Chapter

Effect of Heavy Metal Pollution on Invertebrates

Samir Ghannem, Sonia Daouadi and Samir Touaylia

Abstract

Metal pollutants are widespread in air, soil and water causing a decline in invertebrates worldwide. The increase of environmental pollution by heavy metals has a negative impact to organisms and influence their diversity, distribution, physiology and behavior. Contrary to other pollutants, metals are non-degradable and can potentially bio-accumulate and be biomagnified in the trophic chain. Because soil invertebrates tend to be strongly affected by environmental disturbances, high concentrations of these metals can become hazardous to invertebrates. Noxious effects can affect all biological levels, and toxins affect all ecological interactions. In this brief chapter, we have tried to develop a comprehensive understanding of the influence of metal contamination on ecosystem disturbance. We give examples of studies on the effects of pollutants on invertebrates.

Keywords: impact, heavy metals, invertebrate decline, environmental pollution, bioindicators

1. Introduction

Pollution is a mixture of contaminants in the environment that has serious consequences on the environment. It can originate from chemicals in any environmental medium, with deleterious consequences on living species. The damage expressed to living organisms can lead to damage to their health or interference with the ecological systems of which they are a part [1]. However, the ecological risk of heavy metals has become a major concern in developing countries. The multiple industrial, domestic, agricultural, medical and technological applications of heavy metals have led to their wide distribution in the environment, raising increasing concerns about their potential effects on human health and the balance of ecosystems. Heavy metals are also part of the contaminants that can be found in residential areas. Heavy metals present in the atmosphere, soil and water today come from various sources such as landfills, domestic and industrial waste, mineral and oil extraction sites as well as atmospheric pollution. These products are accumulated by the fauna and flora and magnified along the food chain, which multiplies the impact of this pollution. Each year the levels of pollutants, in particular metals, increase and cause environmental threats that lead to the imbalance of the natural system [2]. Toxic chemicals in the environment can enter ecosystems and end up throughout the biosphere. Ecosystems can be affected by chemical contamination, disturbing the activities of living organisms or changing the physical properties of ecosystems [3].

Disturbed environments and excessively loaded with metals, can lead to a decrease in the biological activity of invertebrates. Currently the regression of the diversity of invertebrates in the world is very remarkable. According to [4], the effects of toxic metal pollution on terrestrial invertebrate species appear to be extremely significant and widespread. Such a revision of regulatory thresholds to better protect terrestrial invertebrates, which seem more sensitive to metal pollution than vertebrates, is necessary and needs urgent attention from scientists and stakeholders [5]. The pollution of ecosystems exposes an astonishing danger to invertebrate's species through direct and/or indirect contact with contaminated soil [6, 7]. The contamination of ecosystems is the complication that strongly affects biodiversity, the environment and human health all over the world via soil and water pollution [8]. Bioindicators have been considered an essential tool for monitoring and discovering changes in the environment [9]. The choice of the most adequate indicator is linked to the objective of the survey and the characteristics of the indicator. In the invertebrate group, several species or groups of species have been used as indicators. Bioindicators species are sensitive to environmental changes, such as high levels of metals [10, 11], this sensitivity is expressed by the absence or presence of species, or by altered physiological and/or morphological changes [12]. The relationships between toxic chemical elements, the biotope and living organisms can cause the degradation of these compounds during their modification, leading to transformations of the environment and leading to harmful damage to living organisms [3]. The response of invertebrates to metal pollution is closely related to species as well as pH conditions, exposure time, and metal form and concentration, which are deeply tied to metal bioavailability [13, 14]. It should be noted that the use of certain species of invertebrates as better indicators than others is linked to their different ecological requirements. As part of biomonitoring programs, measurements of metal residues have been established on invertebrate species [15]. The utility of studies performed on invertebrates can be essential for such an assessment of the impact of toxic metal pollution on environmental behavior and the strategies adopted to anticipate such an effect [10]. They show an important role in terrestrial environments linked to their vast abundance, biomass, and diversity [16]. Invertebrates living in polluted environments have been regularly revealed to accumulate heavy metals [17–19]. Using sensitive species invertebrates lets for an improved valuation of the threats environmental and for more informative and efficient test systems [10].

