Onyango *et al.*
Environmental Systems Research (2024) 13:1
https://doi.org/10.1186/s40068-023-00326-3

Environmental Systems Research

RESEARCH

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Effects of combined nutrient and pesticide exposure on algal biomass, and *Daphnia magna* abundance

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Abstract

Fertilisers and pesticides are increasingly used in agriculture to improve productivity and protect crops from fungi and insects. However, these farm inputs may lead to adverse effects on aquatic biodiversity through eutrophication and pesticide toxicity. This study aimed to establish the effects of nutrient-only, pesticide-only, combined nutrients and pesticides, and control on the abundance of *Daphnia magna*, and algal biomass. In each of the treatments, different concentrations of nutrients and pesticides residues were added separately or in combination. Responses were measured every 24 h, and the experiments ended after 168 h of exposure. The experiment was set in four concentration treatments comprising high, moderately high, moderately low, and low concentrations. Data analysis was done using Multiple Analysis of Variance (MANOVA) and ANOVA to determine the effect of time, concentrations and the interaction of time and concentrations for each of the treatments on *D. magna* abundance, and algal biomass. Higher concentrations of pesticide additives were associated with lower abundance of *D. magna*, and higher algal biomass over the exposure periods. There was a significant reduction in the abundance of *D. magna* in the combined treatment indicating the toxic effect of pesticide addition. Determination of effect concentrations based on combined nutrients-pesticides experiments becomes important in setting water quality standards, and monitoring the quality status, to avoid underestimating the ecological implications of combined contamination.

Keywords *Daphnia magna*, Algal biomass, Exposure experiment, Combined nutrients and, Pesticides

Introduction

Fertilizers contain mainly nitrogen and phosphorus (Folberth et al. 2014) and their presence alters the trophic state of aquatic ecosystems (Durand et al. 2011). The primary fate of nutrients in aquatic ecosystems is the uptake by primary producers, with nutrient limitation inhibiting productivity in water bodies (Chen and Graedel 2016; Howarth and Marino 2010). Eutrophication occurs when a limiting nutrient (mostly nitrogen or phosphorus) is introduced in excess to a water body (FAO 1996; Rabalais 2009). Eutrophication increases primary productivity in aquatic ecosystems through bottom-up trophic control (Camargo and Alonso 2006; Mesner, N. and Geiger 2010). However, increased productivity results in greater oxygen demand due to respiration at night by primary producers (Durand et al. 2011). Very high oxygen demand facilitates anoxic conditions resulting in the death or increased susceptibility to diseases and infections of aquatic biota (Schoumans et al. 2014).

The environmental impact of pesticides is often greater than intended, with estimates that over 98% of sprayed

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insecticides and 95% of herbicides reach a destination other than their intended target (Ritter et al. 2002). Prominent pesticide families include the organochlorines, organophosphates, carbamates, pyrethroids, phenoxy and benzoic acids, and triazines (Kamrin 2010). Each pesticide family works differently, which results in different effects on the target and non-target organisms (Verbruggen & Van den Brink 2010). Pesticide residues in water bodies may cause mortality or have non-lethal effects, inclduing the formation of cancers, tumours and lesions; cause reproductive inhibition or failure; and disruption of the endocrine system of aquatic organisms (Skurlatov et al. 2015). The effects of pesticide active ingredients and their degradants in aquatic ecosystems is dependent on their chemical properties such as solubility in water, volatility, lipophilicity, degradability and particle affinity (Weston et al. 2007). Organochlorine pesticides have low solubility, high volatility, high lipophilicity, high particle affinity and are highly persistence in the environment, and therefore have high risk of impact to aquatic organisms (Aktar et al. 2009).

Contamination of aquatic ecosystems with a combination of nutrients and pesticides has the potential to modify the primary effects of either nutrients (e.g. eutrophication) or pesticides (e.g. ecosystem poisoning) (Aragón-Noriega and Calderón-Aguilera 2000; FAO 1996; SCHER et al. 2012; UNEP 2001; Weston et al. 2007). For example, the Lake Naivasha agricultural catchment in Kenya is a catchment of concern owing to the high concentration of nitrogen and phosphorus (Kitaka et al. 2002; Ndungu et al. 2013; Phillips et al. 2017), and also pesticides contamination (Gitahi et al. 2002; P. Otieno et al. 2015; P. O. Otieno et al. 2012). The interaction between nutrients and pesticides in aquatic ecosystems such as in Lake Naiavasha and its catchment accelerates the primary effects of nutrients or toxicity of pesticides in the environment, resulting in effects higher or lower than would be expected (Kortenkamp et al. 2009). For instance, Roessink et al. (2008) document that the introduction of toxic pesticides in oligotrophic ecosystems poisons aquatic organisms in the upper trophic levels of the aquatic food chain, destabilizing the top-down trophic control, and resulting into *pseudo*-eutrophication.

The complexities in combined effects of nutrients and pesticides in aquatic ecosystems presents ecological and aquatic concerns. The effect on aquatic ecosystems from combined effects of nutrients and pesticides are under studied and hence appreciated (Bainbridge et al. 2009; Brack et al. 2015; Ritter et al. 2002; SCHER et al. 2012; Van Maanen et al. 2001; Verbruggen and Van den Brink 2010). This is despite the inevitable combined occurrence of nutrients and pesticides in aquatic ecosystems, especially in agricultural catchments. Some of the approaches that have been put forward to explain the potential mixture effects include concentration addition (Deneer 2000), independent action (Verbruggen and Van den Brink 2010), and interaction (SCHER et al. 2012). Very few studies have attempted to estimate effects of nutrients and pesticides in combination (Cornejo et al. 2019; Polazzo et al. 2022), and practically none in sub-Saharan Africa. Furthermore, existing studies have used mainly modelling rather than laboratory or field observations. The knowledge gap is a reason for studies and experiments that focus on the responses of indicator organisms to mixtures of nutrients and pesticides residues (Camargo and Alonso 2006; Roessink et al. 2008). It was the aim of this study to determine the response of aquatic ecosystems to independent and combined nutrient-pesticide contamination. It evaluated (a) the effects of varying concentrations of contaminants on the abundance of *Daphnia magna*, and algal biomass; and (b) the effects of combined nutrients and pesticides compared with pesticide- or nutrients-only exposure on the abundance of *D. magna* and algal biomass.

Materials and methods

The study was carried out in the laboratory. The experiment was based on the water quality (nutrient pollution and pesticide contamination) of Lake Naivasha, Kenya, as reported in Onyango et al. (2015a, b). The independent and combined effects of nutrients and pesticides on the aquatic biota (zooplankton and phytoplankton) were determined across varying concentrations and treatments over time. In the experiment, the idependent variables were the varying concentrations (low, moderately low/high, high), time (24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h), and the types of treatment (control, nutrient-only, pesticides-only, and combined nutrients-pesticides treatments). The dependent variables were the algal biomass, and abundance of *D. magna*.

