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# CONTENTS

# HAEMATOLOGICAL RESPONSES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS 1758) TO EXPOSURE TO EFFLUENT FROM PALM OIL MILLS

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**ABSTRACT**

Pollution by palm oil mill effluent (POME) is of great concern in Indonesia. POME pollution of the water can adversely affect aquatic organisms, especially fish. This study aims to analyse the effect of POME on the haematology of tilapia (*Oreochromis niloticus*), including red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit. A random experimental design was used (CRD) consisting of four treatments and five replications, including a Control (0% POME): Treatment A (10% of $LC_{50}$-96 hours: 1.565 mg $l^{-1}$), Treatment B (15% of $LC_{50}$-96 hours: 2.347 mg $l^{-1}$) and Treatment C (20% of $LC_{50}$-96 hours: 3,130 mg $l^{-1}$). The analysis of blood of fish exposed to POME for 15, 30 and 45 days revealed significant effects ($p < 0.05$) only on Hb and MCH on day 15 and WBC on day 30. This study indicates that exposure to POME can reduce the WBC value of tilapia recorded on day 30 in treatment C to a level lower than that recorded in other treatments. The Hb value recorded for tilapia exposed to POME on day 15 in treatment C was significantly higher than in the other treatments. Furthermore, POME caused a significant decrease in MCH recorded on day 15, with the lowest value recorded in treatment A. Based on the results of this study, POME can cause a significant decrease in WBC recorded on day 30 and MCH on day 15 and increase in Hb on day 15 in tilapia.

**Keywords:** fish; haematology; POME; pollutant

## Introduction

Indonesia is one of the world's largest palm oil producers (Mukherjee and Sovacool 2014; Tyson et al. 2018) and palm oil mill effluent (POME) is having an adverse effect on the environment, especially the aquatic environment. Rivers are very vulnerable to POME contamination because almost every palm oil industry is located in watersheds (Amalia et al. 2013; Syahza and Asmit 2020). The adverse effects of this waste water can be direct or indirect depending on the volume produced and the level of contamination (Guedenon et al. 2012). POME has a significant negative effect on aquatic biota (Neoh et al. 2013), including phytoplankton (Muliari and Zulfahmi 2016), anaerobic bacteria (Arisht et al. 2020), bivalves (Zieritz et al. 2016) and fish (Hashiguchi et al. 2021).

Comprehensive studies on the toxicity of POME to aquatic organisms, especially fish, are still needed in Indonesia. Territorial waters around Indonesia are very vulnerable to POME contamination (McCarthy and Zen 2010). One of the important and interesting physiological parameters to be studied is fish haematology. There are few studies on the toxicity of POME to fish (Owolabi et al. 2021), particularly Nile tilapia (*Oreochromis niloticus* L.), which is widely distributed in Indonesian waters and is able to adapt to highly polluted environments, including water close to oil palm plantations (Rahim et al. 2013; Sarong et al. 2013; Dekar et al. 2018; Irhami et al. 2018).

Haematological studies have been used as indicators of the health of fish in terms of infection with bacteria and viruses, and effect of pollutants (Svobodova et al. 1991). Furthermore, the research of Sahetapy (2013) indicates there is a reduction in the haematocrit value in the grouper (*Epinephelus fuscoguttatus*) exposed to lead at 6.86 mg $l^{-1}$ for 30 days. In addition, Witeska et al. (2011) report haemolysis, nuclear deformation and chromatin condensation in red blood cells of carp (*Cyprinus carpio*) exposed to cadmium at 0.65 mg $l^{-1}$ for 4 weeks. The results of Jahanbakhshi et al. (2014) reveal that there is an increase in the concentration of neutrophils and decrease in the concentration of lymphocytes in *C. carpio* exposed to crude oil waste. The same is also reported for catfish (*Clarias* sp.) exposed to potassium permanganate and *Hoplias malabaricus* to methylmercury and inorganic lead (Darwish et al. 2001; Ribeiro et al. 2006). Decreased haematocrit values and increased erythropoietic levels also occur in fish after exposure to heavy metals (Witeska 2005). However, only the effect of POME on reproduction (Muliari et al. 2018; Zulfahmi et al. 2018; Muliari et al. 2020a) and ontogenesis (Muliari et al. 2020b) in tilapia is reported and the effect of POME on its haematology has yet to be studied. Therefore, a haematological investi-

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gation is required to determine the effect of POME on the physiology and homeostasis of *O. niloticus*.

## Materials and Methods

### Research location and time

This research was carried out in 2021, including the sub chronic exposure of fish to POME, which was done at the Aquaculture Laboratory, Almuslim University and the measurement of haematological parameters at the Research Clinical Laboratory, Banda Aceh.

