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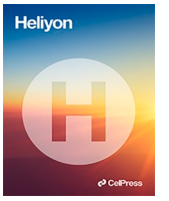

Assessment of the impact of the biological larvicide VectoMax G: Combination of *Bacillus thuringiensis* and *Lysinibacillus sphaericus* on non-target aquatic organisms in Yaoundé-Cameroon

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ABSTRACT

There has been a renewed interest for larviciding during the recent decade. Although biological larvicides are considered not to be harmful to non-target organisms, there is still not sufficient data on the effect of new long-lasting larvicide formulations such as VectoMax G combining *Bacillus thuringiensis israelensis* and *Lysinibacullus sphaericus* on the environment especially on non-target organisms. The present study aimed to assess the possible influence of VectoMax G on the diversity and abundance of the aquatic fauna cohabiting with mosquito larvae in breeding habitats during a larviciding trial in the city of Yaoundé.

Twelve districts of the city of Yaoundé divided into 6 intervention and 6 control sites were chosen for the study. In each district 4 semi-permanent or permanent aquatic habitats were followed. VectoMax G application was done once every two weeks during 6 months and aquatic organisms were collected 48 h after each treatment. All collected organisms were brought to the laboratory for identification. Physico-chemical parameters were recorded as well.

A high diversity of the zooplankton was recorded in the intervention areas with 28 species collected against 14 species in the control areas. Cladocerans were the most represented group in both sites while Ostracods were found only in control sites. A total of 19 macro-invertebrates species were recorded in the control areas vs 16 species in the intervention areas. Gasteropods were the most represented groups of macro-invertebrates. Vertebrates such as larvivorous fishes and amphibians larvae were also found in approximately similar densities in both sites.

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The study indicated no significant influence of larviciding with VectoMax G on the diversity and abundance of the non-target aquatic fauna in the city of Yaoundé.

1. Introduction

The primary focus of malaria vector control interventions is the reduction/stoppage of malaria transmission through the reduction of adult densities or longevity. Many approaches including physical, insecticidal or biological methods could be used for controlling vector populations [1,2,3]. At present, malaria vector control relies heavily on the use of insecticide based interventions with long-lasting insecticidal nets (LLINs) and/or indoor residual spraying (IRS) as main tools [4]. However, widespread use of insecticide-based interventions has resulted in the emergence and expansion of insecticide resistance in malaria vectors to all classes of insecticides [4,5]. As a result of insecticide selection pressure, malaria vectors have changed their biting and resting behavior from indoor to outdoor [6,7,8]. Shift or replacement of species by others has also been reported [9]. For an effective control of vector populations, there is a need to use alternative control measures to complement existing control measures to improve malaria vector control.

Larviciding through the use of microbial larvicides such as *Bacillus thuringiensis* var. *israelensis* (Bti) and Lysini*bacillus sphaericus (formerly Bacillus sphaericus)* (Ls) is increasingly being used for controlling malaria vectors. This method has been reported to selectively kill mosquito larvae with negligible effect on non-target organisms cohabiting with mosquito larvae in aquatic habitats [10]. Different organisms such as zooplankton, macroinvertebrates and vertebrates cohabit with mosquito larvae in aquatic habitats [11,12, 13]and play critical role in regulating the aquatic stages of mosquitoes through predation and competition [13,14,15]. These aquatic organisms also play key role in the food chain.

Previous studies suggested that microbial larvicides such as Bti and Ls were harmless to almost all non-target organisms when applied at recommended doses [16,17]. However, the development of new larvicide formulations containing Bti toxic crystals lasting longer in the environment, has raised some concerns about intensive applications that could lead to accumulation of toxins with adverse effect on non-target organisms [18,19,20,21]. Additional studies monitoring the long-term impact of biocontrol agents on the ecosystem and species diversity are therefore necessary.

In the present study, we investigated whether the application of VectoMax G, a larvicide combining Bti var. *israelensis* and L. *sphaericus* in aquatic habitats has affected the abundance and diversity of non-target aquatic fauna such as zooplanktons, macroinvertebrates, fishes and amphibians larvae in the city of Yaoundé.