Experimental design

The factorial experiment was based on three factors (treatment, concentration, time), measured across different levels (four treatments, four concentrations, seven time steps). This resulted in 112 possible experimental conditions. The experimental conditions were replicated four times for the algal biomass and *D. magna*.

The treatments in the experiment included nutrient-only, pesticide-only, combined nutrients and pesticides, and control. In each of the treatments, concentrations of nutrients and pesticides residues, separately or in combination were added. No nutrients or pesticide residue concentrations were added to the control treatment. The experiment was set to four concentration

levels comprising high, moderately high, moderately low, and low concentrations. The terms used as high, moderate or low were used in the study as qualitative references to the various concentrations. Depending on the experiment treatments, concentrations were added as presented in Table 1. Nutrient treatments were done using laboratory grade nitrate and phosphate solutions comprising potassium nitrate (KNO3,) and disodium hydrogen phosphate (Na_2HPO_4), respectively. Pesticides additions were done using laboratory grade Organochlorine pesticide (OCP) mix comprising of γ-HCH, α-HCH, α-endosulfan, aldrin, and pp-DDT dissolved in ethanol.

Considering the concern in Lake Naivasha regarding potential effects of combined nutrients and pesticides contamination (P. Otieno et al. 2015; Phillips et al. 2017), the added concentrations were based on their ecological importance, and abundance of occurrence in the environment (Gitahi et al. 2002; Kitaka et al. 2002; Ndungu et al. 2015; Onyango, Irvine, et al. 2015; Outa et al. 2014). The study used the Lake Naivasha catchment concentrations as a reference ecosystem to determine the quantities of nitrogen, phosphorus and sum of organochlorine pesticides (OCP). The measurements of algal biomass and *D. magna* abundance was done every 24 h for seven days.

Pyrex glass jars (250 ml) were placed randomly in a laboratory glass chamber with regulated temperature and light. Each jar represented one of the 112 experimental conditions. During the experiment, the temperature in the medium was maintained at 25 ± 2 °C, and the treatments exposed to a light:dark cycle of 16:8 h at a light intensity of 100 µmol m^{-2} s^{-1}.

Determination of algal biomass and *D. magna* abundance

Twenty litres of a water sample collected from the pelagic zone of Lake Naivasha was filtered through a 30 µm sieve and the filtrate used as the medium in the experiment. The residue from the filtered water was suspended into 200 ml vials with filtered lake water. *D. magna* individuals were isolated from the concentrate and incubated in 500 ml of the filtrate water. The density of *Daphnia magna* in the 500 ml pyrex incubation jar was monitored until a stable density was achieved without extra feeding. Two hundred millilitres (200 ml) of the medium was added to the 250 ml experimental jars, and doses of the nutrients and pesticides to achieve the concentrations as in Table 1 were added to the jars. Two hundred *D. magna* individuals were inoculated into each experimental jar with the medium providing an initial density of 1000 Ind.L^{-1}. Samples for enumeration were taken every 24 h for seven days. The enumeration involved counts of *D. magna* individuals per volume of subsample. Subsamples were taken four times from each jar at each time interval, without being replaced.

Algal biomass was estimated from chlorophyll-*a* measurements. To determine chlorophyll-a concentration, 5 ml samples were collected from the experimental jars set up every 24 h for seven days. Chlorophyll-a was extracted and determined using the acetone method after Talling and Driver (1963). The chlorophyll-a acetone method is a commonly used technique for the determination of Chlorophyll-a concentration from water bodies. The water sample was filtered through a 0.45 µm Whatman Grade GF/C Glass Microfiber Filter Paper, to collect the phytoplankton containing Chlorophyll-a. The filter, containing the concentrated phytoplankton, was

Table 1 Added concentrations in the experiment based on the various treatments. Abbreviation: Organochlorine pesticide (OCP)

Treatment	Concentrations	Nitrogen (mmol/L)	Phosphorus (mmol/L)	Sum OCP (nmol/l)
Nutrients-only treatment	High	0.93	0.11	
	Moderately high	0.700	0.08	
	Moderately low	0.47	0.05	
	Low	0.23	0.03	
Pesticide-only treatment	High	–	–	4.86×10^{-7}
	Moderately high	–	–	2.53×10^{-7}
	Moderately low	–	–	1.18×10^{-7}
	Low	–	–	5.61×10^{-8}
Combined nutrients and pesticides treatment	High	0.93	0.11	4.86×10^{-7}
	Moderately high	0.700	0.08	2.53×10^{-7}
	Moderately low	0.47	0.05	1.18×10^{-7}
	Low	0.23	0.03	5.61×10^{-8}
Average concentrations recorded by Onyango et al. (2015a, b)	*Average*	*0.47 ± 0.07*	*0.05 ± 0.01*	$1.18\times10^{-7}\pm1.42\times10^{-9}$

transferred to a centrifuge tube. Acetone was added to the tube to extract the Chlorophyll-a from the phytoplankton biomass. The acetone extract was then analysed using a GENESYS10S Spectrophotometer. The absorbance of the extract was measured at a wavelength of 663 nm and 750 nm and Chlorophyl-*a* concentration calculated using Talling and Driver (1963). formulae to determine the Chlorophyll-a concentration.

The chlorophyll-a concentration was calculated as:

$$\text{Chla}\left(\mu g.L^{-1}\right) = \frac{11.40 \times ((E663 - E750) \times V_1)}{V_2 \times L}$$

where 11.40 is the adsorption coefficient of Chla; V_1 is the volume of extract in mL; V_2 is the volume of the filtered water sample in L; L is the light path length of cuvette in cm; and E663 and E750 are the optical densities of the sample read as absorbance from the spectrophotometer.

The algal biomass was estimated for phytoplankton which is less than 30 µm using the ratio between algal biomass and chlorophyll-a in terms of mg algae/µg chlorophyll-a (ACHLA) with a chlorophyll-a conversion factor of 0.05 (Schmid et al. 1998).

Data analysis

The data from the experiment (see Additional file 1) was tested for normality using the Shapiro–Wilk test. The data on algal biomass and *D. magna* abundance were not normally distributed and were log transformed for analysis.

To determine the effects of different concentrations and exposure time, the study tested three hypotheses for each of the treatments. The hypotheses were: (i) the main effect of varying *concentrations* in the treatments had no significant effect on *D. magna* abundance, and algal biomass; (ii) exposure *time* in the treatments had no significant effect on *D. magna* abundance, and algal biomass; and (iii) the effect of the different *treatments* in the experiment had no significant effect on *D. magna* abundance, and algal biomass. The data was analysed using One Way ANOVA to determine the effect of time, concentrations, an interaction of time and concentrations for each of the treatments, and the effect of treatments (control, nutrients-only, pesticides-only, and their combination) on *D. magna* abundance, and algal biomass. In the analysis, time, concentrations, and treatments were the independent variables, while the dependent variables were the measures of *D. magna* abundance, and algal biomass. All analyses were made using R statistics software (R Core Team 2022). Post hoc analyses using Turkey LSD tests were done to determine statistical variations within independent variables using the Agricolae R package (de Mendiburu 2021). The tables to present the results were generated using the writexl package (Ooms 2021), while the graphical presentations were made using the ggplot2 R package (Wickham 2016).