### Experimental animals

A total of 300 fish (*O. niloticus*) of a weight range of 28–33 g (30.45 ± 0.34) and length range of 10–13 cm (11.62 ± 1.43) were obtained from the Fish Seed Centre (BBI) Batee Iliek, Bireuen Regency. The acclimatization period lasted for seven days and then healthy fish were selected for the experiments (200 fish). A total 30 l of POME was collected from the Bireuen Regency area, then diluted to 10%, 15% and 20% $LC_{50}$ concentration (15.65 mg $l^{-1}$) of POME (Zulfahmi et al. 2017). This study was approved by the Animal Research Ethics Committee of the Aquaculture Department, Faculty of Agriculture, Almuslim University, Indonesia (Ethic Code No. 007/Aquaculture-FP.Umuslim/VIII/2021).

### Experimental design

A completly randomized design (CRD) was used that consisted of four treatments that were replicated five times. The sub-chronic concentration of POME used in each treatment is based on the $LC_{50}$-96 hours of POME for *O. niloticus* reported in a previous study, which is 15.65 mg $l^{-1}$ (Zulfahmi et al. 2017). Control Treatment (0% POME), Treatment A (10% of $LC_{50}$-96 hours: 1.565 mg $l^{-1}$), Treatment B (15% of $LC_{50}$-96 hours: 2.347 mg $l^{-1}$) and Treatment C (20% of $LC_{50}$-96 hours: 3.130 mg $l^{-1}$). The fish were kept in aquaria (60 × 40 × 30 cm) containing 43 litres of aerated water. There were ten fish per container (total of 200 fish). During the period they were exposed the pollutant fish were fed commercial feed twice a day.

Blood samples were collected from five fish taken at random from each replication on days 15, 30 and 45, while anesthetized with clove oil to avoid suffering. A 1 ml syringe was used to draw 2–4 ml of blood from the caudal fin, which was stored in an EDTA solution. Blood samples were collected at the Aquaculture Laboratory, Almuslim University and transported to the Research Clinical Laboratory, Banda Aceh, Indonesia for further analysis.

Haematological features of the fish were recorded on days 15, 30 and 45, including numbers of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit. Measurements of haematological parameters were done using a haematology analyser (Mindray BC-30s). MCH was determined by comparing the average amount of haemoglobin in erythrocytes. In addition, measurements of water quality, including dissolved oxygen (DO), temperature and pH, were also recorded.

### Statistical analysis

Fish haematological parameters including RBC, WBC, Hb, MCHC, MCH, MCV and haematocrit were analysed statistically (one-way ANOVA) with a 95% confidence level using SPSS software version 22 (IBM SPSS Statistics, IBM, Chicago, USA, Macintosh Version).

## Results

### Red blood cells (RBC)

When compared to the control group, there was no significant difference in RBC values recorded for the different treatments and durations of exposure. The RBC value of *O. niloticus* exposed to POME recorded on day 15 was higher in each treatment compared to the control, especially treatment C (1.74 ± 0.19 $10^6/\mu l$). On day 30 RBC values recorded for each treatment was lower than in the control, with the lowest value recorded for treatment C (1.01 ± 0.32 $10^6/\mu l$). A higher RBC value was recorded in each treatment on day 45, but not higher than that for the control (1.55 ± 0.22 $10^6/\mu l$) (Fig. 1A).

### White blood cells (WBC)

Exposure to POME had a significant effect ($p < 0.05$) on WBC recorded on day 30, but not on days 15 and 45. The lowest WBC values were recorded on day 15 in treatment C (64.10 ± 30.00 $10^3/\mu l$) and treatment A (64.32 ± 10.32 $10^3$ µl),

