Toxicity assessment of the biogenic amines cadaverine and putrescine in aquatic organisms

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Abstract

Cemeteries are among the most polluting sources of groundwater and surface water in the world. Necro-leachate, a liquid released during the putrefaction of corpses, is the main culprit of such pollution. Among the compounds in this liquid are the potentially toxic amines cadaverine and putrescine, which are still poorly understood in terms of the environmental health risks they pose. This study evaluated the acute toxicity of cadaverine and putrescine, using efficient contamination bioindicators of aquatic environments. *Danio rerio* and *Daphnia magna* were exposed to both amines individually or in a mixture. Acute toxicity (LC_{50}) was then observed in both organisms after exposure to the amines individually. Cadaverine showed higher lethality for *D. magna* (LC_{50} – 9.5 mg. L^{-1}) and *D. rerio* (LC_{50} - 335.5 mg. L^{-1}) than did putrescine (LC_{50} - 36.7 mg. L^{-1} and LC_{50}- 452.6 mg. L^{-1}, respectively). In embryotoxicity tests with *D. rerio* (hatching rate and malformations), delayed hatching, vertebral column malformations, and pericardial edema were observed after exposure to an amine mixture. However, co-exposure to cadaverine and putrescine was not toxic to *D. magna*. The results of this study confirmed the efficiency of the bioindicators used to assess contaminants in cemeteries and raised awareness of the toxic potential of cadaveric decomposition by-products.

1. Introduction

One of the socio-environmental problems that most concern the world today is water pollution. The waste generated by a range of human actions contributes to this problem, waste that is currently considered the primary source of this contamination (Afangideh and Udokpoh 2022).

Within the last decade, the burial of bodies in cemeteries has aroused the interest of scientists, due to its polluting potential (Gonçalves et al. 2022). According to Batista et al. (2022), better planning is necessary regarding burial practices due to the risk of groundwater pollution from this type of activity. The risks pertaining to this type of pollution must be evaluated, as the degree of understanding with regard to the polluting loads of cemeteries remains insufficient (Franco et al. 2022). Studies carried out in several countries have found soil and groundwater around cemeteries contaminated with necro-leachate (Afangideh and Udokpoh 2022; Idehen 2020; Netto et al. 2021; Oliveira et al. 2022; Rodrigues and Pacheco 2003). These studies point to the need for better monitoring of cemetery areas and to assess, in detail, the toxic effects of by-products coming from cadaveric decomposition (Batista et al. 2022). Contamination from cemeteries has also been identified as a cause for concern by the World Health Organization (WHO) and the National Council for the Environment (CONAMA-Brazil), due to its impacts on the biological environment, soil, groundwater, and human health (Viegas et al. 2022).

Most necropolises were built without prior studies assessing the impacts on the environment and the local community (Neckel et al. 2021). According to Gonçalves et al. (2022), the increasing number of burials, especially during the SarsCov-2 pandemic, led to a worsening of the pollution scenario and to an alert about the potential ecotoxicological risks derived from the presence of necro-leachate in cemeteries (Gonçalves et al. 2022).

Cadaveric decomposition is the most relevant activity relating to the contamination of cemetery environments (Idehen and Ezenwa 2019; Zychowski and Bryndal 2015). During the decomposition of bodies, in addition to gases, a liquid called necro-leachate is generated (Batista et al. 2022). Necro-leachate is composed of water, mineral salts and organic substances, including the biogenic amines (BAs) cadaverine (CAD) and putrescine (PUT); these substances are considered toxic and responsible for the fetid odors that can be found in bodily fluids (Crisanto-Perrazo et al. 2022). Gómez et al. (2022) mention that the adult cadaver releases about 0.4–0.6 L/kg in body weight of necro-leachate. The authors also warn that the physical-chemical conditions of the soil can facilitate the percolation of this liquid and the subsequent contamination of the water table. The necro-leachate may also carry within it microbiological agents and chemical compounds used in life by the body (Afangideh and Udokpoh 2022); in addition to this, substances used in the embalming process and in the construction of coffins are also released, both potentially raising the level of contamination in cemeteries (Silva et al. 2020).

CAD and PUT are by-products naturally synthesized in living organisms by the decarboxylation of amino acids. CAD ($C_5H_{14}N_2$) is a by-product of the amino acid lysine, and PUT ($C_4H_{12}N_2$), of ornithine (Franco et al. 2022; Gonçalves et al. 2022). In endogenous concentrations, these amines participate in important physiological processes, such as the synthesis of proteins, hormones, and nucleic acids. CAD and PUT are also related to cell proliferation and the maintenance of blood pressure and body temperature (Abuhlega and Ali 2022). In addition, BAs influence membrane stability, in addition to acting as neurotransmitters.

However, when found in high concentrations, they can be toxic to different organisms, and pose a potential risk to the environment and public health (Dasa et al. 2022; Wójcik et al. 2020). The toxic level of BAs is difficult to determine, due to their specific characteristics, as well as their concomitant presence with other amines. However, a maximum allowable level of total BAs has been suggested at 750–900 mg/kg body weight (Abuhlega and Ali 2022).

The zebrafish (*Danio rerio*) has been used in several ecotoxicological studies. The species emerged as a vertebrate model in the investigation of changes in embryonic development and the genotoxic, mutagenic and carcinogenic potential of aquatic contaminants (Anifowoshe et al. 2022). The main advantages of this model include its short life cycle, rapid reproductive maturity, extrauterine development, high egg production and transparent embryos, which permits easy phenotypic observation (Lee et al. 2022). Furthermore, according to Howe et al. (2013), the species' genetic makeup is 70% orthologous with that of human beings.

The planktonic water flea, *Daphnia magna*, is also an important bioindicator of freshwater toxicity, mainly due to its sensitivity to pollutants, genetic stability, and representativeness in the food chain. In addition, it allows the assessment of the acute and chronic effects of different chemical substances (Belaid and Sbartai 2021). *D. magna* are able to filter large amounts of water and suspended particles, thus allowing a greater potential to be affected by the ingestion of xenobiotics when compared to other aquatic organisms (Lovern and Hart 2022).

Considering that the environmental impacts caused by necro-leachate are still poorly understood, the objective of this study was to evaluate the effects of the acute toxicity of BAs CAD and PUT, isolated, or associated with each other. The ecotoxicological study was carried out with two important bioindicators of environmental quality (*D. rerio* and *D. magna*); the LC_{50} in aquatic bioindicators and embryotoxicity in the fish was also evaluated. The results of this study have expanded our understanding with regard to BAs originating from cadaveric putrefaction, data that may serve to assist both in the elaboration of better norms for monitoring the effects of cemetery contaminants, as well as in the decision making of environmental agencies, regarding the regulation of installations and licensing of new cemeteries.

2. Material And Methods

2.1 Chemical substances evaluated

The BAs cadaverine (1,5-Diaminopentane, molecular formula $NH_2(CH_2)_5NH_2$, molecular weight 102.18 g/mol, CAS: 461-94-2 – purity ≥ 97%, Sigma Aldrich®) and putrescine (1,4-Butanediamine, molecular formula $C_4H_{12}N_2$, molecular weight 88.15 g/mol, CAS: 110-60-1 – purity ≥ 99%, Sigma Aldrich®) were evaluated individually and associated with each other.

2.2 Maintenance of the bioindicators

2.2.1 Danio rerio

Breeding-stage *D. rerio* (wild-type line) were kept under laboratory conditions appropriate to conductivity (400 ± 0.2 µS), temperature (28 ºC ± 0.2), and pH (7.0 ± 0.2) (Rack Hydrus, ZEB-40, Alesco®). Conductivity was maintained using Red Sea Salt®, and the pH controlled using Acid Buffer™ and Alkaline Buffer™ (Seachem® buffers). The zebrafish embryos were maintained in reconstituted water prepared according to the USEPA (2002). The procedures used in this study were approved by the Committee on Ethics in the Use of Animals (CEUA), from Embrapa Environment (CEUA-Embrapa Meio Ambiente, protocol nº 002/2022).