The analysis revealed large error bars, especially in the treatments (with added nutrients and pesticides) as a result of high variability of the recorded data in the experiment, compared with the control. Other recent studies point out sample heterogeneity where sub-samples are taken from the experiment jars and not replaced to be a source of continuous variations (Cornejo et al. 2019; Polazzo et al. 2022). For future research, increasing replicates will solve the increased variability.

Results

Experiment control

In the control treatment of the experiment, there were no added nutrients or pesticides. The control was exposed to the same environmental conditions as the experimental treatments. Therefore, the results (Table 2) indicate the status of *D. magna* abundance, and algal biomass, with no additives.

The findings indicate that abundance of *D. magna* increased over exposure time with the highest abundance of 4583 ± 1700 Ind L^{-1} at 120 h of exposure. A One-Way ANOVA was performed to compare the effect of exposure time on the abundance of *D. magna*. The One-Way

Table 2 Control results for *D. magna* abundance, and algal biomass

Time Step	D. magna Abundance (Ind/L) n = 32			Algal Biomass (mg/L) n = 32		
0 h	1278	±	161	0.051	±	0.008
24 h	1917	±	629	0.028	±	0.001
48 h	1750	±	479	0.027	±	0.012
72 h	3083	±	1380	0.145	±	0.060
96 h	2667	±	882	0.022	±	0.007
120 h	4583	±	1700	0.015	±	0.002
144 h	4084	±	1980	0.02	±	0.005
168 h	3584	±	1200	0.029	±	0.013

ANOVA revealed that there was no statistically significant difference in the abundance of *D. magna* with exposure time ($F(7,24) = 0.957$, $p = 0.483$).

A One-Way ANOVA performed on the algal biomass over exposure time revealed that there was a statistically significant difference in the algal biomass over exposure time ($F(7,24) = 3.687$, $p < 0.01$). Further Tukey's HSD Test for multiple comparisons revealed a difference between the 72 h exposure time-step compared with 24 h ($P = 0.020$, 95% CI [0.0125, 0.221]), 48 h ($P = 0.019$, 95% CI [0.0132, 0.221]), 96 h ($P = 0.013$, 95% CI [− 0.226, − 0.0182]), 120 h ($P = 0.008$, 95% CI [− 0.2338, − 0.0251]), 144 h ($P = 0.011$, 95% CI [− 0.2291, − 0.0203]), and 168 h ($P = 0.022$, 95% CI [− 0.2198, − 0.0111]). Algal biomass showed a dynamic change over exposure time, with highest algal biomass (0.145 ± 0.06 mg L^{-1}) recorded within 72 h.

Nutrients only treatment

For the nutrients-only treatment, nitrogen and phosphorus were added to the medium. The abundance of *D. magna* fluctuated during the exposure period, with the highest abundance of 2333 ind L^{-1} recorded after 48 h of exposure within the high concentration of nutrients in the treatment (Fig. 1). This peak explains the fluctuations in the algal biomass for the high concentration treatment, which showed a peak reduction in biomass with peak in *D. magna* abundance (Fig. 2). The findings further indicate that as nutrients concentration increased, the peak *D. magna* abundance was recorded earlier, while the lowest algal biomass was delayed with reduced nutrients. Statistically however, there was no significant difference in *D. magna* abundance across the nutrient concentrations ($F_{(3,122)} = 1.311$, $p = 0.274$) and exposure times ($F_{(7,118)} = 1.825$, $p = 0.0886$). Similarly, One-Way ANOVA indicated that there was no statistically significant difference among the exposure concentrations ($F_{(3,124)} = 0.283$, $p = 0.838$) and over the exposure time for algal biomass ($F_{(7,120)} = 1.007$, $p = 0.43$).

A simple linear regression was used to test if algal biomass significantly predicted *D. magna* abundance. The fitted regression model was: Algal biomass $= 8.885 \times 10^{-2} - 1.911 \times 10^{-5} \times$ *D. magna* abundance. The overall regression was statistically significant ($R^2 = 0.06$, $F_{(1,\ 124)} = 9.025$, $p < 0.01$). It was established that algal biomass in the nutrients-only treatment significantly predicted *D. magna* abundance ($\beta = - 1.911 \times 10^{-5}$,