A

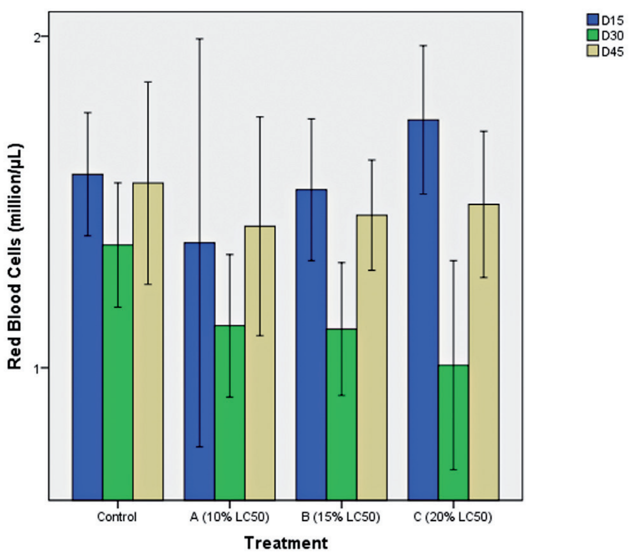
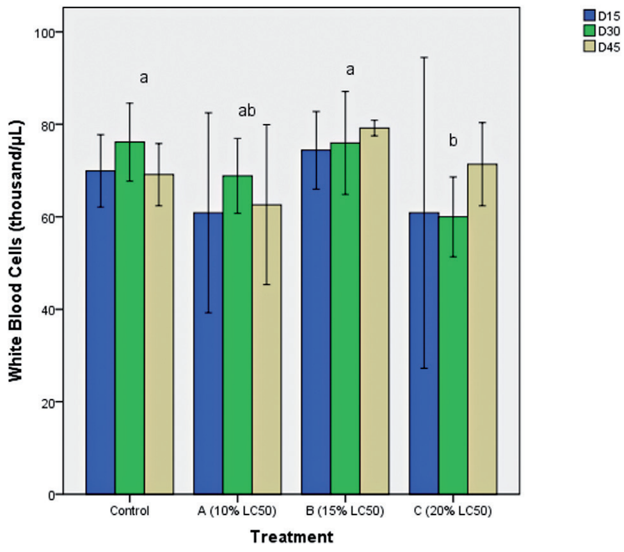
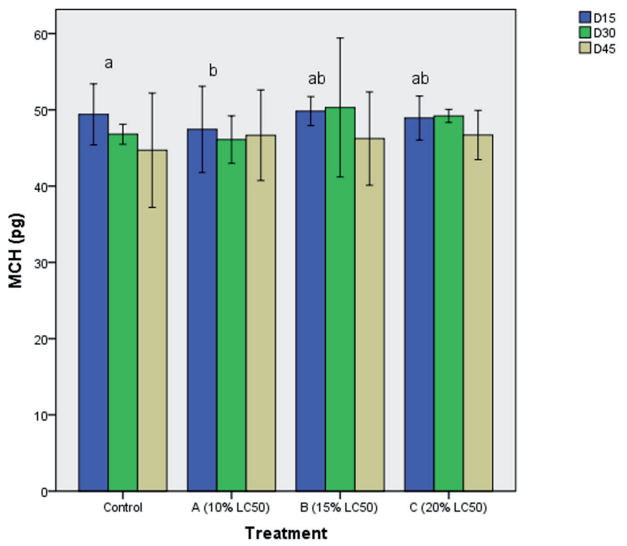



**Fig. 1** Graph of (A) RBC, (B) WBC, (C) haemoglobin, (D) MCHC, (E) MCH, (F) MCV and (G) haematocrit values recorded for *Oreochromis niloticus* exposed to POME at different concentrations and periods of time.

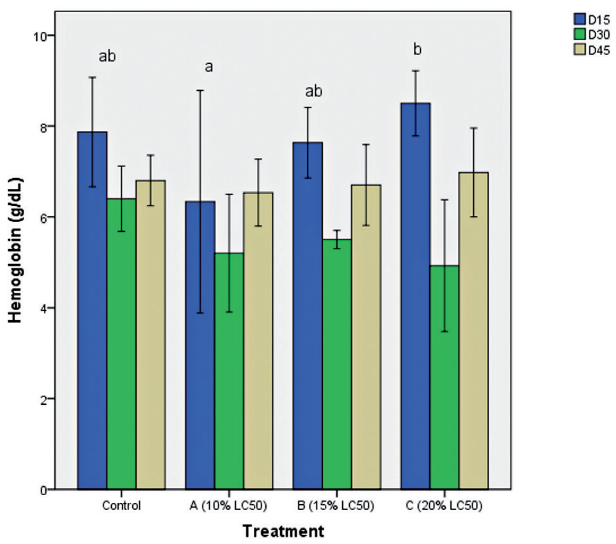
B


White Blood Cells (thousand/µL)
100
80
60
40
20
0
a
ab
a
b
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

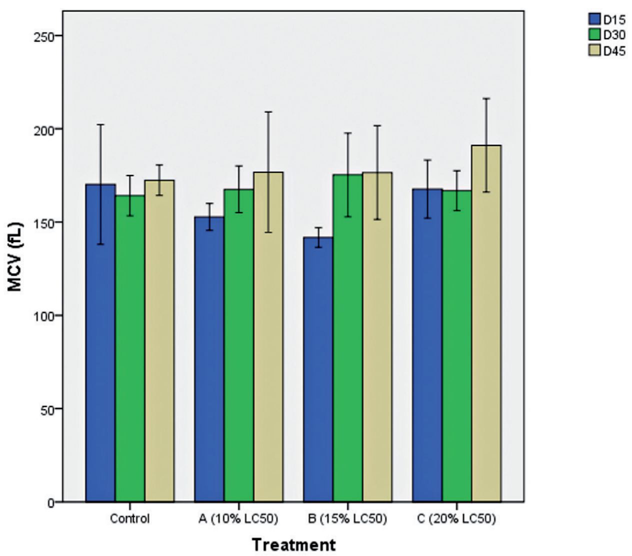
E


MCH (pg)
60
50
40
30
20
10
0
a
b
ab
ab
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