2. Materials and methods

This study focuses to evaluate the potential heavy metals impacts on invertebrates. The results cited come from the literature of 26 years from 1997 until 2022.

3. Bioindicators concept

In recent decades, the use of alternative biological activities for monitoring the state of the environment has been increased due to the high cost associated with traditional instrumental measurement methods. The use of bio-indicators is considerably more economical since it generally makes it possible to avoid using expensive technological equipment and to save time [20, 21]. Bio-indication therefore refers to a process of analysis of various biological indicators that is part of the biomonitoring of the quality of ecosystems [22]. The indicators that can provide ideas on the qualitative state of the environment are defined as bioindicators. These bioindicators are frequently used in scientific investigations to assess the progressive impacts of pollutants due to anthropogenic activities. Bioindicators are sensitive to changes and disturbances in biotopes. However, the quality of an environment can be assessed by living species, which have an essential role in monitoring its disturbances [23]. Moreover, certain bio-indicators for their capacity of bioaccumulation, allow an early detection of pollutants or disturbances [24]. Furthermore, bio-indicators provide information "on the bioavailability of pollutants rather than on their total concentration in the environment" [20]. This distinction is significant when looking at the effects of pollutants on organisms [25]. Finally, unlike instrumental measurements which take parameter values instantaneously and locally, bio-indicators provide an integrated indication of the spatiotemporal effects of pollutants on biota since they "reflect the total time of exposure to the pollutant" [20]. According to [26], a bioindicator (or bio-indicator or biological indicator) is an organism or set of organisms which, by reference to biochemical, cytological, physiological, ethological or ecological variables, makes it possible, in a practical and safe way, to characterize the state of an ecosystem or an ecocomplex and to bring to light as early as possible their modifications, natural or provoked". The choice of indicator species is based on their easy monitoring, and that they can predict the conditions of the environment to which they belong [27]. In addition, indicator species must be easily collected, very abundant, widely distributed, available all year round, easily recognized by non-specialists, possess a high sensitivity to environmental constraints and should be profitable [3].

4. Effect of heavy metal pollution on invertebrates

Metallic elements are considered systemic toxins known to induce neural and multi-organ damage, and contribute to cancer in animals and humans, even at low levels of exposure. Their toxicity depends on several factors, including dose, route of exposure and chemical species, as well as the age, sex, genetics and nutritional status of those exposed. However, in some invertebrates, physiological and ecological changes following pollution by heavy metal can be detected by neurological dysfunctions and sensory, endocrine or metabolic disturbances [28]. Following exposure to a series of soils contaminated with different concentrations of cadmium and zinc on the talitrid amphipod species *Orchestia gammarellus*, [29] detect significant changes in the morphology and in the ultrastructural organization of hepatopancreatic cells as well as the rhythm of locomotor activity. However, the results show that the damage observed at depends on the concentration at which the individuals were expressed. Concerning the behavioral response, a great intervariability of the locomotor rhythm was observed. Patterns were in majority bimodal for the uncontaminated individuals and became unimodal and multimodal when exposed under Cd and the Zn respectively. In addition, the circadian period lengthened after Zn exposure. Referring to [30] pollution intensities could have negative effects on the restriction of the distribution area or even the disappearance of certain species. In a polluted environment morphological, behavioral, tissue or physiological alterations, as well as the abundance, reproductive success and mortality of animal species are observable and measurable variables reflecting the state of their habitat [12, 24, 25]. In line with that, toxic metals interfere with cytoplasmic membranes and lead to pathological results [31, 32]. These pathological ramifications contain the nuclear and cytoplasmic corruptions. The follicular epithelial cells showed signs of damage which is a remarkable appearance seen in the ovarian cells from the polluted site. This can delay oocyte maturation and result in imperfect yolk deposition [33, 34]. The distorted brush borders of the microvilli may block the passage of the materials toward the oocyte. Also, the deformity of yolk granules could obstruct vitellogenesis and result in a lower fecundity and egg viability [34].