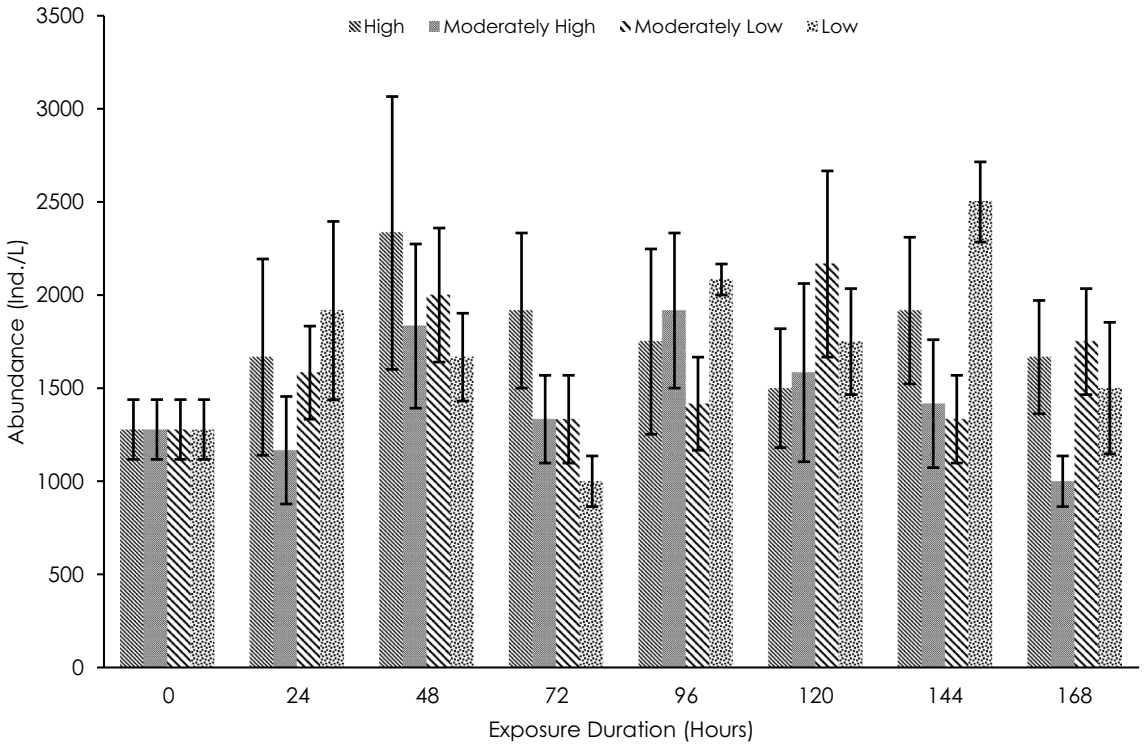

Fig. 1 Abundance of *D. magna* across the Nutrients-only treatment (error bars indicate standard error of mean). Nutrient-only treatment with low (0.233 mmol L^{-1} nitrogen and 0.026 mmol L^{-1} phosphorus), moderately low (0.467 mmol L^{-1} nitrogen and 0.053 mmol L^{-1} phosphorus), moderately high (0.7 mmol L^{-1} nitrogen and 0.079 mmol L^{-1} phosphorus) and high (0.933 mmol L^{-1} nitrogen and 0.105 mmol L^{-1} phosphorus) concentrations

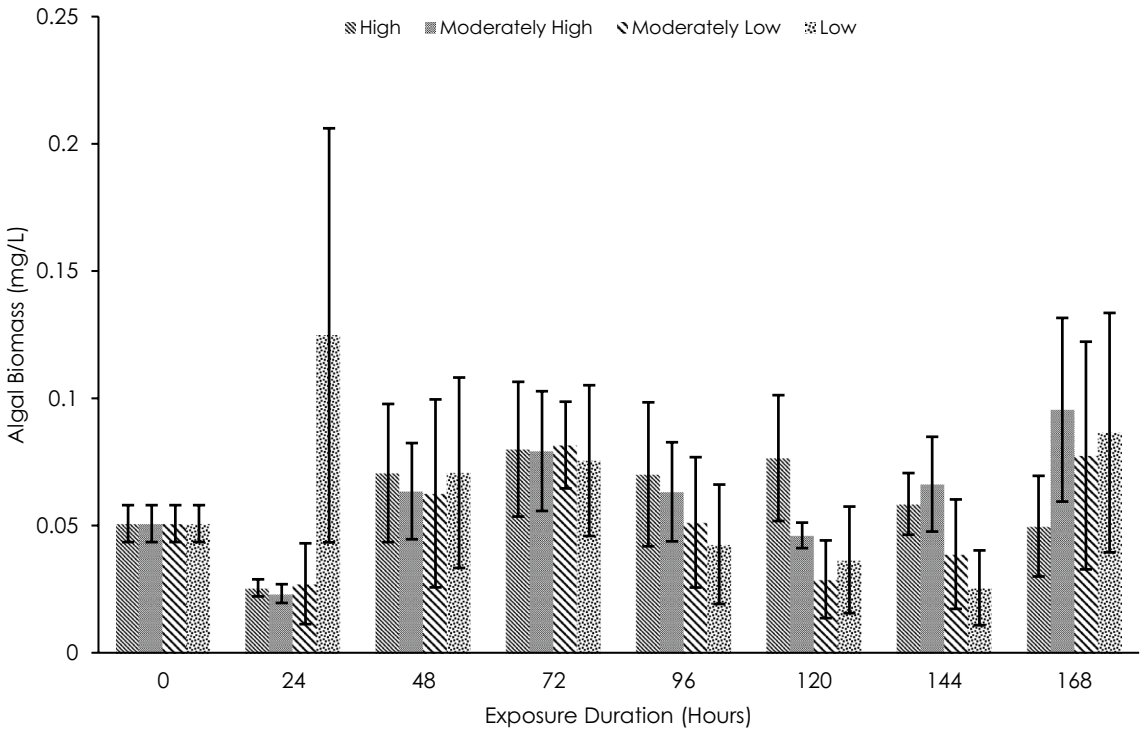

Fig. 2 Algal biomass average in the Nutrients-only treatment (error bars indicate standard error of mean). Nutrient-only treatment with low (0.233 mmol L^{-1} nitrogen and 0.026 mmol L^{-1} phosphorus), moderately low (0.467 mmol L^{-1} nitrogen and 0.053 mmol L^{-1} phosphorus), moderately high (0.7 mmol.L^{-1} nitrogen and 0.079 mmol L^{-1} phosphorus) and high (0.933 mmol L^{-1} nitrogen and 0.105 mmol L^{-1} phosphorus) concentrations

$p < 0.01$), suggesting that the higher the *D. magna* abundance, the lower the algal biomass.

Pesticides-only treatment

In the study, the response of *D. magna* abundance, and algal biomass against a pesticides-only treatment was carried out, with results presented in Figs. 3 and 4. The findings indicate that the higher the concentration of pesticide additives, the lower the abundance of *D. magna* (see Fig. 3), while the abundance increased over exposure time irrespective of the exposure concentration. On the other hand (see Fig. 4), with increased exposure time, the algal biomass reduced, while higher pesticides concentrations stimulated algal biomass.

A One-Way ANOVA analysis to compare the abundance of *D. magna* over exposure time, and varying exposure concentrations revealed that there was a statistically significant deference in the mean of *D. magna* over the exposure concentrations ($F_{(3,\ 124)} = 4.447$, $p < 0.01$), while there was no significant deference in the means of *D. magna* abundance over the exposure time ($F_{(7,\ 120)} = 1.931$, $p = 0.0704$). Turkey's HSD Test for multiple comparisons found that the mean value of *D. magna* abundance was significantly different between the low and high concentrations in the nutrients-only treatment ($p = 0.009$, 95% CI [343.074, 3302.737]).

On the other hand, a One-Way ANOVA analysis to compare the algal biomass over exposure concentrations revealed no significant differences in the mean of algal biomass ($F_{(3,\ 124)} = 2.197$, $p = 0.091$). Analysis to compare algal biomass over exposure time, however, revealed statistically significant differences in the mean of algal biomass over time ($F_{(7,\ 120)} = 11.71$, $p < 0.000$). Turkey's HSD Test for multiple comparisons indicated that the mean algal biomass was significantly different between the 24 h exposure timestep compared with 0 h ($P = 0.000$, 95% CI [0.0805, 0.2435]), 48 h ($P = 0.000$, 95% CI = [− 0.2517, − 0.0887]), 72 h ($P = 0.000$, 95% CI = [− 0.2387, -0.0757]), 96 h ($P = 0.000$, 95% CI [− 0.2703, − 0.1073]), 120 h ($P = 0.000$, 95% CI = [− 0.2727, − 0.1098]), 144 h ($P = 0.000$, 95% CI = [− 0.2676, -0.1046]), and 168 h ($P = 0.000$, 95% CI = [− 0.2654, − 0.1025]).