2. Material and methods

2.1. Study sites

The study was conducted in 12 districts of Yaoundé (3°43′00″ to 3°58′00″ N and 11°24′30″ to 11°34′30″ E). Yaoundé is the capital city of Cameroon covering a surface area of 304 km^2 with about 3 million inhabitants. Yaoundé is situated between 700 and 750 m above sea level. The city is drained by several permanent streams and is situated within the Congo-Guinean phytogeographic zone [22]. The climate is of equatorial type with four seasons (two rainy seasons [September–November and March–June], two dry seasons [December-February and July–August]) [23]. The average rainfall in Yaoundé is estimated at 1688 mm/year, the average annual temperature is 26.31 °C varying between 16 and 33 °C depending on the season. The average humidity is 80% and varies during the day between 35 and 98% [24]. The city is exposed to frequent humid winds blowing south-west to west or north to west [25].

2.2. Sampling of permanent and semi-permanent aquatic habitats

The study was conducted in 12 districts of Yaoundé divided into 6 intervention districts and 6 control districts. In each district, 4 semi-permanent or permanent aquatic habitats were selected. Sampling of breeding habitats was undertaken twice a month from July to December 2020. Most of the breeding habitats were situated in lowland areas and closed to rivers.

2.3. Physico-chemical characterization of breeding sites

Total dissolved solids, pH, temperature, concentrations of sulfates, organophosphates, Hydrogen peroxide (H_2O_2), turbidity, iron and calcium were measured as described in Djamouko-Djonkam et al. [26].

2.4. Larvicide application

The larvicide used for the treatments was VectoMax G (Valent Biosciences Corporation, USA) a granule formulation (CG) containing as active ingredients both *Bacillus thuringiensis israelensis* (Bti), strain AM65-52 (45 g/kg) and *Lysinibacillus sphaericus* (Lsph) strain ABTS-1743 (27 g/kg). VectoMax G is considered having 50 Bsph international toxic units per mg of the product. Dose use for the treatment of water collection varied from 500 to 1500 mg/m^2 as recommended by WHO [27]. The larvicide was applied once every

two weeks in the intervention districts. Non-target aquatic fauna were collected 48 h after each application in both non-intervention and intervention areas.

3. Evaluation of the impact of VectoMax G on non-target aquatic fauna

3.1. Sampling, identification and conservation of the zooplankton

A volume of 50–100 L of water depending on the habitats size varying from 1 m^2 to 10 m^2 was collected using a clear bucket of 10 L rinsed with tap water. Water samples collected were then filtered using a sieve of 10 μm mesh. The filtered organisms were removed from the filter mesh using 100 mL of sampled water and then stored in glass containers. Filtrates were fixed with 5% formalin and species were identified and counted under a microscope.

The process included identification and counting of zooplankton individuals using a Wild M5 binocular magnifier at 250× and 500× magnifications and an Olympus CK2 UL WCD 0.30 microscope; 10 ml of sample were taken after homogenization with a bulb and introduced into a Dollfus cell for zooplankton counting [28].

3.2. Identification of Rotifera

For some species belonging to the Rotifera class who could not be easily identified morphologically, we used the mastax by dissolving the tissues with sodium hypochlorite (bleach) following the techniques described elsewhere [29,30].

3.3. Identification of Copepoda and cladocerans

The identification of Copepoda was conducted by detaching the abdomen, the thoraxic appendages the antennae and the antennules of each individual which play a fundamental role in systematics. In some cases, it was necessary to observe the hip or the distal articles of these appendages in order to study the ornaments as done by Van der Velde [31] and Dussart [32]. Concerning cladocerans, individuals were dissected and appendages, post abdomen and the cephalic carapace were observed using the binocular magnifying glass WILD M5 according to Smirnov [33] and Dodson [34].

3.4. Quantification of the zooplankton

Counts were made following the technique of Frontier [35], Legendre and Watt [36]. Dolfuss cells, rectangular glass chambers (100 × 200 mm) whose inner bottom is subdivided into 200 cubic concavities each measuring 5 mm on a side for a volume of 25 ml were used to count zooplankton individuals.

The concavities (5 × 5 mm) and small squares were small enough to be observed through 1 or 2 fields of microscopic vision with the WILD M5 binocular loupe with which the counts were made. These counts were made at least in triplicate on samples taken with a bulb. This gives an estimation of 20–60% of the sample counted [37].

When the bottoms of the tanks were crowded, dilutions to 1/4 or 1/5 were made. Whenever possible during the enumeration, at least 200 individuals were counted per replicate. If not, additional fractions were added until the sample is exhausted. Lund et al. [38] estimated that a count of 16 individuals would give 50% accuracy and concluded that counting 100 individuals would be more than enough per sample.

