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Herbicidal impacts on freshwater zooplankton

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A B S T R A C T

Application of herbicides is an essential part of agriculture for more crop yields. But their overuse raises the risk of non-target species extinction from freshwater ecosystems. Phytoplanktons, the primary producers in aquatic systems are highly susceptible to herbicide contamination, resulting in disturbance of trophic interactions and energy flow in meta-communities. Persistence of current-use herbicides, dalapon (2 to 3 days), paraquat and diquat (4 to 6 weeks), chlorthiamid (3 months), terbutryne and diuron (>3 months) etc.in pond and river ecosystems can adversely affect plankton community. Interestingly, desphenyl-chloridazon, the metabolite of n-chloridazon is more persistent (>98 days) and more toxic to aquatic planktons. Herbicides such as benfluralin, bensulide, dacthal, ethalfluralin, oxadiazon, pendimethalin, triallate, and trifluralin potentially accumulate in sediments and aquatic biota. Zooplanktons are affected by herbicides directly and indirectly. Herbicides like glyphosate are more toxic to non-target organisms such as Daphnia magna. Some herbicides are potential lethal to amphipods, cladocerans, copepods, malacostracans, and rotifers. Herbicides interfere negatively on the growth, behavior, reproductive potential and population dynamics of zooplanktons. Herbicides (i.e., atrazine) reduce the species abundance and biomass of zooplankton. Atrazine at very low concentration can alter the sex ratio of *Daphnia pulicaria*. The phenylamide herbicide, propanil interferes growth, survival and reproduction efficiency of *D. magna* at 0.08 mg/l concentration and its metabolite 3,4-dichloroaniline (3,4-DCA) creates acute lethal inhibition on reproduction rate in *D. magna*. The sub-lethal effects of herbicides can alter the demographic parameters of zooplanktons. Herbicides inhibit enzyme catalysis, mRNA expression, gene induction and grazing rate of freshwater zooplanktons. Herbicide concentration in freshwater body is very vital for ecosystem productivity and presence of microalgae can reduce the toxicity. So, our review focuses on the harmful effects of several herbicides on different zooplanktons in freshwater ecosystems. Stress enzyme assay and development of more sensitive biomarker are our future scope of study to depict herbicidal effect more precisely.

Keywords: Freshwater zooplankton · Herbicide toxicity · Population dynamic · Reproductive potential · Community structure

1. Introduction

Modern agriculture without herbicides is impossible, but their overuse poses a risk of non-target species extinction from both the agro- and aquatic ecosystems. Water seepage across the soil carries herbicides to groundwater and contaminates it. Herbicides applied to crop lands are washed away by runoff water in ponds, canals, rivers and lakes. The physico-chemical characteristics and community structure of aquatic ecosystems are degraded. Planktons are the base of aquatic food chain and are the critical factor for productivity in the ecosystem. Any alteration in the physico-chemical properties of water owing to chemical contamination like herbicides is reflected in the increase or decrease in population density and diversity of plankton species. The phytoplankton diversity changes rapidly to pollutant. Ostracods, the excellent water quality and sediment quality indicators are highly sensitive to herbicide contamination in freshwater ecosystems [1-3]. The zooplankton species *Brachionus* sp. is abundant and dominant in organically polluted water [4]. The low diversity index of plankton is a clear indication of water pollution [4]. Herbicides affect plankton communities in ponds [5]. Phytoplanktons are highly susceptible non-target organisms in herbicide-contaminated aquatic ecosystems, and their effects on phytoplankton may initiate a series of consequences in metacommunities through trophic interactions [6]. Herbicides can pose direct toxicity to aquatic phytoplankton, which may lead to indirect impacts such as mortality [7], starvation, growth, and reproduction inhibition [8-9], alteration of swimming behavior [10], competition [11], and predator escaping capacity [12] on primary consumers such as zooplankton [13]. It can affect zooplankton populations by reducing their survival rate, impairing egg hatching and reducing their specific wealth and diversity [12]. It selectively decreases primary producers, leading to a bottom-up reduction in consumer abundance [11]. The study provides a comprehensive understanding of the effects of several herbicides on the zooplankton communities in aquatic ecosystems and recommendations for their bioremediation strategies.