The fitted regression model to test whether algal biomass in the pesticides-only treatment significantly predicts *D. magna* abundance was: Algal biomass = $7.680 \times 10^{-2} - 6.617 \times 10^{-6} \times$ *D. magna* abundance. The overall regression was not statistically significant ($R^2 = 0.01$, $F_{(1,\ 126)} = 3.571$, $p = 0.061$). It was established

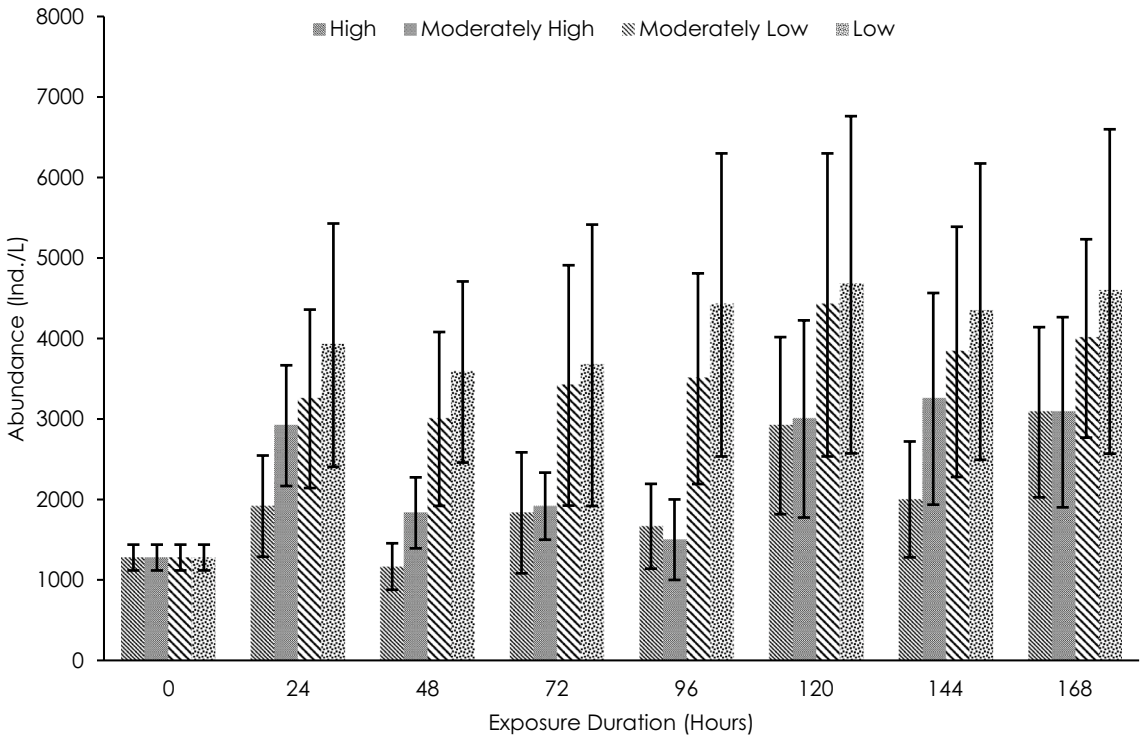

Fig. 3 Abundance of *D. magna* across the Pesticides-only treatment (error bars indicate standard error of mean). Pesticides-only treatment with low (5.61×10^{-8} nmol L^{-1} sum OCP), moderately low (1.18×10^{-7} nmol L^{-1} sum OCP), moderately high (2.53×10^{-7} nmol L^{-1} sum OCP) and high (4.86×10^{-7} nmol L^{-1} sum OCP) concentrations

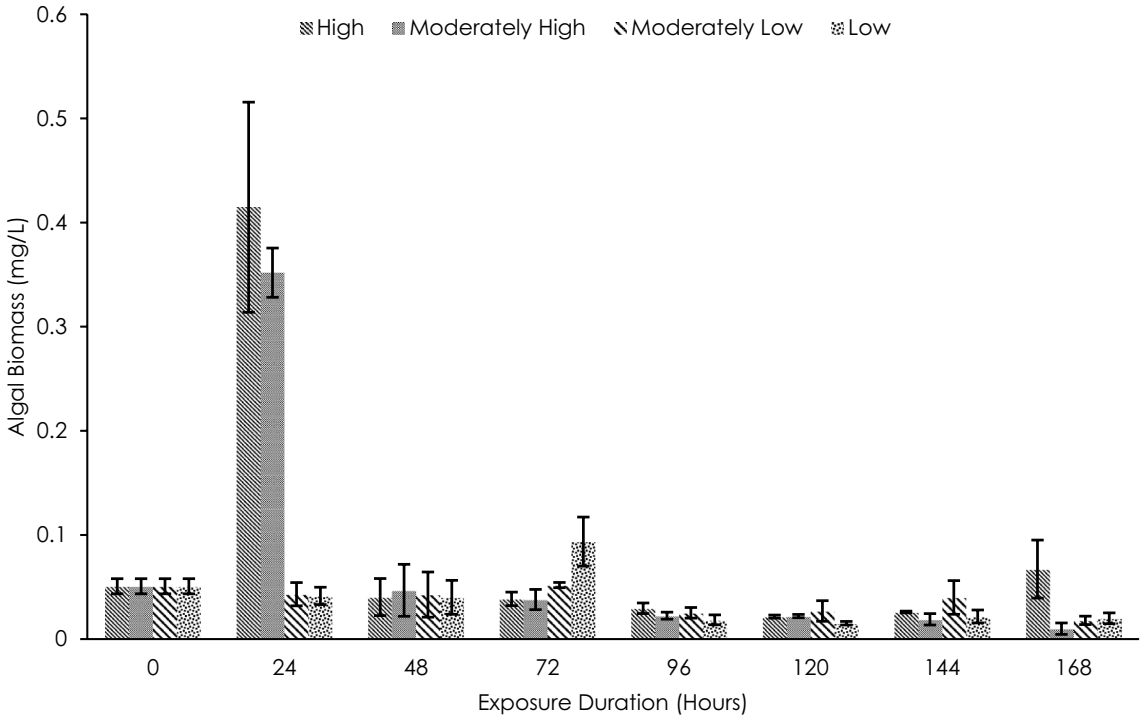

Fig. 4 Algal biomass across the Pesticides-only treatment (error bars indicate standard error of mean). Pesticides-only treatment with low (5.61×10^{-8} nmol L^{-1} sum OCP), moderately low (1.18×10^{-7} nmol L^{-1} sum OCP), moderately high (2.53×10^{-7} nmol L^{-1} sum OCP) and high (4.86×10^{-7} nmol L^{-1} sum OCP) concentrations

that algal biomass in the pesticides-only treatment was not able to significantly predict *D. magna* abundance ($\beta = -6.617 \times 10^{-6}$, $p = 0.061$) suggesting that when using pesticides-only, *D. magna* abundance, would not be predicted from algal biomass.