2.2.2 Daphnia magna

Individuals of the species *D. magna* were cultivated in aquariums measuring 40 x 25 x 15 cm, containing water reconstituted with nutrients prepared according to USEPA (2002). The reconstituted water in the aquariums was changed twice a week. The organisms were kept in an acclimatized room under a controlled temperature (20 ± 2º C) and fed daily with algae of the species *Raphidocelis subcapitata*.

2.3 Toxicity assays

2.3.1 Danio rerio: LC_{50} and embryos (FET test)

Preliminary tests were performed to determine the average lethal concentration for 50% of the exposed organisms (LC_{50} – mg. L^{-1}). The concentrations used to obtain the LC_{50} of CAD and PUT were 200, 400,

600, 800, and 1000 mg. L^{-1}. The bioassays with a BAs mixture were performed using the concentrations in Table 1.

The FET test was performed with four different combinations of these CAD and PUT amines. In this assay, 3-hour post-fertilization (hpf) embryos were exposed to the mixtures for 96 hours, according to OECD protocol 236 (2013). A negative control was carried out with the embryo medium (35 mg of $NaHCO_3$; 60 mg of $MgSO_4$; 4 mg of KCl; 60 mg of $CaSO_4 2H_2O$, in a final volume of 1L of water), and a positive control was performed with dichloroaniline (3,5-Dichloroaniline ≥ 98%, Sigma Aldrich®, D55792).

Exposure was performed in 24-well polystyrene plates (*n* = 24 organisms per treatment). Embryos were individually maintained in 2 mL of test solution, under a 14/10h light/dark cycle at 26 ± 0.2 ºC in a B.O.D incubator. Embryos and larvae were evaluated and photographed every 24h using a stereomicroscope (Model SMZ 2 LED, software Optika View Version 7.1.1.5, Optika®), to determine the occurrence of lethality and malformations (spine, tail, pigmentation, edema, formation of somites, absorption of the yolk sac, and heartbeat). The hatching rate was calculated as the relation between the successful hatching of embryos and the total number of embryos in each replica.

Table 1
Cadaverine (CAD) and putrescine (PUT) concentrations (LC_{50} mg. L^{-1}) used in the toxicity evaluations of mixtures of these biogenic amines for *Danio rerio* and *Daphnia magna*.

CAD and PUT Association	Concentrations mg. L^{-1}
	CAD and PUT, respectively
M1	1/10 LC_{50} + 1/10 LC_{50}
M2	LC_{50} + 1/10 LC_{50}
M3	1/10 LC_{50} + LC_{50}
M4	LC_{50} + LC_{50}

CAD: cadaverine, PUT: putrescine; M1. CAD and PUT association 1 tested; M2. CAD and PUT association 2 tested; M3. CAD and PUT association 3 tested; and M4. CAD and PUT association 4 tested, LC_{50}. Lethal concentration at 50%.

2.3.2 Daphnia magna acute toxicity test

D. magna neonates, which were younger than 24h old, were exposed for 48h to the following treatments: control (only reconstituted water), CAD, PUT and a mixture of both substances (CAD + PUT). Preliminary tests (0; 0.1; 1; 10 and 100 mg. L^{-1}) were performed to determine the mean lethal concentration for 50%

of the individuals (LC_{50} – mg. L^{-1}). After the preliminary tests, the following concentrations were those definitive to obtain the LC_{50}: 10; 16; 25.6; 40.96; 65.54; 104.86 mg. L^{-1}, respectively C1 up to C6. The concentrations for the BAs mixtures are shown in Table 1.

Organisms were distributed on 12 polystyrene well plates in a total volume of 5 mL of test solution per well. For each treatment, the study used one plate, each containing two organisms, thus totaling 24 replicates per treatment. After a period of 24 and 48 h of exposure, the organisms were evaluated and compared to the control group with regard to their immobility.

2.4 Statistical analysis

To determine the average lethal concentration (LC_{50}) of CAD and PUT, as well as their confidence intervals (95%), the “Probit Analysis” method was used. Data were evaluated using Statgraphics Centurion XVII software, Version 1.17.04 (Stat Point Technologies 2014). The data obtained from the BA mixture were evaluated using the STATISTICA 7.0 program for the analysis of normality (Shapiro-Wilk) and homogeneity of variance (Levene). For the analysis of means comparison between treatments and control, this study used the GraphPad Prism 5.0 software. Data were submitted to the parametric ANOVA (Analysis of Variance) test, followed by the Dunnet post-test ($p < 0.05$).

3. Results

3.1 Acute toxicity bioassays with zebrafish embryos

3.1.1 Lethal concentration (LC_{50}) calculation

The lethality of embryos exposed to the BAs CAD or PUT was determined by the clotting of the embryo or by the absence of heartbeats. Data from negative and positive controls validated all the tests performed (OECD, 2013). The observed lethality after 24h of exposure was < 10% for the control group and 100% for the positive control.

The obtained results showed that the lethality rate was directly proportional to the increase in CAD concentration. All tested concentrations induced lethality in zebrafish embryos. After 96h of exposure, the lowest concentration tested (200 mg. L^{-1}) induced 21% lethality, while the highest (1000 mg. L^{-1}) induced 100% lethality. From these results, the average lethal concentration (LC_{50} – 96h) of CAD was calculated (Fig. 1A), the value of which was 329.65 mg. L^{-1}.

Embryos exposed to putrescine presented lethality for all tested concentrations, with dose-response–dependent characteristics. After 96h of exposure, the concentration of 200 mg. L^{-1} caused 8% embryo lethality. The highest concentrations tested (C3–600 mg. L^{-1}; and C4–800 mg. L^{-1}), showed a lethality of 25% and 100%, respectively. The LC_{50} of PUT (96h exposure) was of 449.56 mg. L^{-1} (Fig. 1B).

Table 2

Mean lethal concentration LC_{50-10} (mg. L^{-1}) to different concentrations of CAD and PUT after 96h; 95% confidence interval (n = 24/concentration). Statistical test determined by the "Probit Analysis" module.

LC_{50-10} 96 h (mg. L^{-1})	CAD	PUT
LC_{50}	329.65	449.56
LC_{40}	282.36	388.81
LC_{30}	231.76	323.81
LC_{20}	172.54	247.74
LC_{10}	90.42	142.25

LC_{50-10}. Lethal concentration for 50 – 10% of individuals tested; CAD: cadaverine; PUT: putrescine.

The increase in lethality was significant in all treatments with embryos exposed to CAD and PUT concomitantly (Fig. 2). Effects were observed within the first 24h of exposure. Treatment M2 (LC_{50} CAD + 1/10 LC_{50} PUT) increased the lethality of individuals by 50% when compared to that of the control group. The highest concentration (M4), equivalent to LC_{50} CAD + LC_{50} PUT, showed a 96% lethality for embryos. According to the results observed for M2 (LC_{50} CAD + 1/10 LC_{50} PUT) and M3 (LC_{50} PUT + 1/10 LC_{50} CAD), it may be inferred that cadaverine was more toxic than putrescine.

3.1.2 Hatching delay and malformations

Table 3 displays data on the embryonic development stages (24–96 hpf) of the zebrafish. The control group organisms started hatching at 72 hpf. At the end of the 96-h exposure, the hatching rate of the control group was greater than 90%.

The different concentrations of CAD or PUT evaluated in this study did not induce changes in the hatching parameters of zebrafish eggs, when compared to those of the control group. However, every mixture of CAD and PUT caused morphological changes and a decrease in the egg hatching rate, except for M4, which had such a high lethality rate (> 90%) that it prevented the evaluation of these parameters (Fig. 3, 4, 5, 6).