3.5. Sampling of non-target aquatic macrofauna

Sampling of macrofauna was done according to the multi-habitats approach as described by Stark [39] which consists of sampling the substrate, the water column, and the aquatic plants [40] over the entire area of the water plan, using square-shaped traps of 15 cm on each side, each fitted with a conical net with a mesh opening of 400 μm. Organisms retained by the mesh of the net were collected with forceps and fixed in polyethylene bottles containing 10% formalin and brought back to the laboratory. Sampling of macrofauna was conducted five times: one on each side of a squared pond and one on the middle.

3.6. Identification and enumeration of aquatic macroinvertebrates

In the laboratory, samples of aquatic macroinvertebrates fixed in the field were rinsed with running water. After rinsing, these samples were preserved in 70% alcohol for conservation and subsequent identification. The collected individuals were grouped according to their morphological resemblance, and then identified to taxa using a Leica binocular magnifier and the identification key to Tachet [41].

3.7. Identification of aquatic vertebrates

Aquatic vertebrates considered here were amphibian larvae and larvivorous fishes. They were identified through direct observations. The estimation of the density of these vertebrates was done by counting the number of organisms present in the water pool of a known size.

3.8. Data analysis

The Microsoft Excel 2010 Software was used to draw Histograms of zooplanktons and macrofauna classes abundance in each study sites. The Student T-test was performed to compare the mean concentrations of physico-chemical elements in the water of the breeding sites in control and intervention sites using MedCalc statistical software version 15.8 (MedCalc software bvba, Ostend Belgium; https://www.medCalc.org; 2015). At the significance level of 5%. The Shannon-Weaver diversity index, the Pielou equitability index and the Sorensen similarity indexes were calculated using Past software 3.12 (Paleontological Statistics software package for education and data analysis, 2001. Paleontogia Electronica. 4(1): 9pp). The R software version 3.5.2 (R Core Team (2018) R Foundation for Statistical Computing, Vienna, Austria URL https://www.R-project.org/) was used to do the principal component analysis and compare the diversity of zooplanktons and macrofauna between control and intervention sites. The significance level was fixed at $\alpha < 0.05$.

4. Results

4.1. Physico-chemical characteristics of breeding sites in control and intervention sites

Up to sixteen physico-chemical parameters were measured (Table 1). Water temperature, pH, and oxydability were significantly higher in the breeding sites situated in the control areas compared to breeding habitats situated in intervention sites ($t = -3.468$; $p < 0.05$). No significant variation in the concentrations of physicochemical parameters was observed in aquatic habitats situated in both, control and intervention areas.

4.2. Zooplankton abundance in control and intervention sites

Four classes of zooplanktons were collected and identified: Rotifera, Cladocera, Ostracoda and Copepoda. Ostracoda were recorded only in the control sites whereas the others classes were recorded in both sites. The abundance of Rotifera and Copepoda were much higher in the intervention area whereas cladocerans were much high in aquatic habitats from control sites (Fig. 1).

4.3. Zooplanktons species abundance in control and intervention sites

In general, high species diversity and abundance were observed in the intervention sites (Table 2). *Moina micrura* and *Moina macrocopa* were the most abundant in the intervention sites. Species such as *Rotaria rotatoria*, *Brachionus patulus*, *Moina micrura* and *Moina macrocopa* were predominant in control sites.

4.4. Comparison of zooplankton abundance and diversity between control and intervention sites

Data from all aquatic organisms encountered were transformed into independent variables (principal components) in order to assess whether breeding sites in control and intervention sites differ in their zooplankton abundance and diversity. A logistic regression analysis showed that breeding habitats in control and intervention sites differed in species composition. The ordination diagram was constructed with the first two axes explaining about 100% of the total variance (Fig. 2). The first axis was found to be positively correlated with *Thermocyclops* sp., *Rotaria rotatoria*, *Brachionus patulus*, *Diaphranosoma brachyunum*, *Blapertura affinis*, *Microcyclops* sp.,

Table 1
Comparison of physico-chemical parameters of aquatic habitats in control and intervention sites.