Combined nutrients and Pesticides treatment

The results from the combined treatment with both nutrients and pesticide additives are presented in Figs. 5 and 6. The results presented in Fig. 5, from the combined treatment, indicate that with increased exposure time, there is reduced *D. magna* abundance. At the same time, while higher concentrations recorded higher *D. magna* abundance with increased exposure period, there was no significant difference in the mean of *D. magna* abundance among the different exposure concentrations ($F_{(3, 77)} = [0.711]$, $p = 0.548$), nor in the varying exposure durations ($F_{(7, 73)} = 2.016$, $p = 0.064$) based on One-Way ANOVA analysis.

Although algal biomass (see Fig. 6) showed no observable specific trend over exposure time, One-Way ANOVA analysis revealed a significant differences in the mean of algal biomass over experiment duration ($F_{(7, 120)} = 2.763$, $p < 0.05$), with the Turkey's post hoc test revealing significant mean differences between 72 h time step compared with 24 h ($P = 0.014$, 95% $CI = [0.0069, 0.1077]$), and 120 h ($P = 0.025$, 95% $CI = [-0.1045, -0.0037]$). However, there was no significant difference of the algal biomass mean, over different exposure concentrations ($F_{(3, 124)} = 0.6$, $p = 0.616$) based on One-Way ANOVA analysis.

At the same time, algal biomass reached a peak after 72 h and after 144 h, coinciding with reduction of *D. magna* abundance. This supports the findings from simple linear regression fitted to use algal biomass to predict *D. magna* abundance. The fitted model was Algal biomass $= 9.741 \times 10^{-2} - 3.049 \times 10^{-5} \times$ *D. magna* abundance. The overall regression was statistically significant ($R^2 = 0.05$, $F_{(1, 79)} = 5.72$, $p = 0.019$). It was established that algal biomass in the combined treatment was able to significantly predict *D. magna* abundance ($\beta = -3.049 \times 10^{-5}$, $p < 0.05$) suggesting that when using combined nutrients and pesticides pollution concentrations, an increase in *D. magna* abundance, would be predicted by a reduction of algal biomass.

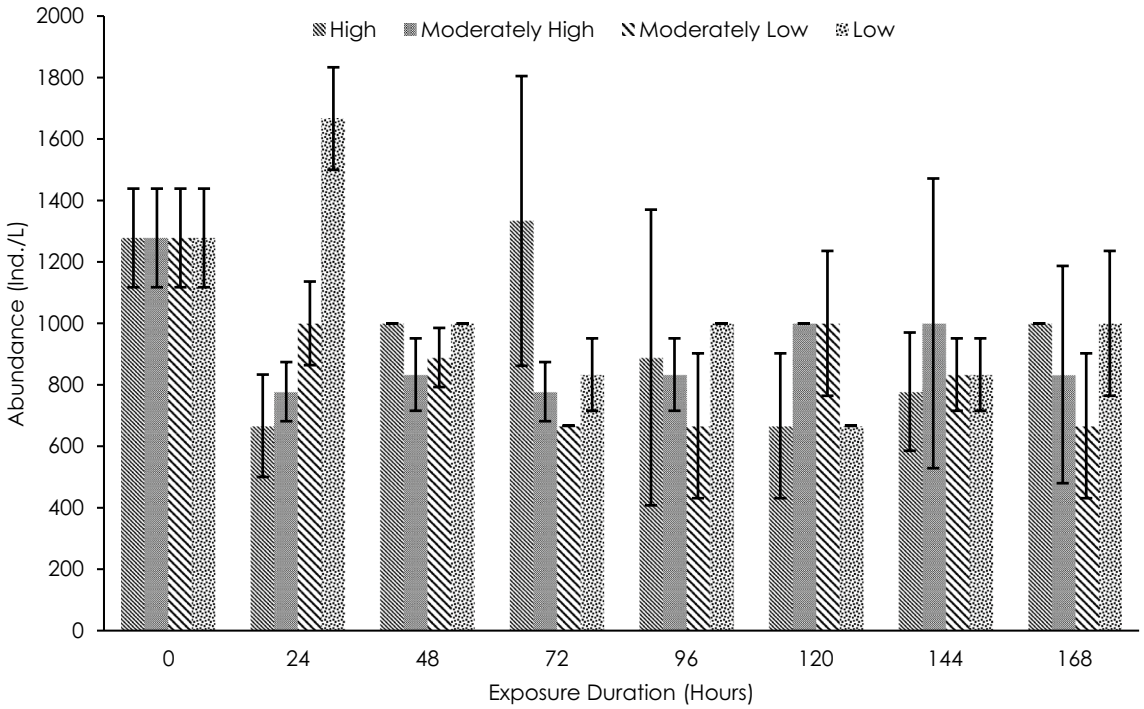

Fig. 5 Abundance of *D. magna* across the Combined Nutrients and Pesticides treatment (error bars indicate standard error of mean). Combined Nutrients and Pesticides treatment with low (0.233 mmol L^{-1} nitrogen, 0.026 mmol L^{-1} phosphorus and 5.61×10^{-8} nmol L^{-1} sum OCP), moderately low (0.467 mmol L^{-1} nitrogen, 0.053 mmol L^{-1} phosphorus and 1.18×10^{-7} nmol L^{-1} sum OCP), moderately high (0.7 mmol L^{-1} nitrogen, 0.079 mmol L^{-1} phosphorus and 2.53×10^{-7} nmol L^{-1} sum OCP) and high (0.933 mmol L^{-1} nitrogen, 0.105 mmol L^{-1} phosphorus and 4.86×10^{-7} nmol L^{-1} sum OCP) concentrations