All CAD + PUT mixtures evaluated in this study led to the delayed hatching of *D. rerio* eggs. The embryonic development stages (24–96 hpf) and zebrafish hatching delay images are shown in Fig. 3. Treatment M1, referring to the combination of lower concentrations (1/10 LC_{50} CAD + 1/10 LC_{50} PUT) had less influence on this reduction in the hatching rate by 10%, when compared to the control group. On the other

hand, the treatment that showed the greatest toxicity was a combination of the highest concentration of CAD with the lowest concentration of PUT (M2 - LC_{50} CAD + 1/10 LC_{50} PUT). This treatment reduced the hatching of embryos by 55% after 96h of exposure (Fig. 4).

The control group did not show significant morphological changes in any of the bioassays. The same occurred for the treatments carried out with isolated CAD and PUT, since none of the concentrations analyzed induced malformations in the embryonic zebrafish. The treatment performed with the M2 mixture (LC_{50} CAD + 1/10 LC_{50} PUT) showed the highest embryotoxicity. At this concentration, 21% of zebrafish embryos tested (FET test – 96h of exposure) were carriers of morphological changes: pericardial edema and spinal deficiency malformations were the most observed. The M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT) and M3 (LC_{50} PUT + 1/10 LC_{50} CAD) treatments induced malformations in 4%, including pericardial edema and spinal deficiency (Fig. 5). It was not possible to obtain malformation data for M4, as this concentration was toxic to *D. rerio*. The malformations found in the acute toxicity assessment for zebrafish exposed to the CAD and PUT mixture are displayed in Fig. 6.

3.2 Assessment of acute toxicity in *D. magna*

The results of the toxicity for *D. magna* exposed to the BAs CAD and PUT showed that CAD is highly toxic to the microcrustacean. All evaluated concentrations of these BAs were lethal for *D. magna*, when compared to that of the control group. The observed lethality for the control group was < 20%.

The lowest concentration tested (C1–10 mg. L^{-1}) caused a lethality of 65% of the individuals. Treatments C2 (16 mg. L^{-1}) and C3 (16 and 25.6 mg. L^{-1}) showed 55 and 65% lethality, respectively. The treatments with the highest concentrations (C4, C5 and C6) caused a lethality of 100% of the individuals. From these results, the mean lethal concentration (LC_{50}) of CAD was calculated after 48h of exposure, the value of which was 9.51 mg. L^{-1} (Fig. 7A).

The results obtained with PUT also showed lethality for *D. magna*. However, the acute toxicity of PUT was lower than that of CAD. Treatment C1 (10 mg. L^{-1}) resulted in 20% lethality, while C2 and C3 caused 10% lethality when compared to the control group. The treatments with the highest concentrations (C4, C5 and C6) caused 100, 65 and 70% lethality, respectively. The LC_{50} value (36.74 mg. L^{-1}) obtained in the 48h of exposure assays appears in Fig. 7B.

The lethality results obtained with *D. magna* for the treatments with the mixture of CAD and PUT amines were not statistically different from those of the control. Concentrations M1 and M3 induced 25% lethality; M2, 8%; M4, 29%; and the control, 17%. The results are shown in Fig. 8.

4. Discussion

Cemeteries are among the most silent and continuous sources of soil and groundwater contamination. The decomposition of human as well as of animal carcasses should be considered a natural phenomenon. However, during the decomposition process, toxic metabolites are produced that can pose a risk both to the physical environment (water and soil) and to the biology of the contaminated site, in addition to threatening human health (Zhou et al. 2021). The necro-leachate generated from this process can infiltrate the soil and contaminate groundwater, in addition to contributing to the increase of microbiological contamination from heavy metals and toxic organic pollutants (Franco et al. 2022).

From the results of the tests carried out with *D. rerio* and *D. magna*, exposed separately to BAs, it was possible to infer that CAD was more lethal than PUT, although the hatching delay values, and morphological changes, were not statistically different from those of the control group.

The assays performed with *D. rerio* exposed to the CAD and PUT mixtures show that these amines, when associated with each other, can increase the lethality rate, and induce delays in hatching and malformations in zebrafish larvae. Some authors also cite that CAD and PUT mixtures are more toxic than isolated amines, due to synergistic effects occurring between them, which potentiates the toxicity of other BAs such as histamine and tyramine (Donato et al. 2022; Saad et al. 2022). Among the morphological changes in zebrafish co-exposed to the BAs evaluated in this study, most notable are the pericardial edema and spinal deficiencies.

Pericardial edema is identified by increased fluid in the pericardium. This malformation is a cardiovascular abnormality resulting from both an increased permeability of blood vessels and the accumulation of contaminants in tissues (Vallim et al. 2022; Wu et al. 2019). According to Cooman et al. (2022), another common phenotype observed in embryotoxicity assays is spinal deformities after exposure to different xenobiotics. Studies showed that tail curvature may be associated with neuroinflammation caused by environmental pollutants (Iqubal et al. 2020; Massei et al. 2019).

The cytotoxic effect of CAD and PUT during zebrafish embryogenesis may be explained by its nucleophilic and polycationic nature at physiological pH. According to Pelka et al. (2017), the lipophilic characteristics, the electrical charge, and the structural conformation of a molecule, can all play an important role in the absorption of chemicals through the chorion of the zebrafish embryo. Furthermore, they may interact with negatively charged chemical species of DNA, RNA, and proteins (Kumar et al. 2021). This effect was reported by Del Rio et al. (2019) for HT29 intestinal cell cultures exposed to these BAs. The authors correlated the cytotoxicity of CAD and PUT with the possibility of amine interaction with phospholipids (of an anionic nature) and by the destabilization of cell membranes. This information may explain the appearance of malformations in the present study, but further studies are necessary to confirm this hypothesis.

The *D. magna* organisms showed greater sensitivity to an isolated BA when compared to that of the acute toxicity tests using zebrafish. CAD also showed a higher level of toxicity for this organism when

compared to PUT (CAD LC_{50} of 9.51 mg. L^{-1} and PUT LC_{50} of 36.74 mg. L^{-1}). However, the BA mixture did not significantly increase the lethality of exposed individuals at any of the evaluated treatments.

Although there are no scientific references regarding an assessment of the acute toxicity of these BAs in *D. magna*, studies by Spadotim (2015) carried out using water samples near a cemetery in the city of Limeira, São Paulo State, Brazil, confirmed the acute toxicity of these samples in relation to the microcrustaceans *D. magna* and *Ceriodaphnia dubia*. The authors also related this toxic effect on the test organisms to the high zinc concentration recorded in the evaluated waters. According to Wieczorek et al. (2021), CAD and PUT increased the lethality of the aphid *Cinara cupressi*, as well as significantly reducing the fecundity of these organisms in up to five generations.

According to Netto et al. (2021) and Oliveira et al. (2022), ecotoxicological tests performed with non-target organisms is an attractive strategy to estimate tolerable concentrations of toxic compounds present in the environment. Thus, the tests carried out here with the bioindicators *D. rerio* and *D. magna*, to evaluate the toxicity of BAs in cemetery environments, present important data that may assist in providing consistent information about the risks and environmental tolerances of this type of contamination.

A study presenting results from with the BAs CAD and PUT is unprecedented because although the toxicity of these amines in food is well known, little is known about the environmental impacts and effects on human health of BAs derived from putrefactive processes in cemeteries. Considering the high and continuous production of these amines in necropolises, the increase in burials in the last two years, resulting from the SARS-CoV-2 Pandemic Gonçalves et al. (2022), as well as the absence of management and treatment of effluents emitted by decomposing bodies, and the possibility of contamination of groundwater near cemeteries (Neckel et al. 2017), these amines should be further investigated, regarding their risks to the environment and human health. The originality of this study also lies in the type of evaluation carried out, with bioindicators and biomarkers of recognized efficiency for monitoring quality and/or environmental impacts (Belaid and Sbartai 2021; Anifowoshe et al. 2022; Lee et al. 2022; Howe et al. 2013) indicated by important environmental agencies around the world (e.g. OECD, USPEA, CONAMA-BR).