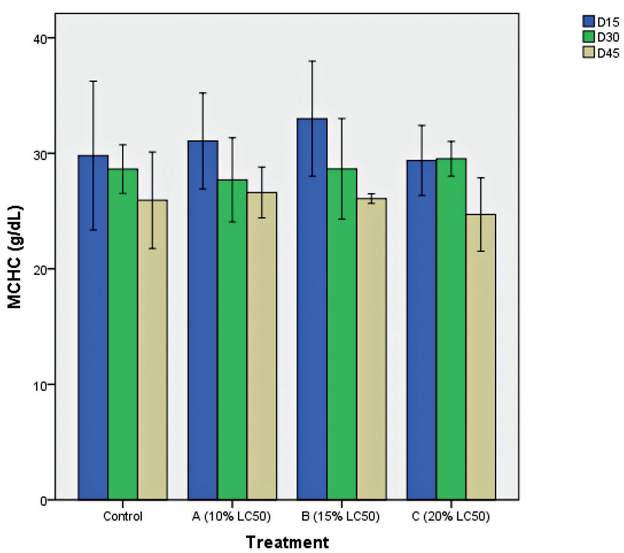
C


Hemoglobin (g/dL)
10
8
6
4
2
0
ab
a
ab
b
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

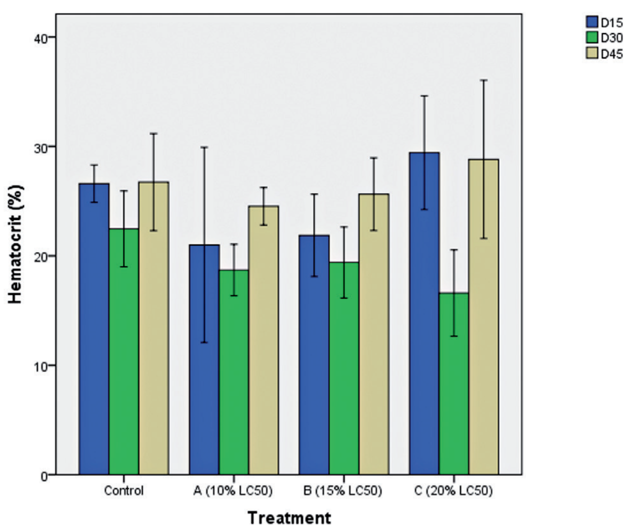
F


MCV (fL)
250
200
150
100
50
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

D


MCHC (g/dL)
40
30
20
10
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

G


Hematocrit (%)
40
30
20
10
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

while the records for the other two treatments were slightly higher. The WBC value for *O. niloticus* in treatment C on day 30 was lower, 59.98 ± 8.61 $10^3$/µl, while that recorded for the other three treatments tended to be higher, with the highest value recorded for the control (76.13 ± 8.45 $10^3$/µl). The WBC level recorded for treatment C (72.70 ± 7.98 $10^3$/µl) on day 45 was higher, while in the other three treatments it was lower, with the lowest value recorded for treatment A (66.84 ± 13.89 $10^3$/µl) (Fig. 1B).

#### Haemoglobin (Hb)

Exposure to POME had a significant effect ($p < 0.05$) on Hb recorded on day 15, but not on days 30 and 45. The highest Hb value was recorded on day 15 for treatment C (8.64 ± 0.69 g/dl) and the lowest for treatment A (6.86 ± 1.88 g/dl). The Hb value recorded on day 30 for treatment C was much lower at 4.93 ± 1.45 g/dl as it was in the other three treatments, for which the values were 6.40 ± 0.72 g/dl for the control, 5.50 ± 0.20 g/dl for treatment B and 5.20 ± 1.30 g/dl for treatment A. On day 45, higher values of Hb were record for all treatments, with the highest value recorded for treatment C (7.14 ± 0.92 g/dl) (Fig. 1C).

#### Mean corpuscular haemoglobin concentration (MCHC)

The MCHC values recorded during the 45 days of this study showed a downward trend in all but treatment A, for which it fluctuated; 29.48 ± 4.58 g/dl on day 15, 27.70 ± 3.64 g/dl on day 30 and 28.20 ± 2.89 g/dl on day 45 (Fig. 1D). The highest MCHC values recorded on days 15, 30 and 45 were recorded for treatment B 33.84 ± 4.71 g/dl, treatment C 29.53 ± 1.50 g/dl and treatment A 28.20 ± 2.89 g/dl.