Referring to [12], following pollution by Cd, Ni and Zn, the response of invertebrate species of *Pterostichus oblongopunctatus* (ground beetles) results in severe intestinal degeneration. In the case of Zn pollution, a decrease in the number of eggs laid by *Poecilus cupreus* has been observed [35]. Furthermore, the results obtained by [36] indicate an increase in egg production and a reduction in egg quality (hatching) in zones polluted by metals compared to unpolluted areas. In [37], it was shown that in polluted areas the body weight of *P. oblongopunctatus* is higher compared to unpolluted areas. It was explained by advanced metal tolerance [38]. Several publications have appeared in recent years documenting that exposure of invertebrates to toxic elements causes sublethal consequences, sometimes difficult to evaluate, such as impaired fertility observed in (grasshoppers: [39]; springtail: [40]; earthworm: [41], resistance to pathogens; ant: [42]; honey bee: [43]), developmental abnormalities fly [44]; moth: [45]; ant: [46]) and similarly altered feeding behavior (aphid: [47]; honey bee: [48]). In addition, metal pollution can cause cell damage or death in the brains of invertebrates, as their nervous systems are small in size and contain relatively few neurons [49, 50]. According to [51], toxic elements, particularly zinc, can disturb molting cycles, alter the digestive glands in isopods and can affect food consumption, reproduction and the composition of the community. Previous studies indicate that metal pollution has severe consequences on pollinator behavior by affecting foraging activity, food perception and memory abilities necessary for energetic foraging [5, 48, 52, 53]. The results obtained by [54] on tenebrionid beetles (*Blaps polycresta* and *Trachyderma hispida*) inhabiting polluted soil shows several abnormalities in oocytes and trophocytes. These baseline abnormalities included exfoliation and vacuolation of follicular epithelium, vacuolated trophocyst, nuclear abnormalities, and morphological changes in cytoplasmic organelles. Co-exposure of honey bees (*A. mellifera*) to cadmium and copper resulted in increased developmental time, high mortality, and decreased food intake and response to sucrose [55]. Referring to [56], the study of the impact of Cadmium on species of Tenebrionidae, reveals a decrease in population density, a reduction in body weight, an increase in the mortality rate and an increase in the sex ratio insects. Moreover, the results also revealed a striking decrease in the body length of polluted insects with a marked increase in the percentage of deformed gonads and digestive tract.

5. Results and discussion

One of the main characteristics of the different bioindicator species is, among other things, the ability to respond in an observable and measurable way to disturbances or to accumulate the pollutant. The distribution of invertebrates in all types of terrestrial and aquatic makes them excellent for ecotoxicological analysis and are efficient biomarkers in detecting environmental pollution. However, the internal study of the compartment of metals in different subcellular fractions, allows us to better interpret the mechanisms of accumulation of toxic elements in organisms and their transfer through trophic chains [12]. In general, toxic metals, even at low concentrations, have a polluting nature with harmful effects for living organisms. Heavy metals have a cumulative effect. Their selective attachment to sensitive organs and tissues can be dangerous when their concentrations are high [57]. It is also likely that heavy metal ions act on Ca^{2+} receptors [58] and metal ions enter the interior of the cell, from where they initiate free radical oxidation [59]. An oxidative stress reaction then develops which in turn influences the lysosomes. Furthermore, variation in the concentration of heavy metals affects the susceptibility of insects to entomopathogenic fungi [60, 61]. According to [62], the humoral immunity of invertebrates could be affected by the stress of toxic elements, and the response of the humoral immunity of insects was found to affect the susceptibility of insects to entomopathogenic microorganisms [62, 63]. In addition, insects reduce their feeding or decrease their digestion and absorption as a result of heavy metals accumulated in food [64]. Referring to [65], a reduction in intermediate carbohydrate metabolism, was observed in chironomid (*Chironomus tepperi*), including glucose 6-phosphate, fructose 6-phosphate and disaccharides following exposure to Zn stress. Thus, to better understand the effect of heavy metal stress on insect disease resistance and their corresponding regulatory mechanisms, studies on several insect species and different types of toxic elements should be done [66].