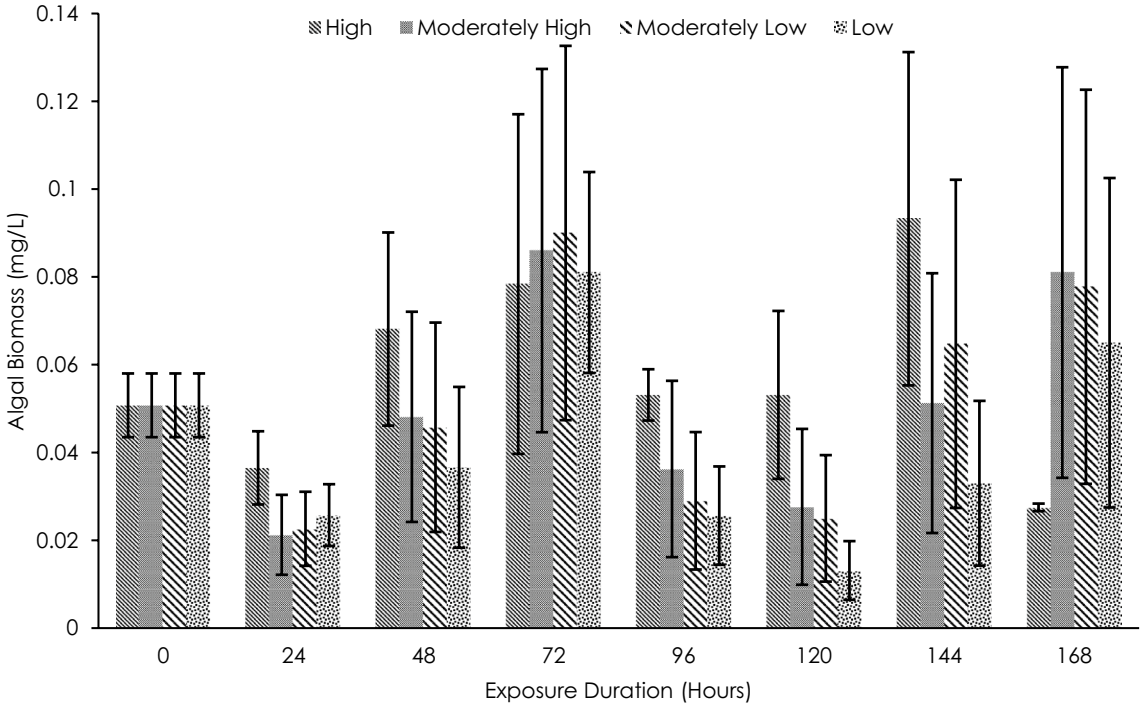

Fig. 6 Algal biomass across the Combined Nutrients and Pesticides treatment (error bars indicate standard error of mean). Combined nutrients and pesticides treatment with low (0.233 mmol L^{-1} Nitrogen, 0.026 mmol L^{-1} Phosphorus and 5.61×10^{-8} nmol L^{-1} sum OCP), moderately low (0.467 mmol L^{-1} Nitrogen, 0.053 mmol.L^{-1} Phosphorus and 1.18×10^{-7} nmol.L^{-1} sum OCP), moderately high (0.7 mmol L^{-1} Nitrogen, 0.079 mmol L^{-1} Phosphorus and 2.53×10^{-7} nmol L^{-1} sum OCP) and high (0.933 mmol L^{-1} Nitrogen, 0.105 mmol L^{-1} Phosphorus and 4.86×10^{-7} nmol L^{-1} sum OCP) concentrations

Relationship among treatments

A multiple ANOVA (MANOVA) analysis was performed to identify any statistically significant differences among the means of the three treatments and the control, for *D. magna* abundance and algal biomass. The results revealed a significant difference among the treatment for *D. magna* abundance ($F_{(3,\ 363)} = 27.97$, $p < 0.000$), with a follow-up Turkey HSD test indicating that the control and the pesticide-only treatment were not significantly different ($P = 1.000$, 95% CI = [− 830.446, 830.368]). The nutrients-only and the combined treatments were significantly different compared with the other treatments (see Table 3).

Table 3 Comparative results from MANOVA of the treatments

Compared treatments		Diff	Lower	Upper	p-adjusted	
Nutrient-only[b]	Control[a]	− 1237.59	− 2069.31	− 405.86	0.0008	***
Combined treatment[c]	Control[a]	− 1891.37	− 2768.64	− 1014.10	0.0000	***
Pesticide-only treatment[a]	Control[a]	− 0.04	− 830.45	830.37	1.0000	
Combined treatment[c]	Nutrient-only[b]	− 653.79	− 1252.15	− 55.42	0.0260	*
Pesticide-only treatment[a]	Nutrient-only[b]	1237.55	710.27	1764.82	0.0000	***
Pesticide-only treatment[a]	Combined treatment[c]	1891.33	1294.80	2487.87	0.0000	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

a – group a (Pesticide-only treatment and Experiment Control)

b – group b (Nutrient-only treatment)

c – group c (Combined Nutrients and Pesticides treatment)

The study indicated no significant difference among the treatments for algal biomass 9. This indicates that across treatments, only marginal differences were recorded on algal biomass

Discussions

Nutrient only treatment

The highest *D. magna* abundance was realised after a short time of exposure to high concentration of nutrients, and coincident with reduced algal biomass. High phosphorus stimulates phytoplankton production (Muylaert et al. 2010), as also demonstrated in this study by the positive correlation of algal biomass with nutrient availability (Gusha et al. 2019; Kim et al. 2016; Stevenson et al. 2006). However, in this study, longer exposure period in higher nutrients concentrations resulted in reduced algal biomass, logically explained by the increase in *D. magna* abundance, and commensurate with increased grazing pressure. Grazing pressure on phytoplankton varies with zooplankton species composition (Kagami et al. 2002). In our experiments reduced concentrations of nutrients delayed attainment of lowest algal biomass likely because of reduced grazing pressure, while grazer densities responded positively to nutrient enrichment (Roll et al. 2005). Furthermore, as phosphorus is a limiting factor to both phytoplankton and *Daphnia* populations, high nitrogen to phosphorus ratio in algal diets may limit the growth of grazing zooplankton (Guo et al. 2019). This can explain the reduction of *D. magna* abundance after longer exposure times at higher N:P ratios.

Autotrophic primary production and heterotrophic respiration are influenced by the availability of nutrients (Dodds and Smith 2016). Within the first 24 h of exposure, the study resulted in a large increase in algal biomass. Simultaneously, increases in algal productivity along with *D. magna* abundance, results in high respiration rates, and reduced dissolved oxygen concentrations, and can have a negative effect on grazer abundance (Munn et al. 2010). Nutrient enhancement led to higher algal biomass in the nutrients-only treatment compared with the control, and the pesticides-only and combined treatments. However, further increase in concentration within the nutrients-only treatment resulted in decreases in algal biomass (Dodds and Smith 2016; Rabalais 2009). That the nutrients-only treatment had lower *D. magna* abundance in comparison with the control, likely reflects the effect of very high algal biomass on inhibiting *D. magna* grazing through clogging of the filtering apparatus or negative reaction of the *Daphnia* through possible algal toxicity (Boudry et al. 2020; Sarnelle et al. 2010). The net result was lower food availability for the grazers with increased trophic state of the water (Chislock et al. 2013; Hiltunen et al. 2021; Pinto-Coelho et al. 2006; Rabalais 2009). In aquatic ecosystems, availability of nutrients, especially nitrogen and phosphorus, favour primary productivity (FAO 1996), but high primary producer densities enhance the respiratory demand on aquatic ecosystems limiting primary productivity (Chislock et al. 2013; Koelmans et al. 2001).