#### Mean corpuscular haemoglobin (MCH)

The MCH values recorded differed significantly ($p < 0.05$) on day 15 but not on day 30 and 45 (Fig. 1E). On day 15 the value recorded for the control was higher than that recorded for the other treatments, with values reaching 51.44 ± 4.06 pg, whereas on days 30 and 45 the values recorded for treatment B were 50.30 ± 9.11 pg and 47.92 ± 4.92 pg, respectively.

#### Mean corpuscular volume (MCV)

The MCV values recorded during the 45 days of the exposure period show an increasing trend (Fig. 1F). The highest values of MCV recorded at each date were for treatment C on days 15 (163.30 ± 16.67 fl) and 45 (189.16 ± 22.12 fl), and treatment B on day 30 (175.27 ± 22.41 fl).

#### Haematocrit

Haematocrit values recorded for all treatments was higher on day 45, than on days 15 and 30, which fluctuated, with that recorded for each treatment on day 30 low and that on day 45 high (Fig. 1G). The highest values were recorded for treatment C on day 15 (28.70 ± 4.79%) and 45 (29.14 ± 6.30%) and for the control on day 30 (22.47 ± 3.47%).

#### Water quality

Dissolved oxygen (DO) values differed significantly ($p < 0.05$) depending on the concentration of POME and when sampled. The DO measurements are negatively correlated with the concentration of POME. Furthermore, concentration of POME is also significantly associated ($p < 0.05$) with temperature and pH (Table 2).

## Discussion

The decrease in the metabolic rate of fish exposed to pollutants can be expressed in terms of haematological features (Palar 2004). The results of this study revealed that exposure to POME are associated with changes in the number of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit of tilapia. Only Hb and MCH were significantly affected on day 15 of the treatments and WBC on day 30. Different results are reported by Owolabi et al. (2021) for Hybrid Catfish *Heteroclarias,* in which exposure to POME has a significant effect on RBC, MCV and MCHC, and Hb, MCH and WBC.

The results show that RBC recorded for the control was lower than that for the three treatments on day 15, but higher on days 30 and 45. Several types of pollutants are also associated with low RBC values, including copper (Cu), cadmium (Cd), insecticides and nickel (Kang et al. 2005; Al-Akel et al. 2010; Sabilu 2010). The low RBC value of fish exposed to heavy metals is thought to be closely related to the increase in blood viscosity accompanied by damage to RBC (Sabilu 2010). According to Alamanda et al. (2007), a low value of RBC can disrupt the supply of nutrients to cells, tissues and organs, resulting in a reduction in metabolism.

The WBC values of *O. niloticus* exposed to POME were significantly different ($p < 0.05$) on day 30 but not ($p > 0.05$) on days 15 and 45. On the day 30, the WBC value of *O. niloticus* in treatment C 59.98 ± 8.61 $10^3$/µl, while in the other three treatments it tended to be higher. Similar results are also reported by Owolabi et al. (2021), with 28 days exposure to POME having a significant effect on WBC values in hybrid catfish. Another study on Tor putitora reports significantly higher values of WBC when these fish are exposed to water polluted by industrial waste.

In terms of Hb concentrations, on day 15 of exposure to POME, the highest value (8.64 ± 0.69 g/dl) was recorded for treatment C and the lowest (6.86 ± 1.88 g/dl) for treatment A. On day 30, for treatment C, the Hb levels were low (4.93 ± 1.45 g/dl), as in the other three treat-