6. Conclusions

The use of invertebrates as bioindicators to assess environmental risks occupies a high priority for researchers and environmental observers due to their efficient and rapid responses to various external causes. However, toxic elements are capable of penetrating the body and accumulating in certain tissues, causing cellular alterations as well as damage to energy metabolism, growth and reproduction. Further study of the issue would be of interest to improve international guidelines for metal pollutants and to develop maintenance plans to protect invertebrates and ecosystem services.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Appannagari RR. Environmental pollution causes and consequences: A study. North Asian International Research Journal of Social Science and Humanities. 2017;**3**(8):151-161

[2] Al-Khazraji HI, Thakir BM, El-Hadeeti SAK. Bioindicators of pesticides pollution in the aquatic environment: A review. Plant Archives. 2020;**20**:1607-1618

[3] Abdel-Aziz N, Shalaby S, El-Bakry A. Using of Bioindicators for Monitoring Pesticides Pollution. 2nd ed. LAP LAMBERT Academic Publishing; 2021. p. 72

[4] Thimmegowda GG, Mullen S, Sottilare K, Sharma A, Mohanta SS, Brockmann A, et al. A field-based quantitative analysis of sublethal effects of air pollution on pollinators. Proceedings of the National Academy of Sciences. 2020;**117**(34):20653-20661

[5] Monchanin C, Devaud JM, Barron AB, Lihoreau M. Current permissible levels of metal pollutants harm terrestrial invertebrates. Science of the Total Environment. 2021;**779**:146398

[6] Ciadamidaro L, Puschenreiter M, Santner J, Wenzel WW, Madejón P, Madejón E. Assessment of trace element phytoavailability in compost amended soils using different methodologies. Journal of Soils and Sediments. 2017;**17**(5):1251-1261

[7] Touceda-González M, Prieto-Fernández A, Renella G, Giognoni L, Sessitsch S, Brader G, et al. Microbial community structure and activity in trace element contaminated soils phytomanaged by Gentle Remediation Options (GRO). Environmental Pollution. 2017;**231**:237-251

[8] Jan S, Rashid B, Azooz MM, Hossain MA, Ahmad P. Genetic strategies for advancing phytoremediation potential in plants: A recent update. In: Plant Metal Interaction. 2nd ed. Elsevier; 2016. pp. 431-454

[9] Rainio J, Niemelä J. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation. 2003;**12**(3):487-506

[10] Ghannem S, Khazri A, Sellami B, Boumaiza M. Assessment of heavy metal contamination in soil and *Chlaenius* (*Chlaeniellus*) *olivieri* (Coleoptera, Carabidae) in the vicinity of a textile factory near Ras Jbel (Bizerte, Tunisia). Environmental Earth Sciences. 2016;**75**(5):1-10

[11] Parmar TK, Rawtani D, Agrawal YK. Bioindicators: The natural indicator of environmental pollution. Frontiers in Life Science. 2016;**9**(2):110-118

[12] Bednarska AJ, Świątek Z. Subcellular partitioning of cadmium and zinc in mealworm beetle (Tenebrio molitor) larvae exposed to metal-contaminated flour. Ecotoxicology and Environmental Safety. 2016;**133**:82-89

[13] Ashraf MA, Maah MJ, Yusoff I. Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. International Journal of Environmental Research. 2012;**6**(1):209-218

[14] Rengel Z. Availability of Mn, Zn and Fe in the rhizosphere. Journal of Soil Science and Plant Nutrition. 2015;**15**(2):397-409

[15] Nico M, Van Straalen NM, Ruslan O, Butovsky RO, Andrei D,

Pokarzhevskii AD, et al. Metal concentrations in soil and invertebrates in the vicinity of a metallurgical factory near Tula (Russia). Pedobiologia. 2001;**45**:451-466