Parameters	Control sites		Intervention sites			
	N	Means ± SE	N	Means ± SE	*t*-test	*P*-Value
Temperature (°C)	83	25.53 ± 1.01	84	25.01 ± 1.26	−2.94	0.004
PH	74	6.8 ± 0.33	77	6.61 ± 0.38	−3.226	0.002
TDS (mg/l)	79	281.01 ± 37.09	82	281.16 ± 36.13	0.026	0.979
Conductivity (μs/cm)	79	425.55 ± 58.97	83	422.24 ± 56.9	−0.353	0.725
Turbidity (FTU)	84	131.35 ± 18.91	80	131.97 ± 19.55	0.206	0.837
Calcium (mg/l)	113	23.52 ± 9.88	106	21.97 ± 9.99	−1.154	0.25
Magnesium (mg/l)	83	70.14 ± 19.6	83	69.8 ± 19.29	−0.116	0.91
Sulfate (mg/l)	113	37.14 ± 2.7	106	37.43 ± 2.59	0.787	0.432
Hydrogen Peroxyde (mg/l)	97	16.91 ± 3.76	94	16.97 ± 3.77	0.11	0.912
Ammoniac (mg/l)	113	42.57 ± 3.87	106	42.89 ± 3.99	0.622	0.535
Nitrate (mg/l)	113	3.29 ± 1.29	106	3.07 ± 1.17	−1.379	0.169
Phosphate (mg/l)	113	54.95 ± 11.03	106	55.76 ± 11.40	0.534	0.594
Dissolved Carbon Dioxyde (mg/l)	49	8.2 ± 2.6	51	8.10 ± 2.48	−0.177	0.86
Oxydability (%)	50	55.13 ± 2.17	55	53.24 ± 3.25	−3.468	0.001
Alcalinity (mg/l)	49	44.8 ± 36.81	53	46.31 ± 34.49	0.214	0.831
Dissolved Oxygen (mg/l)	49	24.31 ± 2.9	52	23.63 ± 2.86	−1.192	0.236

N= Sample size; SE= Standard Error.

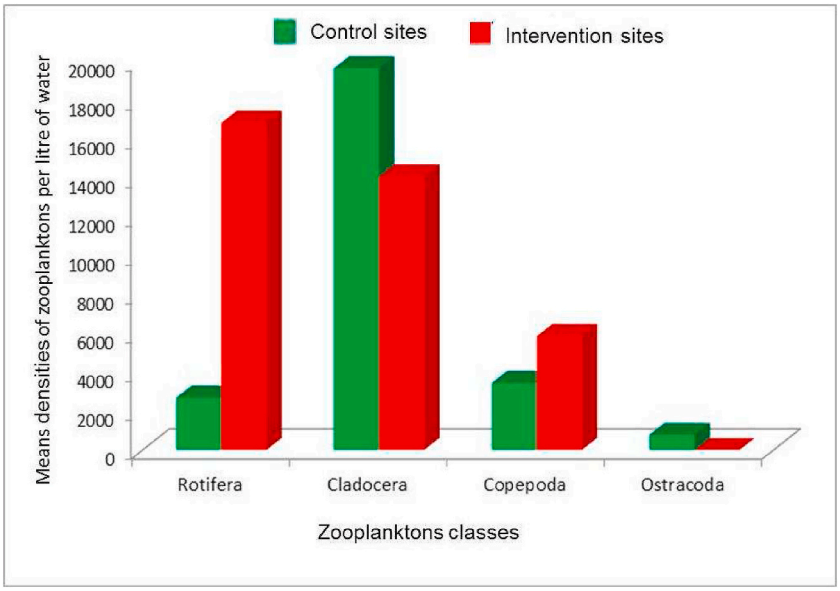

Fig. 1. Abundance of zooplankton in control and intervention sites.

Table 2
Abundance of Zooplankton species in control and intervention sites.

Groups	Species	Control sites	Intervention sites
Copepoda	*Thermocyclops* sp.	+++	+++
	Microcyclops sp.	+	+
	Tropocyclops sp.	+	+
	Mesocyclops sp.	–	+++
Rotifera	*Rotaria rotatoria*	++++	++
	Brachionus patulus	++++	++
	Notholca salina	+	–
	Brachionus ferficala	–	+
	Frola zaralli	–	+
	Brachionus budapestinensis	+	–
	Trichocerca diurella	–	++
	Colurella geophila	+/–	++
Cladocera	*Alona protzi*	–	+/–
	Alona weltneri	+/–	+/–
	Alona guttata	–	+/–
	Alona quadrangularis	–	+/–
	Alonella exugua	–	+
	Daphnia similis	–	+
	Oxyurella terulcaudia	–	+
	Sida crystallina	–	+/–
	Chydorus ovalis	–	+/–
	Disparalona rostrata	–	+/–
	Chydorus piger	–	+
	Oxyurella terulcaudia	–	+
	Alona rectangula	–	+
	Diaphranosoma brachyunum	++	+/–
	Blapertura affinis	+++	–
	Camphocercus rectirostris	–	+
	Ceriodaphnia rotunda	–	+
	Moina micrura	++++	++++
	Moina macrocopa	++++	++++
Ostracoda	*Ostracod* spp.	+/–	–