Pesticides treatment

Pesticide additives reduced the abundance of *D. magna* over periods of exposure. Increased pesticides concentrations poisons and reduces zooplankton abundance, and therefore the potential grazing pressure on the algae. However, over time, the dynamics of the algal population is limited by the nutrients, thereby reducing the algal biomass. Bengtsson et al. (2004) recorded significant reduction in the grazing rate of *D. pulex* with exposure to dichlorordiphenyldichloroethylene (DDE) insecticide and enhanced growth of the algae in response to phosphorus and nitrogen glycophosate excreted by the death of the zooplankton Roessink et al. (2008). The pesticide toxicity on *D. magna* results in higher algal biomass. The pesticides-only treatment showed eutrophication-like effects (Roessink et al. 2008) through poisoning of the grazers and reducing the grazing pressure and increasing algal biomass. This was demonstrated by the increase in algal biomass with increasing concentration in the pesticides-only treatment (Camargo and Alonso 2006; O´Toole and Irvine 2006). The high *D. magna* abundance in the low concentrations of the pesticide-only treatment compared with the nutrients-only and the combined treatment is attributable to pesticides poisoning as a disturbance, triggering proliferation (Hose and Guillette 1995). However, the higher the concentrations, the lower the abundance, suggesting that the disturbance from poisoning is increased, and the ability of *D. magna* to recover diminished as a result of increased poisoning (Czub and McLachlan 2004).

Combined treatment

Hegde et al. (2014) established low zooplankton diversity and density when there is a combination of pesticides with fertilisers. The argument being that pesticides cause selective toxicity in algae which reduces invertebrate feeding. Traas et al. (2004) on the other hand, using ecotoxicology models, indicated that nutrient additions alone caused little effects on the fate of the toxicant and the ecological effects were due to the relatively high rate at which pesticides are distributed in the environment. Pesticides can reduce trophic transfer of energy to grazers and predators (Hanazato 2001) resulting to lower abundance in the higher trophic levels. In contrast, Baker et al. (2016) reported that combination of nutrients and herbicides increased abundance of zooplankton, indicating the possibility of differences between model predictions and field experiments as part of risk assessment in ecotoxicology. In our experiment, a combination of nutrients and pesticides increased algal biomass compared

with the control, probably due to the decrease in the *D. magna*.

The combined nutrient-pesticide residue treatment had lower algal biomass compared with the nutrient-only or pesticides-only treatment. This supports the arguments by Kortenkamp et al. (2009) of nutrients-pesticides interaction in aquatic ecosystems. Further aligns with conclusions by Polazzo et al. (2022) arguing that nutrients enrichment is a key factor influencing the resilience of freshwater ecosystems to multiple stressors. This trend was similar to the pesticides-only treatment; therefore, the effects of combined nutrients and pesticides are attributable more to pesticides, rather than nutrients, in the aquatic ecosystem. Although some studies (SCHER et al. 2012) argue that such interactions are either synergistic or antagonistic, this study concluded that the concentrations of the combined contaminants determine the type of interaction between nutrients and pesticides. Compared with the pesticides-only treatment, an antagonistic interaction was evident at lower concentrations, while synergism developed in higher concentrations. The antagonism at lower concentrations can be attributed to biomass dilution, where nutrients enrichment accelerates the growth of algal biomass which take up or adsorb the pesticides, reducing the expected effect (Skei et al. 2000). Cornejo et al. (2019) in their study focusing on multiple stressors on macroinvertebrates reported that most stressors showed antagonistic interactions (i.e., lower combined effects than expected from their individual effects). With increasing combined concentration however, eutrophication as a result of the higher nutrients concentrations, and eutrophication-like characteristics mediated by pesticides (Roessink et al. 2008), results in nutrient enrichment reducing algal biomass. Compared with the nutrients-only treatment, the algal biomass was lower in combined treatment irrespective of the concentration. The combined nutrients and pesticides show a synergistic interaction with respect to nutrients-only contamination. While an increase in nutrients concentrations results in a reduction of algal biomass, an increase in combined concentration results in an increase. This shows that combined contamination has a higher effect to algal biomass than nutrients-only treatment and determining nutrients-only would be an under estimation of the effects on algal biomass in an agricultural catchment (Koelmans et al. 2001).

In the combined nutrients and pesticides treatment, the abundance of *D. magna* was lower compared with the nutrients-only and the pesticides-only treatments. As the combined concentration increased, there was a reduction in the abundance of *D. magna*. The higher combined concentration resulted in lower food quality and availability for *D. magna* (Roessink et al. 2008). Coupled with *D. magna* poisoning, poor food quality and availability results in the low abundance in the combined nutrients and pesticides treatment, compared with the pesticide-only and nutrient-only treatments (Alexander et al. 2016; Koelmans et al. 2001; Schweiger and Jakobsen 1998). Essentially, combined contamination results in a synergistic effect on *D. magna* abundance compared with the nutrients-only or pesticides-only contamination. As such, effects on *D. magna* abundance focusing on either pesticides or nutrients underestimate the potential effects to the ecosystem.

Conclusions

Ecological processess such as eutrophication and grazing have a significant effect on the outcome of combined nutrients and pesticides contamination through bottom-up and top-down control, respectively. It is important to have studies relating these processes to combined (nutrients and pesticides) contamination both to be able to determine the concentrations at which the aquatic biota are affected (effect concentrations), and the magnitude of such effects.

Determination of effect concentrations based on combined nutrients-pesticides experiments becomes important in setting water quality standards, and monitoring quality status. Without these types of experiments, water quality professionals are not able to monitor the quality of water systems effectively, because of the likelihood of under or over estimation of the effect. This is evidenced by the fact that in this experiment, combined contamination yields different results compared with stand alone nutrients and pesticide treatments. If water quality managers are not able to use this kind of information, it means that the quality that is reported would have undetected ecological effects.

This study makes two important conclusions. First, the estimation of water quality in agricultural catchment requires consideration of both nutrients and pesticides. as these have potential for both individual and combined effects on ecosystems health. Such a combined assessment guides agriculture and water management. Second, current water quality indicators need revision to account for combined contamination to set acceptable water quality thresholds. Single contaminant approaches overlook the interaction and potential cumulative effects of combined contaminants.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40068-023-00326-3.

Additional file 1. Full experiment data for the study with the control and treatments.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Joel Onyango. The first draft of the manuscript was written by Joel Onyango and all authors commented on previous versions of the manuscript. All authors read and approved the revised manuscript.

Funding
This work was supported by Royal Netherlands Academy of Arts and Sciences (KNAW). Author Joel Onyango has received research support from WWF Kenya.

Data Availability
All data supporting the findings of this study are available within the paper and its supplementary information, in the Additional file 1.

Declarations

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Received: 26 September 2023 Accepted: 30 November 2023
Published online: 04 January 2024

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