[16] Zödl B, Wittmann KJ. Effects of sampling, preparation and defecation on metal concentrations in selected invertebrates at urban sites. Chemosphere. 2003;**52**(7):1095-1103

[17] Braun M, Simon E, Fábián I, Tóthmérész B. Elemental analysis of pitfall-trapped insect samples: Effects of ethylene glycol grades. Entomologia Experimentalis et Applicata. 2012;**143**:89-94

[18] Simon E, Harangi S, Baranyai E, Braun M, Fábián I, Mizser S, et al. Distribution of toxic elements between biotic and abiotic components of terrestrial ecosystem along an urbanization gradient: Soil, leaf litter and ground beetles. Ecological Indicators. 2016;**60**:258-264

[19] Ghannem S, Touaylia S, Boumaiza M. Beetles (Insecta: Coleoptera) as bioindicators of the assessment of environmental pollution. Human and Ecological Risk Assessment: An International Journal. 2018;**24**(2):456-464

[20] Markert BA, Breure AM, Zechmeister HG. Bioindicators and Biomonitors. Principle, Concepts and Applications. 2nd ed. Vienne: Elsevier; 2003. p. 997

[21] Carignan V, Villard MA. Selecting indicator species to monitor ecological integrity: A review. Environmental Monitoring and Assessment. 2002;**78**(1):45-61

[22] Chabot B. Les facteurs de sélection des bio-indicateurs de la qualité des écosystèmes aquatiques: Elaboration d'un outil d'aide à la décision. Québec: Université de Sherbrooke; 2014

[23] Asif N, Malik M, Chaudhry FN. A review of on environmental pollution bioindicators. Pollution. 2018;**4**(1):111-118

[24] Kaiser J. Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment. 2nd ed. Hartford: Science Publishers; 2001. p. 304

[25] Kerckhove O. Espèces ou association d'espèces de poissons en tant que bioindicateur de l'état de santé des récifs coralliens. Sherbrooke, Québec: Université de Sherbrooke; 2012

[26] Banaru D, Perez T. Bioindicateurs, Biomarqueurs, Notes de cours. Paris: Université de Marseille; 2010. p. 24

[27] Siddig AA, Ellison AM, Ochs A, Villar-Leeman C, Lau MK. How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. Ecological Indicators. 2016;**60**:223-230

[28] Amiard-Triquet C, Amiard JC. L'écotoxicologie du comportement. In: Amiard JC, Amiard-Triquet C, editors. Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques. 2008. pp. 211-240

[29] Jelassi R, Khemaissia H, Ayari A, Bohli-Abderrazek D, Ghemari C, Raimond M, et al. Physiological and behavioral responses of Orchestia gammarellus (Amphipoda, Talitridae) towards trace elements contamination soil. Microscopy Research and Technique. 2022;**85**(5):1713-1722

[30] Acevedo-Whitehouse K, Duffus AL. Effects of environmental change

on wildlife health. Philosophical Transactions of the Royal Society B: Biological Sciences. 2009;**364**(1534): 3429-3438

[31] Kheirallah DAM, El-Moaty ZA, El-Gendy DA. Impact of cement dust on the testis of *Tachyderma hispida* (Forskal, 1775) (Coleoptera: Tenebrionidae), inhabiting Mariout region (Alexandria, Egypt). Journal of Entomology. 2016;**13**(3):55-71

[32] Shonouda M, Osman W. Ultrastructural alterations in sperm formation of the beetle, *Blaps polycresta* (Coleoptera: Tenebrionidae) as a biomonitor of heavy metal soil pollution. Environmental Science and Pollution Research. 2018;**25**(8):7896-7906

[33] Sreelatha KB, Geetha PR. Disruption of oocyte development and vitellogenesis in Oryctes rhinoceros treated with methanolic extract of Eupatorium odoratum leaves. Journal of Biopesticides. 2010;**3**(Special Issue):253