2. An overview of Herbicidal impacts on zooplankton

The effects of herbicides on freshwater ecosystems occur directly and indirectly. The indirect herbicidal impacts on the freshwater invertebrate consumer populations are observed as a result of their unfavorable consequences on primary producers (e.g., algae and macrophytes) [14]. Due to diquat contamination in ponds, *Simocephalus vetulus* (crustacea) may die because of a lower oxygen supply, which in turn promotes *D. longispina* population growth [15]. Herbicides such as ethalfluralin, benfluralin, dacthal, bensulide, triallate, oxadiazon, pendimethalin, and trifluralin potentially build up in aquatic deposits as well as organisms [16], resulting in bioaccumulation and biomagnification. Various commercial herbicide principles, such as glyphosate, can be more poisonous to off-target organisms than their active components. Glyphosate reduces reproductive capability of the flea *D. Magna* [17].

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Glyphosate and glyphosate-copper complexes decrease the swimming velocity of juvenile of *D. magna* [18]. The Ostracod, *Eucypris* sp. is resistant to glyphosate at concentration up to 6 ppm but its sub-lethal concentration (9.03 ppm) may affect parthenogenesis, survival of neonates and population growth [19]. The freshwater copepod *Acanthocyclops robusta* is also resistant to glyphosate at lower concentration (LC_{50} 19 ppm in 24 h and 13 ppm in 48 h) [19]. The decapod *Caridina nilotica* is sensitive to glyphosate [20]. This herbicide is highly toxic to the copepod *Pseudodiaptomus annandalei* (LC_{50} 11.7 ppm and 10.23 ppm in 24 and 48 h, respectively) and the nauplius of *P. annandalei* poses a deleterious effect of survivorship towards glyphosate (Roundup®) (LC_{50}: 1.10 mg/l, 96 h), whereas adult ones are slightly better tolerant (LC_{50}: 6.31 mg/l, 96 h) [21]. Glyphosate affects the development time of embryos (3 and 8 mg/l), length of juvenile and reproductive phages, usual lifetime, net rate of reproduction (8.0 and 10.50 mg/l), and the inherent rate of population increase of the rotifer *Brachionus calyciflorus in fresh water* [22]. The body length of *D. magna* decreases with tebuthiuron exposition at a concentration of 44.2 mg/l [23]. Trisulphuron has a harmful effect on reproduction and survival of *D. magna* (LC_{50}: 48 mg/l) and regulates *Brachionus platulus* growth at 120 µg/ml [24-25]. Atrazine decreases the abundance and biomass of *D. magna* at a concentration 50 µg/l for 21 days of contact [26]. The phenylamide herbicide propanil interferes survival, growth, and reproduction efficiency of *D. magna* at a concentration of 0.08 mg/l, and the propanil metabolite 3,4-dichloroaniline (3,4-DCA) creates acute lethal inhibition on reproductive rate in *D. magna* [27-28]. The mortality rate of *Simocephalus vetulus* significantly increased at a 0.5 mg/L concentration of triclopyr [29]. The exposure to molinate on parental *D. magna* [30] and *Moina australiensis* [31] *results in* significantly reduced reproduction and decreased number of offspring per adult female in 3 broods, respectively [32]. A drastic fall in population density and complete death of *Brachionus* sp. within four days are observed in cultures fed with 500 nM terbutryn-exposed microalgae [33]. Furthermore, they reported that with increasing herbicide concentrations, the percentage of adult females in populations of the rotifer fed with terbutryn containing microalgae decreases considerably. The lethality of glyphosate and its commercial form Faena® to *Lecane quadridentata* and *D. magna found* occurs in the runoff water at 5.2 mg/l in 1 day at the maximum rate at 8.6 kg/ha of Roundup® [34].