- = Absent = -; +/– = x < 1%; + = 1% < x < 5%; ++ = 5% < x < 25%; +++ = 25% < x < 50%; ++++ = x> 50%; x = density.

Tropocyclops sp., *Moina micrura* and *Moina macrocopa* while, the second axis was positively correlated with *Thermocyclops* sp., *Rotaria rotatoria*, *Brachionus patulus*, *Diaphranosoma brachyunum*, *Blapertura affinis*, *Microcyclops* sp., *Tropocyclops* sp., *Moina micrura* and *Moina macrocopa*.

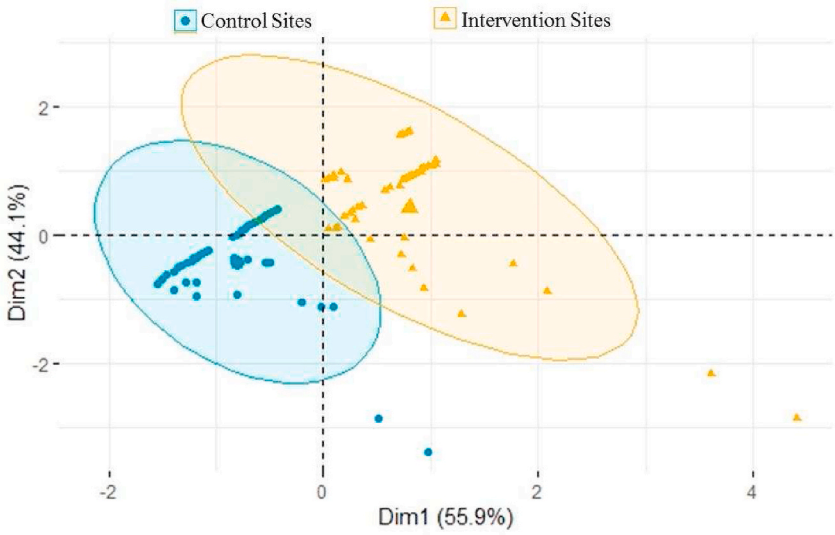

Fig. 2. A principal component regression analysis comparing zooplankton abundance in control and intervention sites.

4.5. Zooplanktons diversity indexes

High diversity and richness of zooplanktons were observed in intervention sites compared to control sites (H = 3.17 and H = 2.3 respectively). Shannon and Weaver index was above 2 and this was in conformity with the predominance of some species over others. Both *Moina micrura* and *Moina macrocopa* were highly represented in samplings done in the control sites. In the intervention sites, the value of the Shannon index was above 3 reflecting a great dominance of *Rotaria rotatoria*, *Brachionus patulus*, *Moina micrura* and *Moina macrocopa* over the other species. A high evenness index of Pielou was observed in breeding habitats situated in the intervention areas (J = 0.84) and suggest well balanced ecosystem and similar distribution of species (Table 3).

4.6. Macro-invertebrates abundance

Fig. 3 presents the distribution of aquatic macro-invertebrates in intervention and control sites. Gastropods (with a percentage of 72.9%) were the most abundant macro-invertebrates found in both sites whereas ephemeropterans (0.03%) were the least represented.

4.7. Abundance of macro-invertebrates species in control and intervention sites

Different species of macro-invertebrates were recorded in both control and intervention sites. Out of the 20 macro-invertebrates species identified, 16 species were found in the intervention sites whereas 19 were recorded in the control sites. The most frequent species in both sites was *Physa acuta*. *Culex* sp. larvae were recorded in both sites. Species such as *Melanoide* sp., *Hydraenopsisripae aurea*, *Ranatra* sp. and *Anopheles* sp. larvae were only encountered in control sites. On the other hand, *Abedus herberti* was only recorded in intervention sites (Table 4).