**Table 1** Haematological values recorded for tilapia exposed to POME at different concentrations and periods of time.

| Haematological values | Period of time (days) | Treatments | | | |
|---|---|---|---|---|---|
| | | Control (0%) | A (10% LC50) | B (15% LC50) | C (20% LC50) |
| RBC ($10^6$/μl) | 15 | 1.46 ± 0.21a | 1.58 ± 0.53a | 1.51 ± 0.21a | 1.74 ± 0.19a |
| | 30 | 1.37 ± 0.19a | 1.13 ± 0.22a | 1.12 ± 0.20a | 1.01 ± 0.32a |
| | 45 | 1.55 ± 0.22a | 1.48 ± 0.28a | 1.33 ± 0.28a | 1.53 ± 0.21a |
| WBC ($10^3$/μl) | 15 | 68.12 ± 18.98a | 64.32 ± 10.32a | 73.32 ± 7.20a | 64.10 ± 30.00a |
| | 30 | 76.13 ± 8.45a | 68.83 ± 8.11ab | 75.97 ± 11.12a | 59.98 ± 8.61b |
| | 45 | 69.64 ± 6.04a | 66.84 ± 13.89a | 73.70 ± 10.37a | 72.70 ± 7.98a |
| Hb (g/dl) | 15 | 7.54 ± 0.96ab | 6.86 ± 1.88a | 7.46 ± 0.60ab | 8.64 ± 0.69b |
| | 30 | 6.40 ± 0.72a | 5.20 ± 1.30a | 5.50 ± 0.20a | 4.93 ± 1.45a |
| | 45 | 6.88 ± 0.64a | 6.86 ± 1.01a | 6.32 ± 1.26a | 7.14 ± 0.92a |
| MCHC (g/dl) | 15 | 32.30 ± 5.73a | 29.48 ± 4.58a | 33.84 ± 4.71a | 30.62 ± 3.83a |
| | 30 | 28.63 ± 2.10a | 27.70 ± 3.64a | 28.65 ± 4.36a | 29.53 ± 1.50a |
| | 45 | 26.00 ± 3.41a | 28.20 ± 2.89a | 26.90 ± 1.88a | 24.92 ± 2.80a |
| MCH (pg) | 15 | 51.44 ± 4.06a | 44.88 ± 5.75b | 49.85 ± 4.88ab | 49.50 ± 2.80ab |
| | 30 | 46.80 ± 1.31a | 46.10 ± 3.12a | 50.30 ± 9.11a | 49.20 ± 0.86a |
| | 45 | 44.88 ± 5.54a | 46.96 ± 4.23a | 47.92 ± 4.92a | 46.70 ± 2.79a |
| MCV (fl) | 15 | 162.64 ± 25.371a | 152.92 ± 8.99a | 148.28 ± 10.57a | 163.30 ± 16.67a |
| | 30 | 164.13 ± 10.76a | 167.50 ± 12.51a | 175.27 ± 22.41a | 166.90 ± 10.68a |
| | 45 | 172.86 ± 7.79a | 168.12 ± 26.14a | 178.40 ± 19.94a | 189.16 ± 22.12a |
| Hematocrit (%) | 15 | 23.78 ± 4.06a | 24.22 ± 8.48a | 22.42 ± 3.93a | 28.70 ± 4.79a |
| | 30 | 22.47 ± 3.47a | 18.70 ± 2.36a | 19.40 ± 3.24a | 16.60 ± 3.94a |
| | 45 | 26.74 ± 3.14a | 24.4 ± 1.76a | 23.78 ± 5.64a | 29.14 ± 6.30a |

Different superscripts on the same line indicate a significant difference ($p < 0.05$).

**Table 2** Water quality in terms of the concentration of POME and period of time.

| Water Quality | Period of time (days) | Treatments | | | |
|---|---|---|---|---|---|
| | | Control (0%) | A (10% LC50) | B (15% LC50) | C (20% LC50) |
| DO | 15 | 7.04 ± 0.90b | 6.06 ± 1.38b | 4.00 ± 0.64a | 3.60 ± 0.63a |
| | 30 | 6.34 ± 1.05b | 6.20 ± 1.43b | 3.90 ± 0.76a | 3.84 ± 0.60a |
| | 45 | 4.34 ± 0.23b | 4.32 ± 0.43b | 4.14 ± 0.80b | 2.54 ± 0.81a |
| Temperature | 15 | 28.20 ± 0.45b | 28.00 ± 0.00b | 27.5 ± 0.50a | 28.20 ± 0.27b |
| | 30 | 29.00 ± 0.00b | 29.00 ± 0.00b | 28.60 ± 0.55a | 29.00 ± 0.00b |
| | 45 | 28.00 ± 0.00ab | 28.00 ± 0.00ab | 28.00 ± 0.00ab | 28.00 ± 0.00ab |
| pH | 15 | 8.00 ± 0.06ab | 8.05 ± 0.14ab | 7.97 ± 0.13a | 8.12 ± 0.03b |
| | 30 | 7.71 ± 0.04b | 7.76 ± 0.15b | 7.57 ± 0.06a | 7.59 ± 0.08a |
| | 45 | 7.86 ± 0.08b | 7.85 ± 0.15b | 7.62 ± 0.05a | 7.58 ± 0.07a |

Different letters on the same line indicate significant differences ($p < 0.05$).

ments. The Hb values on day 45 in all treatments was high, with the highest value (7.14 ± 0.92 g/dl) recorded for treatment C. The Hb value indicates the ability of fish blood to bind and distribute oxygen (Gomez et al. 2020). According to Bastiawan et al. (2001), low haemoglobin values can result in a low metabolic rate and energy utilization. Exposure to other pollutants (e.g. cadmium chloride, arsenic and lead) also reduces haemoglobin levels in tilapia (*Oreochromis niloticus*), catfish (*Clarias batrachus*) and grouper (*Epinephelus fuscoguttatus*) (Sahetapy 2013; Al-Asgah et al. 2015; Kumar and Banerjee 2016).