3. Research methods

Herbicide application is a global practice in agricultural management with the purpose of controlling weeds. The careless application of these herbicides could affect creatures that are not their intended targets. Application of herbicide may cause mortality by reducing the dissolved oxygen supply and may affect in growth and reproduction of freshwater zooplankton. The examination of herbicides' direct effects on freshwater zooplankton yields a complicated mixture of information about lethal and sublethal values from conventional toxicity testing. The current review work was formulated on the basis of some review works related to herbicidal impacts on freshwater zoo plankton. Few herbicides potentially show lethal effects only on some freshwater groups of zooplankton like amphipods, copepods, malacostracans, cladocerans, and rotifers [32]. The sub-lethal impacts of herbicides may alter the demographic parameters (chiefly life tables and “r” value determination) of three freshwater zooplankton species, such as cladocerans, amphipods, and rotifers [32]. The dynamics of freshwater zooplankton population may be altered through herbicides by controlling their survival as well as reproduction, and through varying the sex ratio. The impacts of herbicides develop on the ecosystem and community levels by the following means, a) stimulation of small species dominance, b) enhance richness and diversity of species, and c) food chain elongation and decrease the efficiency of energy transfer from primary producers to top predators [12]. Herbicides inhibit enzyme catalysis, mRNA expression, gene induction and grazing rate of freshwater zooplanktons [32].

4. Results and Discussion

The concentration of herbicides in freshwater bodies is crucial for the productivity of the ecosystem. Therefore, the main emphasis of our review is the detrimental impact that various herbicides have on various zooplanktons in freshwater habitats. Our future research will focus on developing more sensitive biomarkers and stress enzyme assays to better accurately reflect the herbicidal effect.

4.1. Action mechanism of herbicides on freshwater zooplankton

The herbicides can go into freshwater ecosystems through spray drift, run-off, and leaching [35] where the zooplanktons are affected by trophic chain interactions [35-37]. Herbicides in aquatic bodies act on phytoplanktons directly through different mechanisms. The significant impacts of herbicides from various groups are summarized below:

4.1.1 Amino acid synthesis inhibitors

Glyphosate inhibits shikimate pathway of the synthesis of the aromatic amino acids-phenyl alanine, tyrosine and tryptophan through inactivation of the enzyme 5-enolpyruvoylshikimate 3-phosphate synthetase that converts phosphoenol pyruvate and 3-phosphoskimate into 5-enolpyruvylshikimate-3-phosphate [38-39]. Tebuthiuron acts as an inhibitor of acetolactate synthase and ceases the biosynthesis of branched chain amino acids [40].

Glyphosate toxicity can adversely affect *the morphology, reproduction,* development, growth, and various behavioral responses of freshwater zooplankton such as *D. magna* [17], *Eucypris* sp. and *A. robustus* [19], *P. annae* and *C. nilotica* [20]*, P. annandalei* [21], *and B. calyciflorus* [22].

4.1.2 Cell-membrane disrupters

Almost all plankton communities, including zooplanktons such as copepods (*Mesocyclops* sp., *Thermocyclops decipiens*), cladocerans (*Diaphanosom aexcisum),* and various rotifers (*Conochiloide* sp., *Brachionus* *calyciflorus*, *Lecane* sp., *Asplanchna* sp., and *Hexarthra* sp.) are affected by paraquet toxicities such as the reduction of numbers, biomass, and general trophic performances. In fact, the sensitivity of paraquat to *Thamnocephalus decipiens is* dose-dependent [41].

Paraquat induces oxidative stress (enzymatic actions of catalase and superoxide dismutase, lipoperoxidation) through generation of reactive oxygen species leading to cell membrane damage [42] and also interferes with cellular transport of polyamines (putrescine, spermidine, and spermine) in two freshwater invertebrate species: the *Lumbriculus variegates (*oligochaete*)* and the *Biomphalaria glabrata* (gastropod) [43]. Fomesafen deactivates protoporphyrinogen oxidase (PPO) and stimulates production of reactive oxygen species [44].