5. Conclusion

The data from the present study confirm the toxic potential of the BAs CAD and PUT on the aquatic organisms *D. rerio* and *D. magna*. Delays in hatching and malformations during the embryonic development of the fish, as well as an increased lethality for the water flea *D. magna* were observed. Although isolated BAs did not provide significant toxic effects, compared to the control group, CAD showed higher toxicity values than PUT. Mixtures of these substances have already triggered more worrying toxic effects. These data on the toxicity of BA mixtures reinforce the need for studies that assess impacts on the soil and groundwater around cemeteries, as these amines are released concomitantly during the cadaveric putrefaction process.

Given these observations, it is possible to conclude that the test organisms used in the study were efficient in assessing the toxicity of the BAs CAD and PUT. The proven toxicity of these amines highlights the requirement for more efficient environmental management in cemeteries, which should help to control necropolis pollution.

Declarations

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Competing Interests

The authors declare no competing of interests.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ana Paula Andrade Braga. José Henrique Vallim and Rodrigo Fernandes Castanha contributed to the performance of bioassays and data analysis. Vera Lucia Scherholz Salgado de Castro and Maria Aparecida Marin Morales acting in the supervision, conceptualization, and revision of the entire manuscript. All authors read and approved the final manuscript.

Ethical approval

All experimental procedures were approved by the Ethics Committee on the Use of Animals registered nº 001/2022. Embrapa Environment, Brazil.

References

1. Abuhlega, T.A., Ali, M.R (2022) Biogenic amines in fish: Prevention and reduction. J. Food Process. Preserv. 1–15. https://doi.org/10.1111/jfpp.16883
2. Afangideh, C.B., Udokpoh, U.U (2022) Environmental impact assessment of groundwater pollution within cemetery surroundings. Int. J. Eng. 19.
3. Anifowoshe, A.T., Roy, D., Dutta, S., Nongthomba, U (2022) Evaluation of cytogenotoxic potential and embryotoxicity of KRS-Cauvery River water in zebrafish (Danio rerio). Ecotoxicol. Environ. Saf. 233, 113320. https://doi.org/10.1016/j.ecoenv.2022.113320
4. Batista, F.S., Cruz, M.J.M., Anjos, J.Â.S.A. dos, Gonçalves, M.V.P., Porciúncula, D.C.L. da, Andrade, J.J. de S (2022) Geo-Environmental Assessment and Quality of Groundwater in the Complex of

Cemiteries: Quinta Dos Lázaros Salvador, Bahia, Brazil. Int. J. Hum. Sci. Res. 2, 2–25. https://doi.org/10.22533/at.ed.558282201042

5. Belaid, C., Sbartai, I (2021) Assessing the effects of Thiram to oxidative stress responses in a freshwater bioindicator cladoceran (Daphnia magna), Chemosphere. Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2020.128808
6. Cooman, T., Bergeron, S.A., Coltogirone, R., Horstick, E., Arroyo, L (2022) Evaluation of fentanyl toxicity and metabolism using a zebrafish model. J. Appl. Toxicol. 42, 706–714. https://doi.org/10.1002/jat.4253
7. Crisanto-Perrazo, T., Guayasamín-Vergara, J., Mayorga-Llerena, E., Sinde-Gonzalez, I., Vizuete-Freire, D., Toulkeridis, T., Gomez, G.F., Fierro-Naranjo, G (2022) Determination of Empirical Environmental Indices for the Location of Cemeteries—An Innovative Proposal for Worldwide Use. Sustain. 14. https://doi.org/10.3390/su14106284
8. Dasa, F., Bejo, W., Abdo, T (2022) Importance and Toxicity of Biogenic Amines in Fresh and Processed Foods. J. Food Technol. Nutr. Sci. 4, 1–8.
9. del Rio, B., Redruello, B., Linares, D.M., Ladero, V., Ruas-Madiedo, P., Fernandez, M., Martin, M.C., Alvarez, M.A (2019) The biogenic amines putrescine and cadaverine show in vitro cytotoxicity at concentrations that can be found in foods. Sci. Rep. 9, 1–7. https://doi.org/10.1038/s41598-018-36239-w
10. Donato, F., Greco, R., Carpentieri, C., Pittore, B., D'Orlando, C., Donato, A., Malara, N., Donato, G (2022) All ptomaines fault! The strange story of the cadaveric alkaloids, from forensic medicine to molecular biology passing through the asylum. Med. Hist. 6.
11. Franco, D.S., Georgin, J., Villarreal Campo, L.A., Mayoral, M.A., Goenaga, J.O., Fruto, C.M., Neckel, A., Oliveira, M.L., Ramos, C.G (2022) The environmental pollution caused by cemeteries and cremations: A review. Chemosphere 307, 136025. https://doi.org/10.1016/j.chemosphere.2022.136025
12. Gómez, F.G., Crisanto-Perrazo, T., Toulkeridis, T., Fierro-Naranjo, G., Guevara-García, P., Mayorga-Llerena, E., Vizuete-Freire, D., Salazar, E., Sinde-Gonzalez, I (2022) Proposal of an Initial Environmental Management and Land Use for Critical Cemeteries in Central Ecuador. Sustain. 14. https://doi.org/10.3390/su14031577
13. Gonçalves, L.R., Roberto, M.M., Braga, A.P.A., Barozzi, G.B., Canizela, G.S., de Souza Gigeck, L., de Souza, L.R., Marin-Morales, M.A (2022) Another casualty of the SARS-CoV-2 pandemic—the environmental impact. Environ. Sci. Pollut. Res. 29, 1696–1711. https://doi.org/10.1007/s11356-021-17098-x
14. Howe, K., Clark, M.D., Torroja, C.F. et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503. https://doi.org/10.1038/nature12111
15. Idehen, O (2020) A Comparative Investigation of Groundwater Contamination in Typical Dumpsites and Cemetery Using Ert and Physicochemical Analysis of Water in Benin Metropolis, Nigeria. J. Geosci. Environ. Prot. 08, 72–85. https://doi.org/10.4236/gep.2020.81005

16. Idehen, O., Ezenwa, I (2019) Influence of Third Cemetery Location on the Quality of Domestic and Groundwater Resources in Benin City, Nigeria. J. Appl. Sci. Environ. Manag. 23, 5. https://doi.org/10.4314/jasem.v23i1.1
17. Iqubal, A., Ahmed, M., Ahmad, S., Sahoo, C.R., Iqubal, M.K., Haque, S.E (2020) Environmental neurotoxic pollutants: review. Environ. Sci. Pollut. Res. 27, 41175–41198. https://doi.org/10.1007/s11356-020-10539-z
18. Kumar, S., Arockiaraj, M., Esokkiya, A., Sudalaimani, S., Hansda, S., Sivakumar, C., Sulaiman, Y., Khan, M.M., Giribabu, K (2021) Ion-Pair Facilitated Non-Enzymatic Electrochemical Sensing of Cadaverine and Putrescine. J. Electrochem. Soc. 168, 047505. https://doi.org/10.1149/1945-7111/abf263
19. Lee, Y.L., Shih, Y.S., Chen, Z.Y., Cheng, F.Y., Lu, J.Y., Wu, Y.H., Wang, Y.J (2022) Toxic Effects and Mechanisms of Silver and Zinc Oxide Nanoparticles on Zebrafish Embryos in Aquatic Ecosystems. Nanomaterials 12, 1–18. https://doi.org/10.3390/nano12040717
20. Lovern, S.B., Hart, R. Van (2022) Impact of oxytetracycline exposure on the digestive system microbiota of Daphnia magna. PLoS One 17, 1–8. https://doi.org/10.1371/journal.pone.0265944
21. Massei, R., Hollert, H., Krauss, M., von Tümpling, W., Weidauer, C., Haglund, P., Küster, E., Gallampois, C., Tysklind, M., Brack, W (2019) Toxicity and neurotoxicity profiling of contaminated sediments from Gulf of Bothnia (Sweden): a multi-endpoint assay with Zebrafish embryos. Environ. Sci. Eur. 31. https://doi.org/10.1186/s12302-019-0188-y
22. Neckel, A., Costa, C., Mario, D.N., Sabadin, C.E.S., Bodah, E.T (2017) Environmental damage and public health threat caused by cemeteries: a proposal of ideal cemeteries for the growing urban sprawl. urbe. Rev. Bras. Gestão Urbana. https://doi.org/10.1590/2175-3369.009.002.ao05
23. Neckel, A., Korcelski, C., Kujawa, H.A., Schaefer da Silva, I., Prezoto, F., Walker Amorin, A.L., Maculan, L.S., Gonçalves, A.C., Bodah, E.T., Bodah, B.W., Dotto, G.L., Silva, L.F.O (2021) Hazardous elements in the soil of urban cemeteries; constructive solutions aimed at sustainability. Chemosphere 262. https://doi.org/10.1016/j.chemosphere.2020.128248
24. Netto, L.G., Filho, W.M., Moreira, C.A., di Donato, F.T., Helene, L.P.I (2021) Delineation of necroleachate pathways using electrical resistivity tomography (ERT): Case study on a cemetery in Brazil. Environ. Challenges 5, 100344. https://doi.org/10.1016/j.envc.2021.100344
25. Oliveira, A.E. De, Saba, E.D., Saba, J.M., Saba, E.D., Saba, J.M., Mendes, T.A (2022) Groundwater Vulnerability and Cemetery Urbanization Areas Groundwater Vulnerability and Cemetery Urbanization Areas 0–25.
26. Pelka, K.E., Henn, K., Keck, A., Sapel, B., Braunbeck, T (2017) Size does matter – Determination of the critical molecular size for the uptake of chemicals across the chorion of zebrafish (Danio rerio) embryos. Aquat. Toxicol. 185, 1–10. https://doi.org/10.1016/j.aquatox.2016.12.015
27. Rauscher-Gabernig, E., Gabernig, R., Brueller, W., Grossgut, R., Bauer, F., Paulsen, P (2012) Dietary exposure assessment of putrescine and cadaverine and derivation of tolerable levels in selected foods consumed in Austria. Eur. Food Res. Technol. 235, 209–220. https://doi.org/10.1007/s00217-012-1748-1