On day 15, exposure to POME had a significant ($p < 0.05$) effect on the MCH value of *O. niloticus*, but not ($p > 0.05$) on days 30 and 45. Following exposure to POME the MCH value recorded for the control was higher than for the other treatments on day 15 (51.44 ± 4.06 pg) but the value recorded for treatment B was very much higher on days 30 and 45, reaching 50.30 ± 9.11 pg and 47.92 ± 4.92 pg, respectively. MCH is the haemoglobin level divided by the number of red blood cells (Stockham and Scott 2008). Polizopoulou (2010) states that high MCV and MCH values can indicate a regenerative anaemia response caused by the haemolysis of red blood cells. Decreases in MCHC values are also reported in various types of fish exposed to arsenic, insecticides, dichlorophenoxy acetic acid, copper (Cu) and cadmium (Cd) (Kang et al. 2005; Kumar and Banerjee 2016).

Over the 45-day exposure period, the MCV value in *O. niloticus* increased (Fig. 1F). Treatment C had the highest MCV value on days 15 (163.30 ± 16.67 fl) and 45 (189.16 ± 22.12 fl) and treatment B on day 30 (175.27 ± 22.41 fl). The MCV value indicates the performance of erythrocyte production during the erythropoiesis process (Burgos-Aceves et al. 2019). Similar results are also reported by Kumar and Banerjee (2016) of an increase in the MCV value of catfish (*Clarias batrachus*) exposed to arsenic. The increase in the MCV value is thought to be due to an increase in the number of RBC per unit volume of plasma (Zandecki et al. 2007). Ikhimioya and Imasuen (2007) also argue that an increase in MCV can occur due to the release of immature RBC into the blood.

Haematocrit values for all treatments fluctuated, with a low value recorded on day 30 and a high value on day 45 (Fig. 1G). The values were highest for treatment C on day 15 (28.70 ± 4.79%) and 45 (29.14 ± 6.30%) and for the control it was at its lowest on day 30 (22.47 ± 3.47%). This value tends to be positively correlated with the number of red blood cells (Boggs et al. 2022). In addition, this value also indicates the level of hunger and gender of fish (Jawad et al. 2004; Rashmeei et al. 2022). Sahetapy (2013) also reports similar results, as the exposure to lead at a concentration of 6.86 ppm for 30 days results in this value decreasing from 24.70% to 9.66% in Grouper (*Epinephelus fuscoguttatus*). Decrease in haematocrit values following exposure to pollutants is reported occurring in several species of fish including tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*), piava (*Megaleporinus obtusidens*) and rockfish (*Sebastes schlegeli*) (Kim and Kang 2004; Sabilu 2010; Al-Asgah et al. 2015).

## Conclusion

The exposure to POME had no significant effect on the haematology of tilapia (*Oreochromis niloticus*), in terms of the number of red blood cells (RBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit ($p > 0.05$),with the exception of Hb and MCH on day 15 and WBC on day 30. This study indicates that exposure to POME resulted in a low value of WBC for tilapia on day 30 in treatment C, which was lower than in the other treatments. Exposure to POME also resulted in the Hb value recorded for tilapia on day 15 in treatment C being significantly higher than in other treatments. Furthermore, exposure to POME was associated with a low value of MCH on day 15, especially in treatment A. Based on the results of this study, exposure of tilapia to POME was associated with low values of WBC on day 30 and MCH values on day 15, and significantly high values of HB on day 15.

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# POTENTIAL ENVIRONMENTAL AND HUMAN HEALTH RISKS CAUSED BY HEAVY METALS AND PATHOGENS FROM ILLEGAL LANDFILL SITES IN BOSNIA AND HERZEGOVINA

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**ABSTRACT**

In Bosnia and Herzegovina, several illegal waste dumps pose a significant threat to soil and water contamination. The aim of this study was to determine the levels of heavy metal contamination of soils and microbiological contamination of water near investigated landfills. The goal was to identify the harmful effects of illegal landfills on the environment and public health, as well as assessing the potential of contaminants to pollute soil and water resources. Using an Atomic Absorption Spectrometer, the amounts and distribution of four heavy metals (Cd, Co, Pb, and Cr) in soil at four illegal landfills in Central Bosnia were studied. Three water samples collected near the dumpsites studied were tested for microbes. Bacteriological analyses of water included determination of total mesophilic aerobic bacteria, aerobic heterotrophic bacteria, total coliform and fecal coliform bacteria and fecal enterococci. Quantitative results were analyzed using an analysis of variance and Tukey HSD post hoc test. Concentrations of Cd (4.96 mg/kg) and Pb (206.97 mg/kg) recorded in soil at particular sites were above the limits of maximum allowable concentration. Cr and Pb values in soil samples were relatively higher on average than that of Cd and Co. Enrichment factor and pollution load index indicated high concentration of heavy metals in soil, especially Cd. Based on these results, waste from illegal landfills may release considerable amounts of harmful metals and microbes into the environment.