4.1.3 Growth regulators

The 2,4-Dichlorophenoxy acetic acid negatively influences the increase of *Brachionus patulus* population when exposed through food and water [24]. This selective herbicide can disrupt cellular signal transduction and induce reactive oxygen species (ROS) generation [45].

4.1.4 Lipid synthesis inhibitor

The metazachlor and norflurazon can block synthesis of lipids. Their high concentrations in surface waters may affect zooplankton by causing alterations in the habitat composition of species like *Keratella quadrata*, *Lecane* spp., *Acropercus harpae, Alonella excise, Chydorus sphaericus*, *Brachionus calyciflorus*, *Polyathra dolicoptera, Ceriodaphnia quadrangular*, and *Bosmia longirostris* [8, 46]. The chloroacetamide herbicide inhibits the synthesis of long chain fatty acid resulting in cessation of cell division and cell expansion [47].

The norflurazon induces mortality in bio-indicator organisms such as the *Polycelis feline with* morphological, locomotive, and histological modifications (damage of the external mucous layer, absence of rhabdites, epidermis damage, and widespread parenchymal cell damage) [46]. Norflurazon inhibits python desaturase and blocks carotenoid biosynthesis pathway [48].

4.1.5 Pigment inhibitor

Herbicides such as isoxazolidinones (i.e., clomazone), pyridazinones (i.e., norflurazon), fluridone, difunone, amitrole, and *m*-phenoxybenzamides prevent the development of photosynthetic pigments - chlorophylls and carotenoids in leaf tissues by interfering synthesis pathway of chlorophyll and terpenoid [49-51] resulting in the decline of phytoplankton communities, that might cause negative impacts indirectly on the freshwater zooplankton due to a decline in feed accessibility for zooplankton, reducing their abundance, and/or influencing alterations in the zooplankton taxa composition [36-37].

4.1.6 Photosynthesis inhibitor

Photosynthesis inhibitors include triazines (i.e., atrazine), phenylureas (i.e., linuron), uracils, nitriles, and benzothidiazoles [49-51], which bind to the quinone-binding protein (D1 protein) and block the photosynthetic transport of electrons. Diuron inhibits the transfer of electrons during photosynthesis in plants and algae, and it may also influence freshwater zooplankton. Atrazine can lead to chlorophyll destruction and block photosynthesis by blocking electron transport in photosystem II. Inhibitors of photosynthesis have no direct influences on zooplankton of freshwater, instead suffers indirect toxic effects such as reduce in abundance of various taxa, increase of several taxa, both reduce diversity and alters in zooplankton species composition [52,35,12,36,37,53]. Chang et al. observe that the diversity, dominance, and species structure in the community of freshwater zooplankton consisting of rotifers and cladocerans can shift with simetryn (20 and 80 µg/l) application [53]. Atrazine can affect the production of male *Daphnia* and alter the ratio of sex, which exerts control on dynamics of *Daphnia* population [54]. A chronic linuron application (0.5, 5, 15, 50, and 150 µg/l during 28 days) on microcosms of freshwater might have a negative impact on some algae such as cryptophytes, diatoms, while the positive impact on *Chlamydomonas spp.* resulted in a decline of some Rotatoria as well as an increase in Copepoda, and, to a smaller level, Cladocera [35].

The lethal impacts of terbuthylazine on phytoplankton communities reduce the zooplanktonic abundance through limitation of food and lesser dissolved oxygen levels. Roth et al. observes a shift in the zooplanktonic community composition and a decline in abundance of zooplankton in the terbuthylazine treatment [55]. At 15 µg/l, terbuthylazine exert direct influences on primary producers like phytoplankton (e.g., *Pseudokirchneriella subcapitata*; EC_{50}= 12µg/l) which expected to exert effects indirectly on zooplankton (e.g., *Daphnia magna*; EC_{50}= 21,200 µg/l) [56].