28. Rodrigues, L., Pacheco, A (2003) Groundwater contamination from cemeteries cases of study. Environ. 2010 Situat. Perspect. Eur. Union 1–6.
29. Saad, M.A., Abd-Rabou, H.S., Elkhtab, E., Rayan, A.M., Abdeen, A., Abdelkader, A., Ibrahim, S.F., Hussien, H (2022) Occurrence of Toxic Biogenic Amines in Various Types of Soft and Hard Cheeses and Their Control by Bacillus polymyxa D05-1. Fermentation 8, 1–12. https://doi.org/10.3390/fermentation8070327
30. Silva, R.B.P. da, Campos, M.C.C., Silva, L.S., Filho, E.G. de B., Lima, A.F.L. de, Pinheiro, E.N., Cunha, J.M (2020) Concentration of Heavy Metals in Soils under Cemetery Occupation in Amazonas, Brazil. Soil Sediment Contam. 29, 192–208. https://doi.org/10.1080/15320383.2019.1696280
31. Spadotim, R.F (2015) Avaliação Ecotoxicológica e identificação da toxicidade no Ribeirão Pires, Limeira - SP.
32. Vallim, J.H., Clemente, Z., Castanha, R.F., do Espírito Santo Pereira, A., Campos, E.V.R., Assalin, M.R., Maurer-Morelli, C.V., Fraceto, L.F., de Castro, V.L.S.S (2022) Chitosan nanoparticles containing the insecticide dimethoate: A new approach in the reduction of harmful ecotoxicological effects. NanoImpact 27. https://doi.org/10.1016/j.impact.2022.100408
33. Viegas, C., Cervantes, R., Dias, M., Gomes, B., Pena, P., Carolino, E., Twarużek, M., Kosicki, R., Soszczyńska, E., Viegas, S., Caetano, L.A (2022) Six Feet under Microbiota: Microbiologic Contamination and Toxicity Profile in Three Urban Cemeteries from Lisbon, Portugal. Toxins (Basel). 14. https://doi.org/10.3390/toxins14050348
34. Wieczorek, K., Świątek, P., Durak, R (2021) Influence of selected biogenic amines on development and demographic parameters of a temperate population of Cinara (Cupressobium) cupressi (Hemiptera, Aphididae). Arthropod. Plant. Interact. 15, 583–593. https://doi.org/10.1007/s11829-021-09839-z
35. Wójcik, W., Łukasiewicz, M., Puppel, K (2020) Biogenic amines: formation, action and toxicity – a review. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.10928
36. Wu, Y., Zhang, Y., Chen, M., Yang, Q., Zhuang, S., Lv, L., Zuo, Z., Wang, C (2019) Exposure to low-level metalaxyl impacts the cardiac development and function of zebrafish embryos. J. Environ. Sci. (China) 85, 1–8. https://doi.org/10.1016/j.jes.2019.03.019
37. Zhou, R., Yu, Q., Li, T., Long, M., Wang, Y., Feng, T., Su, W., Yang, J., Li, H (2021) Carcass decomposition influences the metabolic profiles and enriches noxious metabolites in different water types by widely targeted metabolomics. Chemosphere 269, 129400. https://doi.org/10.1016/j.chemosphere.2020.129400
38. Zychowski, J., Bryndal, T (2015) Impact of cemeteries on groundwater contamination by bacteria and viruses - A review. J. Water Health 13, 285–301. https://doi.org/10.2166/wh.2014.119