[34] Osman W, Shonouda M. X-ray metal assessment and ovarian ultrastructure alterations of the beetle, Blaps polycresta (Coleoptera, Tenebrionidae), inhabiting polluted soil. Environmental Science and Pollution Research. 2017;**24**(17):14867-14876

[35] Kramarz P, Laskowski R. Effect of zinc contamination on life history parameters of a ground beetle, *Poecilus cupreus*. Bulletin of Environmental Contamination and Toxicology. 1997;**59**(4):525-530

[36] Lagisz M, Laskowski R. Evidence for between-generation effects in carabids exposed to heavy metals pollution. Ecotoxicology. 2008;**17**(1):59-66

[37] Zygmunt PM, Maryański M, Laskowski R. Body mass and caloric value of the ground beetle (Pterostichus oblongopunctatus) (Coleoptera, Carabidae) along a gradient of heavy metal pollution. Environmental Toxicology and Chemistry: An International Journal. 2006;**25**(10):2709-2714

[38] Tőzsér D, Magura T, Simon E, Mizser S, Papp D, Tóthmérész B. Pollution intensity-dependent metal accumulation in ground beetles: A meta-analysis. Environmental Science and Pollution Research. 2019;**26**(31):32092-32102

[39] Schmidt GH, Ibrahim NM, Abdallah MD. Toxicological studies on the long-term effects of heavy metals (Hg, Cd, Pb) in soil on the development of *Aiolopus thalassinus* (Fabr.) (Saltatoria: Acrididae). Science of the Total Environment. 1991;**107**:109-133

[40] Crouau Y, Pinelli E. Comparative ecotoxicity of three polluted industrial soils for the Collembola Folsomia candida. Ecotoxicology and Environmental Safety. 2008;**71**(3):643-649

[41] Konečný L, Ettler V, Kristiansen SM, Amorim MJB, Kříbek B, Mihaljevič M, et al. Response of Enchytraeus crypticus worms to high metal levels in tropical soils polluted by copper smelting. Journal of Geochemical Exploration. 2014;**144**:427-432

[42] Sorvari J, Rantala LM, Rantala MJ, Hakkarainen H, Eeva T. Heavy metal pollution disturbs immune response in wild ant populations. Environmental Pollution. 2007;**145**(1):324-328

[43] Polykretis P, Delfino G, Petrocelli I, Cervo R, Tanteri G, Montori G, et al. Evidence of immunocompetence reduction induced by cadmium exposure in honey bees (*Apis mellifera*). Environmental Pollution. 2016;**218**:826-834

[44] Nascarella MA, Stoffolano JG Jr, Stanek EJ III, Kostecki PT, Calabrese EJ. Hormesis and stage specific toxicity induced by cadmium in an insect model, the queen blowfly, Phormia regina Meig. Environmental Pollution. 2003;**124**(2):257-262

[45] Van Ooik T, Rantala MJ, Saloniemi I. Diet-mediated effects of heavy metal pollution on growth and immune response in the geometrid moth Epirrita autumnata. Environmental Pollution. 2007;**145**(1):348-354

[46] Skaldina O, Peräniemi S, Sorvari J. Ants and their nests as indicators for industrial heavy metal contamination. Environmental Pollution. 2018;**240**:574-581

[47] Stolpe C, Krämer U, Müller C. Heavy metal (hyper) accumulation in leaves of Arabidopsis halleri is accompanied by a reduced performance of herbivores and shifts in leaf glucosinolate and element concentrations. Environmental and Experimental Botany. 2017;**133**:78-86

[48] Burden CM, Morgan MO, Hladun KR, Amdam GV, Trumble JJ, Smith BH. Acute sublethal exposure to toxic heavy metals alters honey bee (Apis mellifera) feeding behavior. Scientific Reports. 2019;**9**(1):1-10

[49] Niven JE, Farris SM. Miniaturization of nervous systems and neurons. Current Biology. 2012;**22**(9):323-329

[50] Klein S, Cabirol A, Devaud JM, Barron AB, Lihoreau M. Why bees are so vulnerable to environmental stressors. Trends in Ecology & Evolution. 2017;**32**(4):268-278