4.1.7 Seedling growth inhibitor

The herbicides dinitroanilines (i.e., trifluralin), acetanilides (i.e., acetochlor), and thiocarbamates (i.e., EPTC) function as inhibitors either of plant root or shoot growth, respectively, by binding tubulin protein (disrupting cell division) and disrupting protein synthesis (weakening the cell wall) [49-50]. These herbicidal impacts are not direct on freshwater zooplankton since their direct harmful impacts, like disruption of cell division, restricting phytoplankton's growth as well as multiplication, decreasing feed accessibility for zooplankton, and reducing their rate of reproduction and their population [11, 37]. The addition of acetochlor and metolachlor at 6-16 ppb has no distinct sign of indirect consequences on zooplankton. Furthermore, the combination of 5 herbicides (acetochlor, metolachlor, glyphosate, atrazine, and 2,4-D) when applied at 6-16 ppb. to the zooplankton taxon (i.e., *Ceriodaphnia* sp.) led to an abundance increase [37]. Acetochlor and other herbicides, such as 2,4-D and atrazine, at a low concentration have no influence on cladoceran survival but can raise their population as a result of their high rate of reproduction [37].

4.2 Herbicides as endocrine disruptors of freshwater zooplankton

The aquatic contamination of atrazine has no influence on the fecundity and survival endpoints of cladocerans, whereas its chronic exposure to *D. pulicaria* at extremely low concentrations (0.5 µg/l) influences the increased male production and changes the sex ratio of the population toward maleness [57, 54].

The lethal impacts of propanil on 21-day exposure decrease the survivorship (0.55 mg/l), number of offspring (0.21 mg/l), numbers and size of brood (0.26 mg/l) per female, and intrinsic growth rate (r) of *D. magna* as concentrations increase in the aquatic medium [28, 33]. After a 5-hour introduction to this herbicide, the filtration as well as intake rates decrease as a result of the paralysis and loss of coordination due to the toxic herbicidal impacts on the nervous system of *D. magna*. The dense *Bracionus* sp. population in the culture medium is reduced, while females are raised with cells of *Chlorella vulgaris* earlier exposed to various terbutryn concentrations for 4 days at 500 nM [33].

5. Conclusion

Herbicides are harmful to both phyto- and zooplanktons in freshwater ecosystems and hinder the energy flow through trophic level interactions, making the ecosystem non-equilibrium. Zooplanktons are adversely affected directly and indirectly (via phytoplanktons) by herbicides. Reproductive cycles of zooplanktons are severely affected and the population dynamics of them are drastically altered towards extinction. Phytoplankton uptake reduces the herbicidal lethality to zooplanktons. The herbicidal toxicity of freshwater zooplankton is mostly centered on studies of the dynamics of population and its effects on the community biodiversity. Toxicity studies on zooplanktons are not sufficient to draw a concrete conclusion. So, we need more studies taking another zooplankton. We need further investigation for new indicator species and for herbicide resistance species. Further study on herbicide and endocrine disruption is indispensable. Different developed and underdeveloped countries are not following the safe standard and good application techniques and the Quantitative Structure/Activity Relationship (QSAR) models are being violated resulting in appearances of excessive quantity of herbicides in water body. Some herbicides can interact with a variety of natural stressors, and synergism among herbicides and other pesticides will be analyzed. Stress enzyme assay will be studied for stress assessment in zooplanktons. Ecotoxicogenomic study on zooplankton will be another future scope. Development of more sensitive biomarker to assess adverse impact on zooplanktons will be a challenge to many recent researchers.

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Conflict of interest

The authors declare that there is no conflict of interest in this manuscript.

Data availability

The authors confirm that all data collected or analyzed during this study are included in this published article.

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