Figures

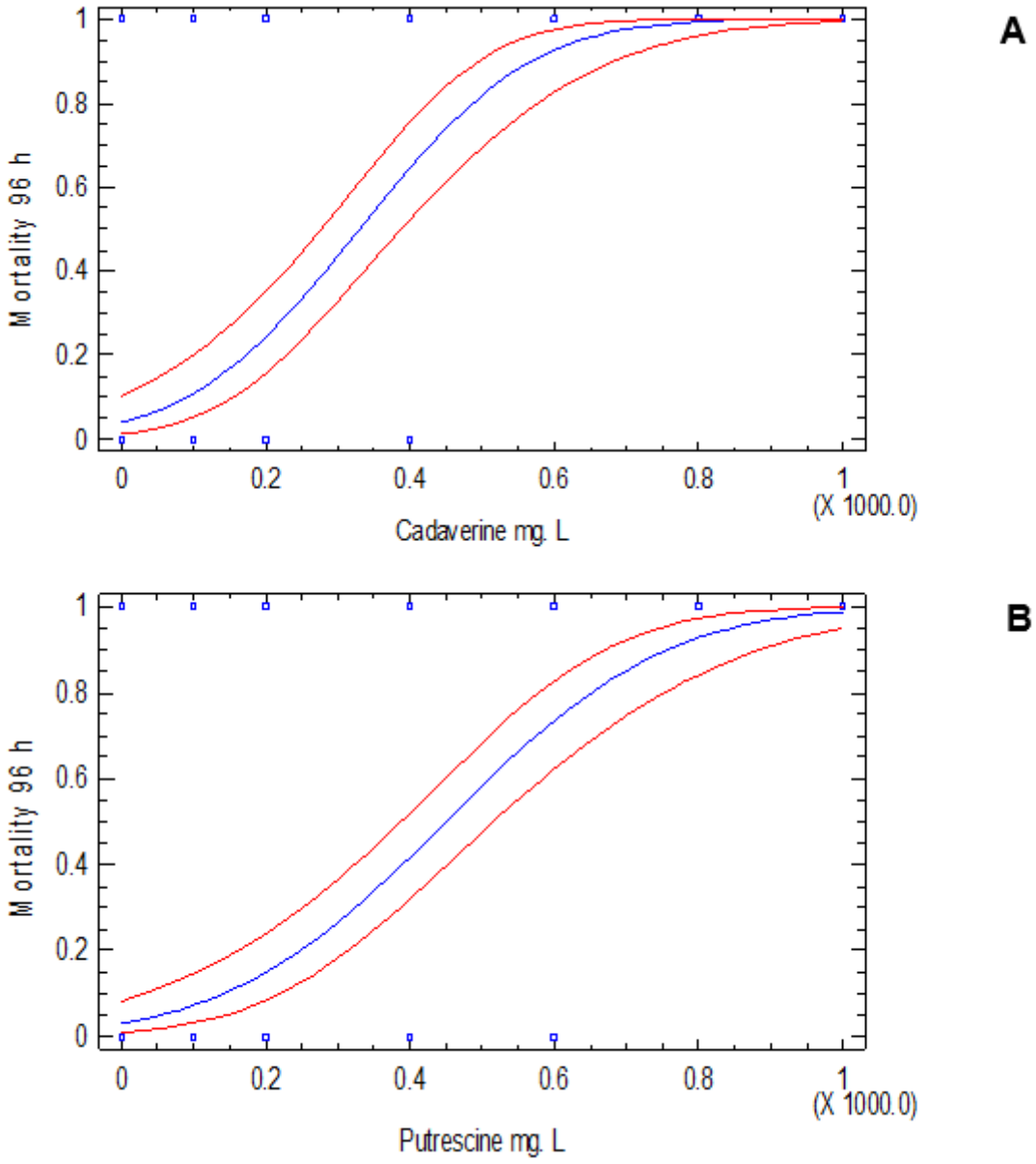

Figure 1

A) Regression analysis for the calculation of cadaverine LC_{50}. **B**) Regression analysis for the LC_{50} calculation of putrescine. Statistical test determined by the “Probit Analysis” module.

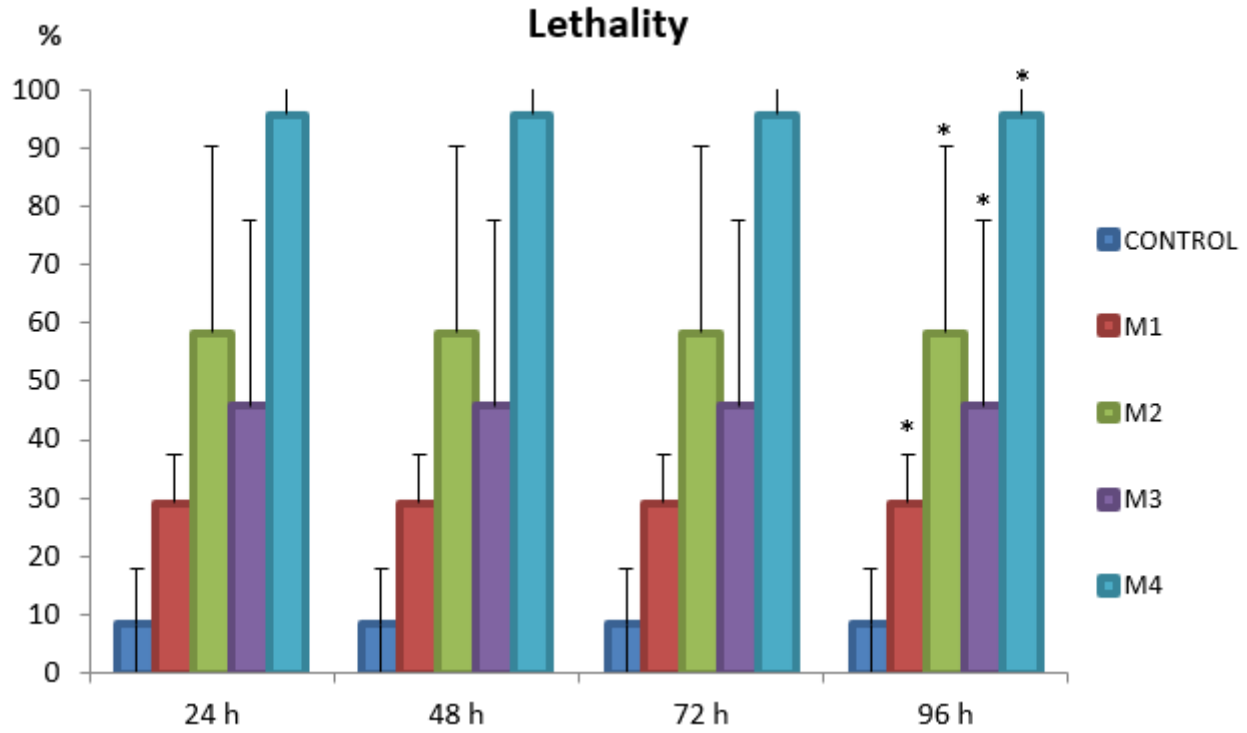

Figure 2

Lethality rate of zebrafish embryos exposed to cadaverine and putrescine mixture. M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT); M2 (LC_{50} CAD + 1/10 LC_{50} PUT); M3 (LC_{50} PUT + 1/10 LC_{50} CAD); M4 (LC_{50} CAD + LC_{50} PUT). ANOVA/Dunnett (* $p<0.05$).

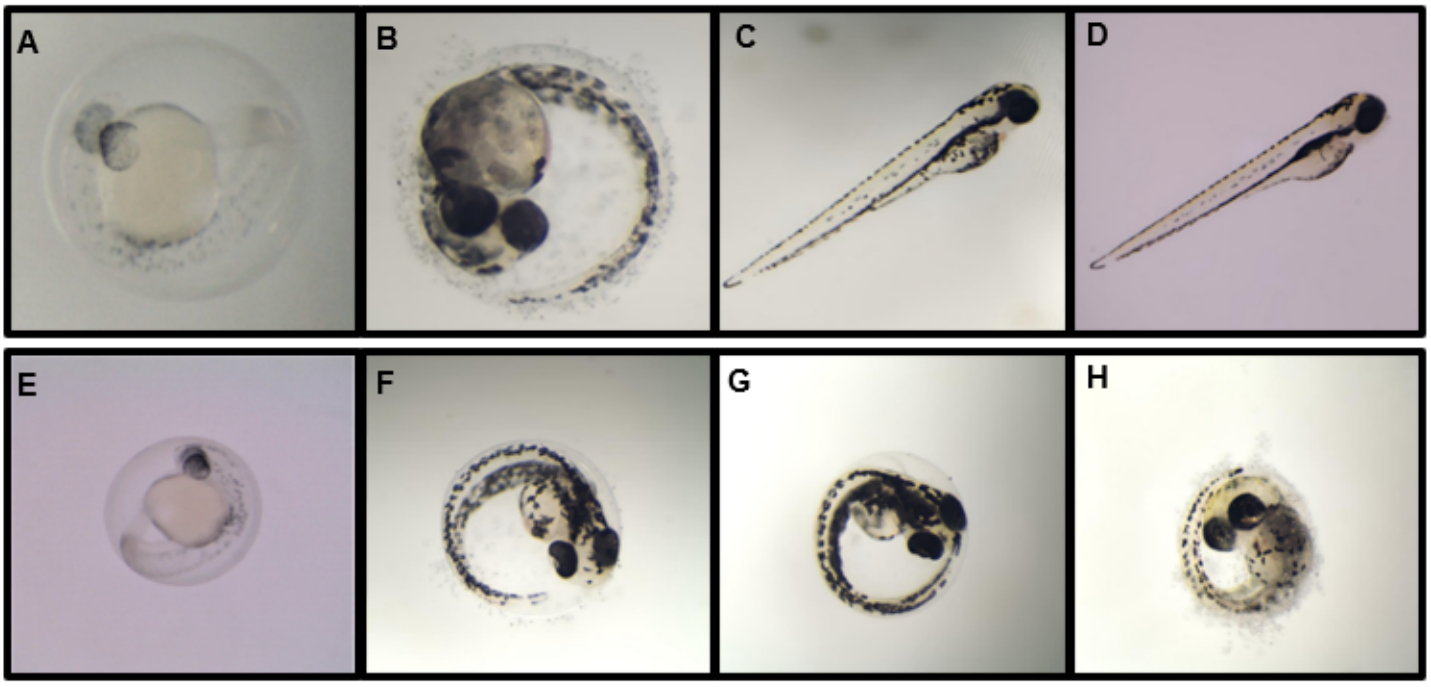

Figure 3

Embryonic development at different stages of zebrafish development (24 to 96 hpf). A-D: Standard development (control) to 24, 48, 72, and 96 hpf, respectively. E-H: Developmental delay observed in zebrafish embryos exposed to the mixture of CAD and PUT at 24 (E), 48 (F), 72 (G), and 96 hpf (H).