4.8. Abundance of vertebrates in control and intervention sites

Vertebrates were divided into two groups: fishes and amphibians. Fishes were exclusively constituted of *Gambusia affinis* while amphibians included frogs and toad tadpoles. Fishes (representing 93.1%) were found at high densities in both sites while amphibians (6.9%) were less abundant (Fig. 4).

Table 3
Diversity and species richness of Zooplankton.

Indexes	Control sites	Intervention sites
Total Richness (S)	30	43
Species abondance (N)	333,936	876,424
Shannon Index (H^a)	2.30	3.17
Similarity index of Sôrensen	0.34	0.56
Evenness index of Pielou (J^b)	0.68	0.84

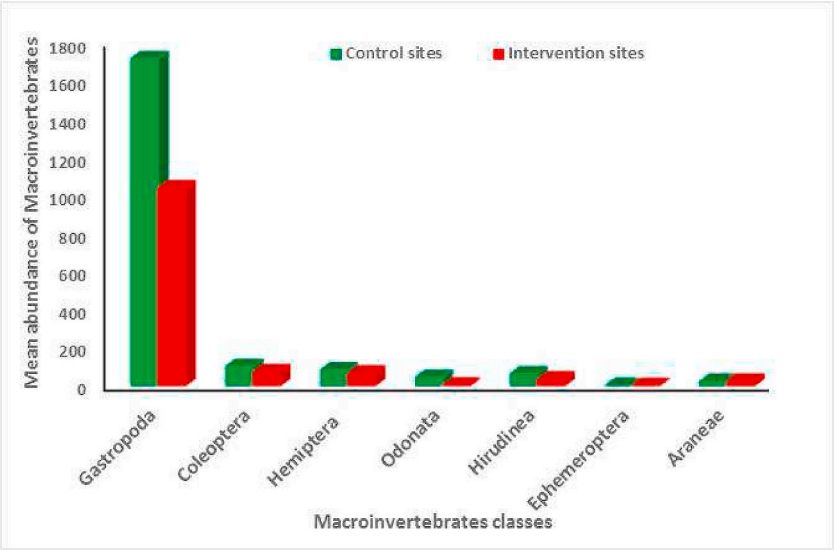

Fig. 3. Abundance of different Macro-invertebrates classes in control and intervention sites.

Table 4
Abundance of macrofauna species in control and intervention sites.

Groups	Species	Control sites	Intervention sites
Gasteropoda	*Physa acuta*	++++	++++
	Radix peregra	++	+
	Radix sp.	+	+
Coleoptera	*Melanoide* sp.	+	–
	Hydraenopsisripae aurea	+/–	–
	Amphiops sp.	+	++
	Agabus sp.	+/–	+/–
	Oreclochilus sp.	+	+
Hemiptera	*Sigara* sp.	+	+/–
	Abedus sp.	+	+
	Abedus herberti	–	+
	Laccotrephes sp.	+/–	+
	Borborophilus sp.	+/–	+
	Ranatra sp.	+/–	–
Odonata	*Ophiogomphus* sp.	+	+
Arhynchobdella	*Erpobdella* sp.	+	+
Ephemeroptera	*Baetis* sp.	+/–	+
Araneae	*Argyroneta* sp.	+	+/–
Diptera	*Anopheles* sp.	++++	–
	Culex sp.	++++	+/–

- = Absent; +/– = 0 < x < 1%; + = 1% < x < 5%; ++ = 5% < x < 25%; +++ = 25% < x < 50%; ++++ = x> 50%; x = density.

4.9. Comparison of the macrofauna abundance and diversity between control and intervention sites

To assess whether breeding sites in control and intervention sites differed, in their macrofauna abundance and diversity, all aquatic macro-organisms encountered were transformed into independent variables (principal components). A logistic regression analysis was applied and no significant difference in species composition between intervention and control sites was recorded. The ordination diagram constructed with the first two axis explaining about 100% of the total variance, showed a non-ambiguous difference between breeding sites of the two study sites (Fig. 5). The first axis was found to be positively correlated with *Physa acuta*, *Radix peregra*, *Amphiops* sp., *Anopheles* sp. *Culex* sp. and larvivorous fishes (*Gambusia affinis*) while, the second axis was positively correlated with *Laccotrephes* sp., *Radix peregra, Physa acuta*, *Amphiops* sp., *Anopheles* sp. *Culex* sp. and larvivorous fishes (*Gambusia affinis*).