[51] Drobne D. Terrestrial isopods—A good choice for toxicity testing of pollutants in the terrestrial environment. Environmental Toxicology and Chemistry: An International Journal. 1997;**16**(6):1159-1164

[52] Sivakoff FS, Gardiner MM. Soil lead contamination decreases bee visit duration at sunflowers. Urban Ecosystem. 2017;**20**(6):1221-1228

[53] Xun E, Zhang Y, Zhao J, Guo J. Heavy metals in nectar modify behaviors of pollinators and nectar robbers: Consequences for plant fitness. Environmental Pollution. 2018;**242**:1166-1175

[54] Kheirallah DA, El-Samad LM. Oogenesis anomalies induced by heavy metal contamination in two tenebrionid beetles (*Blaps polycresta* and *Trachyderma hispida*). Folia Biologica (Kraków). 2019;**67**(1):9-23

[55] Di N, Zhang K, Hladun KR, Rust M, Chen YF, Zhu ZY, et al. Joint effects of cadmium and copper on Apis mellifera forgers and larvae. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2020;**237**:108839

[56] OsmanW,El-SamadM,MokhamerEH, El-Touhamy A, Shonouda M. Ecological, morphological, and histological studies on *Blaps polycresta* (Coleoptera: Tenebrionidae) as biomonitors of cadmium soil pollution. Environmental Science and Pollution Research. 2015;**22**(18):14104-14115

[57] Lallogo H. Concentration de certains métaux d'importance médicale dans les poisons: cas des poissons de la lagune de Lomé. In: Mémoire de Technicien Supérieur en Génie Sanitaire (EAM). 1992

[58] Nemeth EF. Ca2+– Receptor–dependent regulation of cellular functions. News in Physiological Sciences. 1995;**10**(2):1-5

[59] Berridge MJ, Heslop JP, Irvine RF, Brown KD. Inositol trisphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor. Biochemical Journal. 1984;**222**(1):195-201

[60] Dubovskiy IM, Grizanova EV, Ershova NS, Rantala MJ, Glupov VV. The effects of dietary nickel on the detoxification enzymes, innate immunity and resistance to the fungus Beauveria bassiana in the larvae of the greater wax moth Galleria mellonella. Chemosphere. 2011;**85**(1):92-96

[61] Nanda KP, Kumari C, Dubey M, Firdaus H. Chronic lead (Pb) exposure results in diminished hemocyte count and increased susceptibility to bacterial infection in Drosophila melanogaster. Chemosphere. 2019;**236**:124349

[62] Pragya P, Shukla AK, Murthy RC, Abdin MZ, Kar Chowdhuri D. Characterization of the effect of Cr (VI) on humoral innate immunity using Drosophila melanogaster. Environmental Toxicology. 2015;**30**(11):1285-1296

[63] Afify A, Ghezawy NA, Ali MM, El-Tayeb TA. Effects of copper chlorophylline and magnesium chlorophylline on the humeral immune response, ultrastructural changes of midgut and DNA damage to Culex pipiens larvae. African Entomology. 2019;**27**(1):146-158

[64] Li K, Chen J, Jin P, Li J, Wang J, Shu Y. Effects of Cd accumulation on cutworm Spodoptera litura larvae via Cd treated Chinese flowering cabbage *Brassica campestris* and artificial diets. Chemosphere. 2018;**200**:151-163

[65] Long SM, Tull DL, Jeppe KJ, De Souza DP, Dayalan S, Pettigrove VJ, et al. A multi-platform metabolomics approach demonstrates changes in energy metabolism and the transsulfuration pathway in *Chironomus tepperi* following exposure to zinc. Aquatic Toxicology. 2015;**162**:54-65

[66] Jiang D, Tan M, Guo Q, Yan S. Transfer of heavy metal along food chain: A mini-review on insect susceptibility to entomopathogenic microorganisms under heavy metal stress. Pest Management Science. 2021;**77**(3): 1115-1120