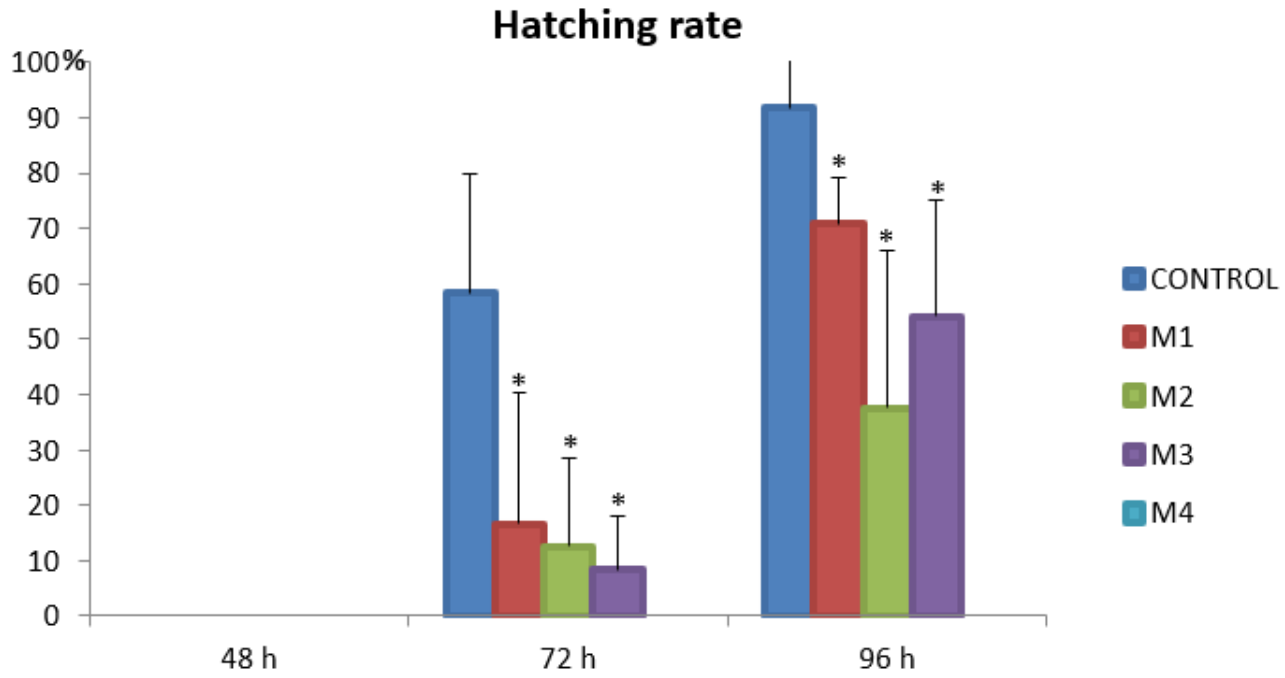

Figure 4

Hatching rate of zebrafish embryos exposed to a cadaverine and putrescine mixture. M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT); M2 (LC_{50} CAD + 1/10 LC_{50} PUT); M3 (LC_{50} PUT + 1/10 LC_{50} CAD); M4 (CL_{50} CAD + LC_{50} PUT). ANOVA/Dunnett (* $p<0.05$).

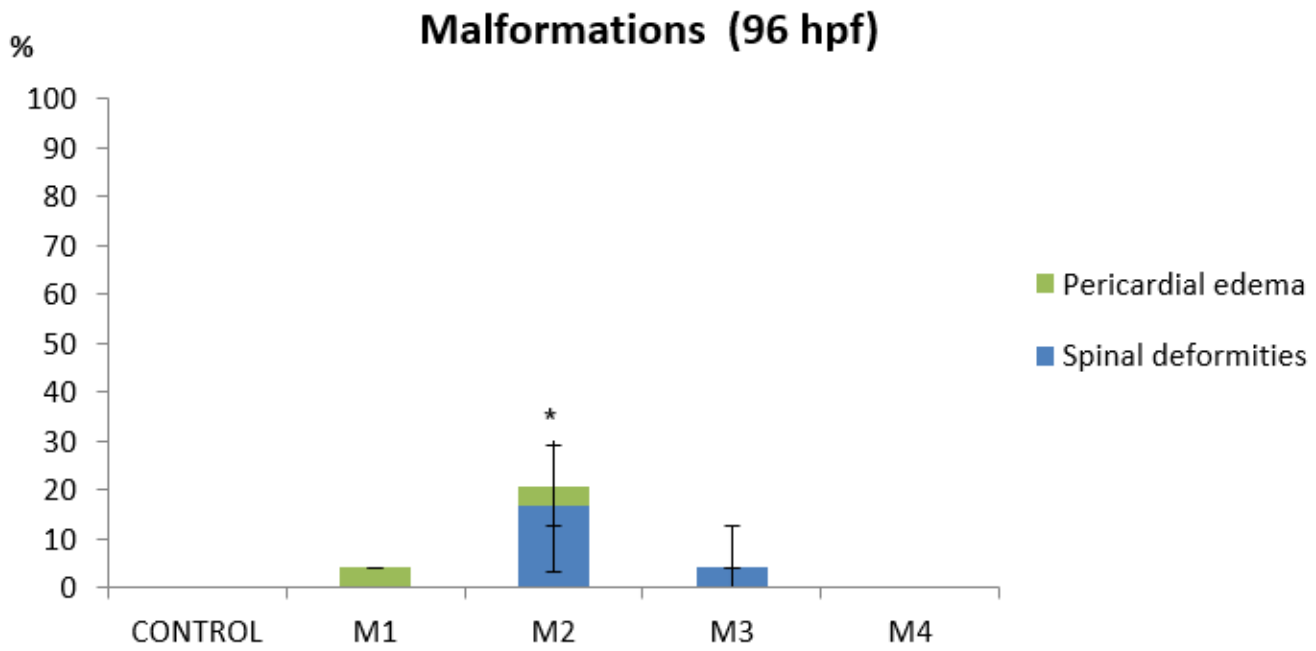

Figure 5

Malformations in zebrafish after 96h of exposure to different concentrations of cadaverine and putrescine mixtures. M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT); M2 (LC_{50} CAD + 1/10 LC_{50} PUT); M3 (LC_{50} PUT + 1/10 LC_{50} CAD); M4 (CL_{50} CAD + LC_{50} PUT). ANOVA/Dunnett ($p<0.05$).