4.10. Macrofauna diversity indexes

Physa acuta, *Anopheles* sp., *Culex* sp. and *Gambusia affinis* were predominant in control sites while *Physa acuta*, amphibian larvae and *Gambusia affinis* were the predominant species in intervention sites. Low Shannon and Weaver and evenness indexes supporting low diversity and non-homogeneous distribution of species were recorded in both sites (Table 5).

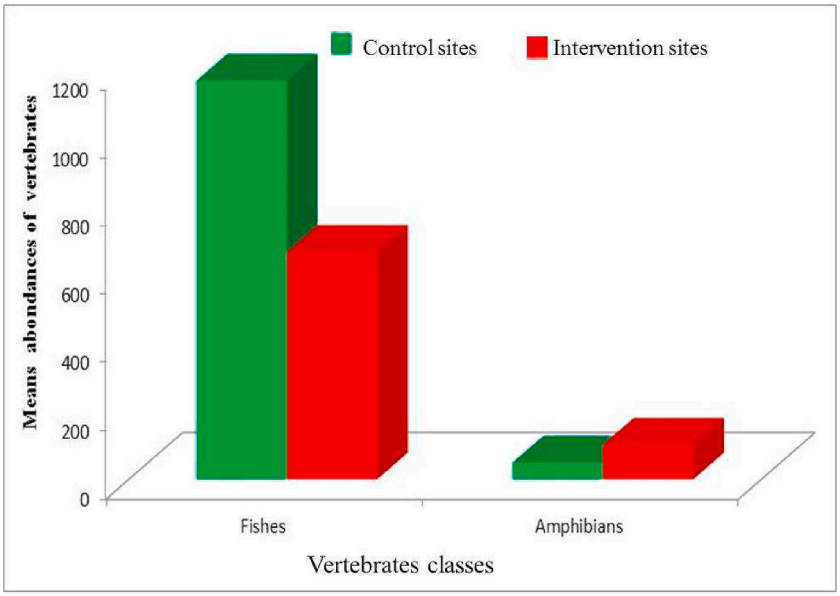

Fig. 4. Abundance of vertebrates in control and intervention sites.

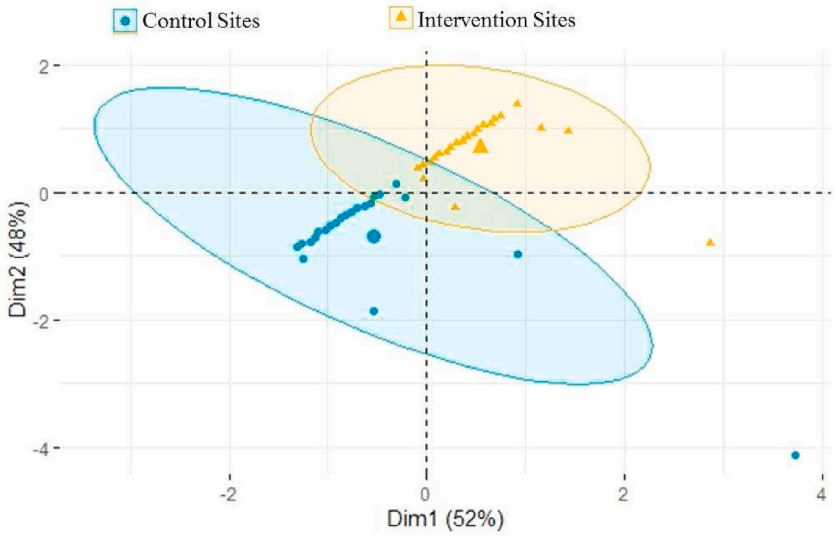

Fig. 5. A principal component regression analysis comparing aquatic macrofauna abundance in both control and intervention sites.

5. Discussion

The main objective of the study was to assess whether the application of VectoMax G, a larvicide combining *Bacillus thuringiensis* var. *israelensis* and *L. sphaericus* also kill non-target aquatic organisms that cohabit with anopheline and culicine larvae in breeding habitats in the city of Yaoundé. The present study is part of a series of studies that assessed the impact of larviciding on mosquito population dynamic in the city of Yaoundé. Our previous published study reported that the application of VectoMax G in breeding habitats was associated with over 60% reduction of anopheline and culicine larvae [42]. In the present study, no significant reduction of the diversity and abundance of the non-target aquatic fauna was recorded.

