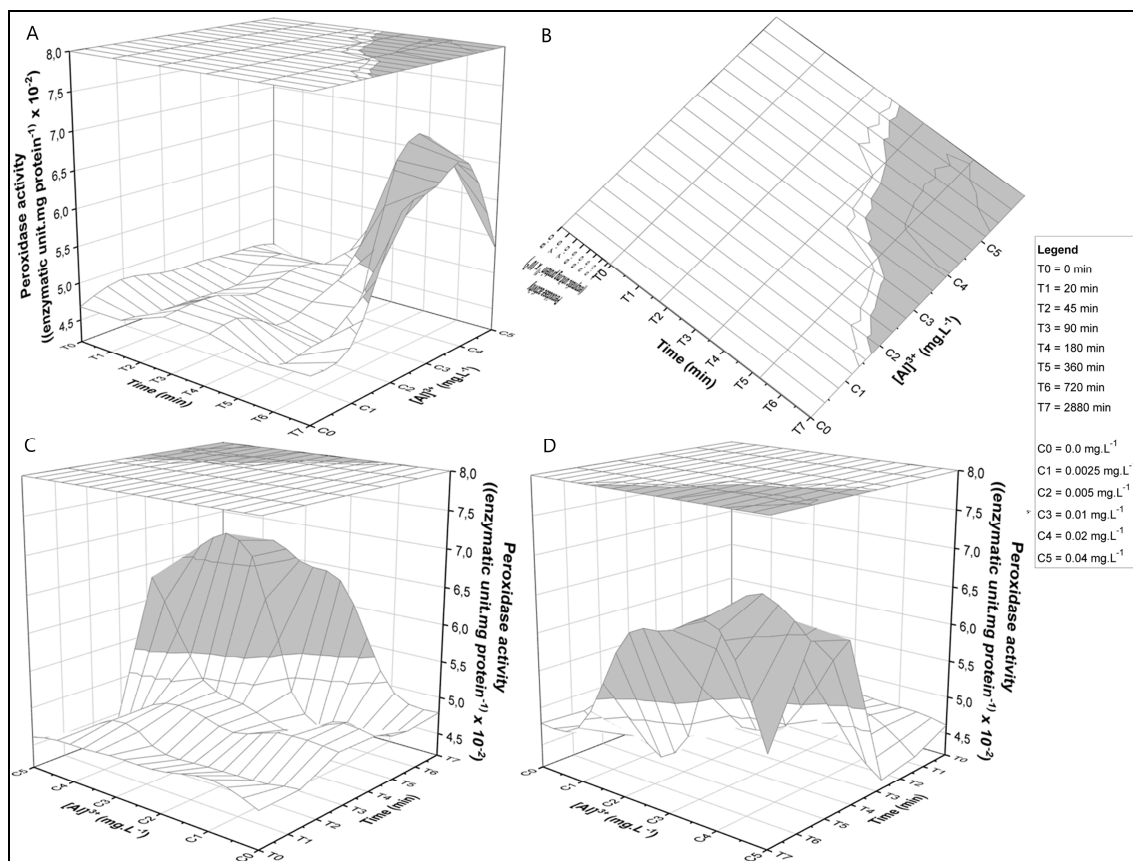


Fig. 4. Different 3D profiles of peroxidase activity measured in roots of *Zea mays* as a function of concentration-time-exposures in roots grown in aluminium (Al^{3+}) solution. Coordinate references were: A (Azimuth= 310; Inclination = 15.8; Roll = 0.2); B (Azimuth= 319; Inclination = 81.0; Roll = 0.8); C (Azimuth= 220; Inclination = 15.8; Roll = 0.2); D (Azimuth= 40; Inclination = 15.8; Roll = 0.2).

Table 1. Comparative values of LOEC for *Zea mays* for different contaminants and endpoints studied in the present work.

Toxicant	Compartment	LOEC for Biomass*	LOEC for Peroxidase activity as a function of time or concentration	
		Concentration (ppm)	Time-Concentration (min - ppm)	Concentration-Time (ppm - min)
Arsenic	Roots	0.04	90 - 0.02	0.002 - 720
	Leaves	0.04	180 - 0.02	0.005 - 720
Aluminium	Roots	0.4	90 - 0.4	0.02 - 720
	Leaves	0.4	180 - 0.4	0.05 - 2880

*2880 minute (48 hour) exposure-time.