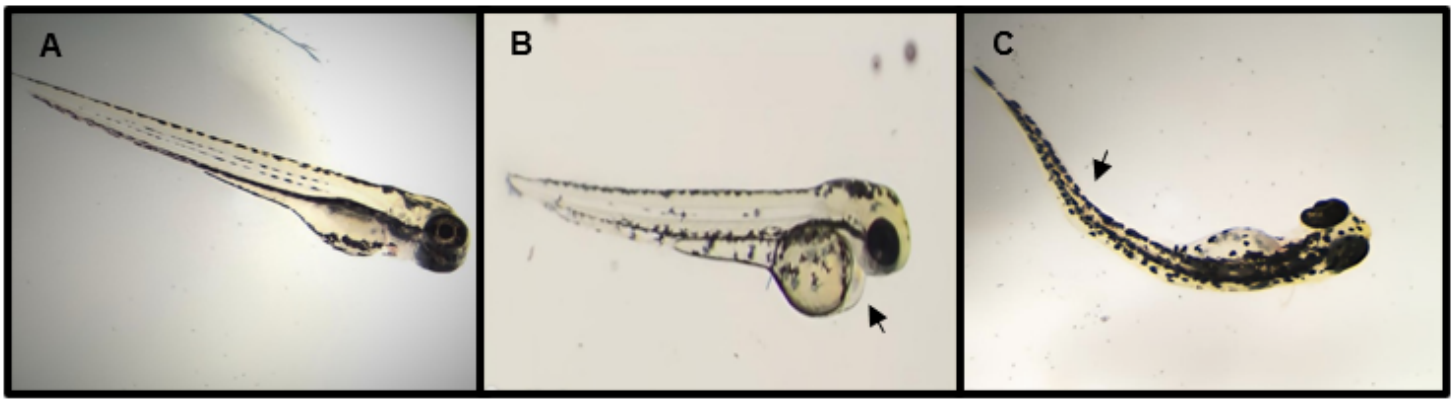

Figure 6

Morphological changes in zebrafish larvae after 96h of exposure to different concentrations of cadaverine and putrescine mixtures. M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT); M2 (LC_{50} CAD + 1/10 LC_{50} PUT); M3 (LC_{50} PUT + 1/10 LC_{50} CAD); M4 (CL_{50} CAD + LC_{50} PUT). ANOVA/Dunnett ($p<0.05$). **A**: control (without malformations). **B**: pericardial edema. **C**: spinal malformation.

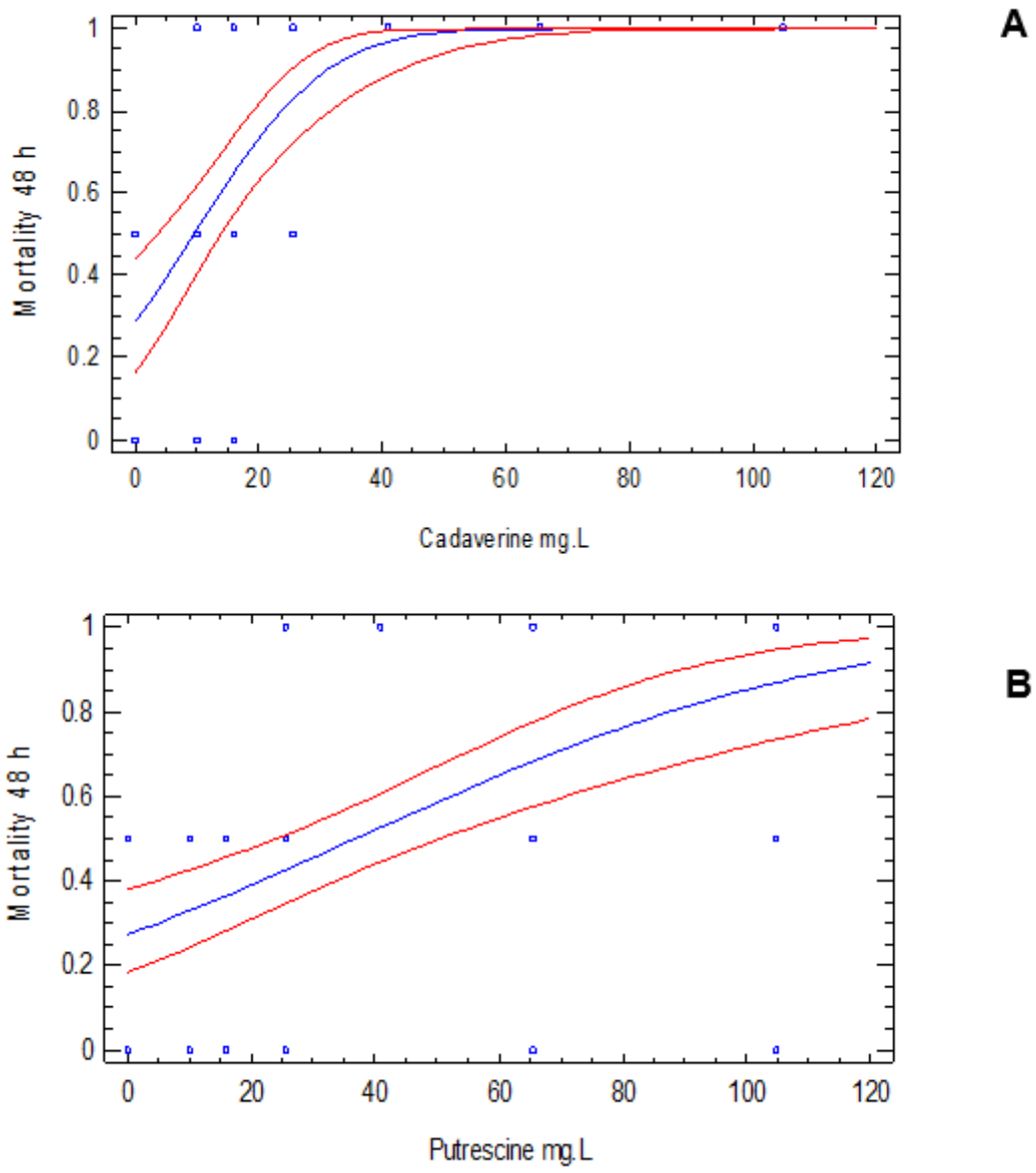

Figure 7

A. Regression analysis for the calculation of cadaverine LC_{50}. **B**. Regression analysis for calculation of the putrescine LC_{50}. Probit Analysis.

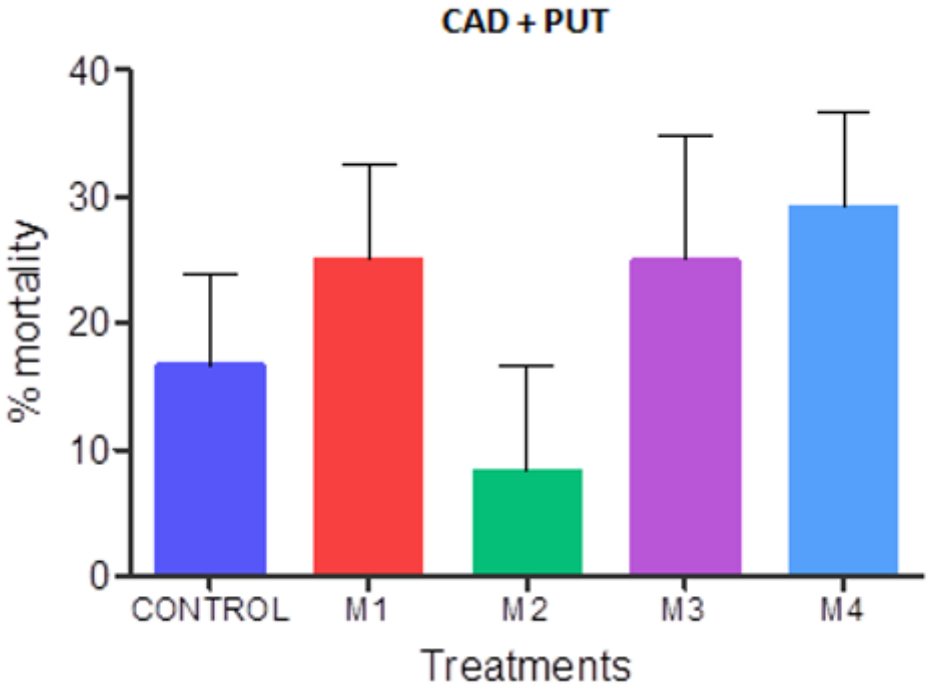

Figure 8

Lethality of D. magna exposed to different concentrations of a cadaverine and putrescine mixture. M1 (1/10 LC_{50} CAD + 1/10 LC_{50} PUT); M2 (LC_{50} CAD + 1/10 LC_{50} PUT); M3 (LC_{50} PUT + 1/10 LC_{50} CAD); M4 (LC_{50} CAD + LC_{50} PUT). ANOVA/Dunnett ($p>0.05$).

Supplementary Files

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- Graphicalabstract.png