VectoMax G is a formulation that persists for at least 20 days in the environment. Problem with formulations that last longer is the accumulation of these particles in the environment and the possible negative effect of this accumulation on beneficial insects that are involved with the regulation of the mosquito densities and the aquatic habitat [43,44]. Anopheline larvae were found to cohabit with a large number of species including species of the zooplankton, macroinvertebrates and vertebrates. A high diversity of the zooplankton was recorded in the intervention areas with up to 28 species collected compared to control areas where 14 species were recorded. The

Table 5
Diversity and species richness of the Macrofauna.

Indexes	Control sites	Intervention sites
Total Richness (S)	19	16
Species abondance (N)	2019	1231
Shannon Index (H[a])	1.21	1.30
Similarity index of Sörensen	0.21	0.22
Evenness Index of Pielou (J[b])	0.44	0.46

high diversity of the zooplankton in the intervention areas supports no harmful effect of VectoMax G on species of the zooplankton. In addition, the diversity of the aquatic fauna recorded here was similar to studies conducted previously in the city of Yaoundé [45]. It is likely that some species of the zooplankton are preferred food for mosquito larvae. The Zooplankton is an important component of the aquatic food chain affecting organisms at different trophic levels either directly or indirectly. They play a significant role in the microbial loop and nutrient recycling [46]. Ecological investigations on zooplankton communities is therefore important when assessing the health of aquatic ecosystems [47].

Macro-invertebrates were also monitored during the larviciding trials. Overall, they were not affected by the application of VectoMax G in breeding habitats. A total of 19 species was recorded in the control sites against 16 species in the intervention sites. Shannon, Sorensen and Pielou indexes were almost similar between the two sites supporting no significant difference in species diversity and richness. Previous studies evaluating Bti effects on non-target organisms reported no direct effect on aquatic organisms other than mosquitoes, blackflies and chironomids larvae [48,49,50]. Aquatic organisms, such as shrimps and mites have generally never been affected by Bti [51,52]. The large safety margin of Bti formulations on non-target organisms support their suitability for mosquito control programmes in areas displaying high insecticide resistance or where protection of the natural ecosystem is important [52,53].

There have been so far many field studies assessing the potential impacts of microbial larvicides on wetland invertebrate communities, and the absence of negative impacts recorded in this study is in line with findings of most of these studies [54,55,56]. The application of VectoMax G did not alter species richness nor community diversity of macroinvertebrates and zooplanktons. The study is in agreement with previous studies supporting a high level of effectiveness and safety of *B. thuringiensis* and *L. sphaericus* when used for mosquito control [57,12]. The high level of safety to non-target organisms makes microbial larvicides ideal for integrated vector control operations. Yet the continuous elimination of target organisms (anopheline and culicine larvae) could in the long-term reduce the ecosystem diversity and richness and affect the structure of the aquatic fauna cohabiting with anopheline and/or culicine larvae and this deserve further attention particularly in conservation units like parks or preserved areas.

6. Conclusion

The present study suggested no impact of VectoMax G on the non-target aquatic fauna. The use of microbial larvicides could be highly indicated for the fight against mosquito communities in urban settings.

Ethical clearance and authorizations

The study was conducted under the ethical clearance N°2016/11/832/CE/CNERSH/SP delivered by Cameroon National Ethics Committee on Human Health. Further informed consent was obtained from the senior division administrator of the city of Yaoundé and each local District Medical Officer. Verbal and written informed consents were obtained from all respondents and the study purpose was explained to them. Permission to carry the trial was given by the Ministry of Public Health of Cameroon (Reference: 631-06-17). All experiments were performed in accordance with relevant guidelines and regulations.

Research and import permit for the use of VectoMax in Cameroon was granted by the Minister of Trade (Reference IF014167; IF021096; IF031126).

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Author contribution statement

Antonio-Nkondjio Christophe and Wondji Charles Sinclair: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper, Djamouko Djonkam Landre and Djepand-Ngognouak Thierry: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper, Mayi Marie Paul Audrey: Analyzed and interpreted the data; Wrote the paper, Tchuinkam Timoléon, Zébazé-Togouet Serge Hubert and Foko Dadji Gisèle: Contributed reagents, Materials, analysis tools or data; Wrote the paper.

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Data availability statement

The authors do not have permission to share data.

Declaration of competing interest

The authors declare no competing interests.

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