**Keywords:** heavy metals; illegal landfills; pathogens; pollution

## Introduction

Rapid population growth, intensive industrialization and urbanization have adversely affected the environment, primarily in terms of pollution of soil and water. The production of solid waste is increasing rapidly and is an important environmental problem worldwide (Williams and Hakam 2016). This has resulted in a dramatic increase in waste and numerous, often illegal and uncontrolled landfills (Biotto et al. 2009). Soil contamination caused by human activity is damaging the environment and ecosystems around the world (Bai et al. 2013; Adamcová et al. 2016; Chen et al. 2016). Studies indicate that exposure to toxic chemicals, such as heavy metals in waste or contaminated water is likely to adversely affect human health (Agusa et al. 2003; Nguyen et al. 2003).

The recent contamination of soil with heavy metals is of major global concern (Dahija et al. 2019). Lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel are the most common heavy metals that contaminate soil worldwide. Heavy metals are the dominant causes of environmental pollution due to the method of production, toxicity, possibility of uptake by plants and inclusion in the human food chain (Dumitrel et al. 2013). Accumulations of heavy metals are highly toxic for biological organisms, such as humans, animals, microorganisms and plants (Njagi et al. 2016).

In addition to these chemical contaminants, landfills are of major concern because they might contain viable pathogenic microorganisms or their toxins that are known to cause disease in animals or humans (Anand et al. 2021). The decomposition of food waste can also result in the production of organic leachate that can significantly change the composition of bacteria and initiate the growth of pathogenic bacteria such as *Salmonella*, *Pseudomonas*, *Enterobacteria* and *Clostridium perfringens* (Wu et al. 2018), which could be dispersed in the environment via water and wind.

Unfortunately, there is less and less time to develop an integrated waste management system in Bosnia and Herzegovina (B&H), as large amounts of waste are generated every day and landfills are the primary way of disposing of waste. Moreover, this is occurring in unregulated landfills, which is likely to have a long-lasting effect on human health and the environment. Thus, the objective of this study was to determine the concentrations of lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu) in soil from four illegal landfills in Central Bosnia and determine the degree of microbiological contamination of water close to these sites.

## Materials and Methods

### Description of the study area

The area studied included four illegal landfills sites, namely: "Cipalo" (S1), "Dolovi" (S2), "Malkin Most" (S3) and "Paljike-Hum" (S4) and is located in Central Bosnia, Bosnia and Herzegovina (Fig. 1). The most common content of these waste dumps is domestic and household waste and construction material and they are

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close to streams. These landfills have an area of 4,355 m$^2$. A map of the area studied is shown in Fig. 1. Water samples were collected from "Cipalo", "Dolovi" and "Malkin most", but not "Paljike-Hum", as there are no nearby streams there.

#### Collection of soil samples

At each of the sites, three quadrats were marked out. In each quadrat, four core soil samples were collected randomly at depths of from 0–20 cm using a soil auger and mixed together to form a composite sample. Three soil samples were collected at each site, making a total of 12 soil samples from the area studied. The control samples (S5) were collected 700 m away from each dumpsite.

At each site, the surface debris was removed and a sample of soil collected using a clean shovel. The soil samples were transferred to clean zip-locked plastic bags, labelled carefully and brought to the laboratory for further treatment and analysis. The samples were mixed, gently homogenized and sieved through a 2-mm-mesh sieve to remove any coarse components. The samples were first air-dried at room temperature, then placed in an electric oven at a temperature of 40 °C for approximately for 30 minutes. The resulting fine powder was stored at room temperature.

#### Heavy metals analysis

Approximately, 0.25 g of the dry soil was placed in a Teflon vessel (100 ml) to which 6 ml HCL (Merk 30%), 2 ml of $HNO_3$ (65% Merck) and 2 ml of HF (Merk 40%) were added and then mineralized using a Milestone, Start D microwave digestion system. After 26 minutes of digestion, samples were cooled for 30 minutes and diluted to 25 ml with distilled deionized water. A blank digest was carried out in the same way. The final solutions were subsequently analyzed for Cd, Co, Pb and Cr by flame atomic absorption spectroscopy (FAAS) using Atomic Absorption Spectrometer Agilent AA 220. The metal concentration is reported as mg/kg dry weight.

#### Methods of assessment of contamination of soils

We used the geoaccumulation index ($I_{geo}$), enrichment factor (EF) and pollution load (PL) to assess the degree of heavy metal contamination at each site.

The index of geoaccumulation ($I_{geo}$) is widely used for assessing contamination by comparing the levels

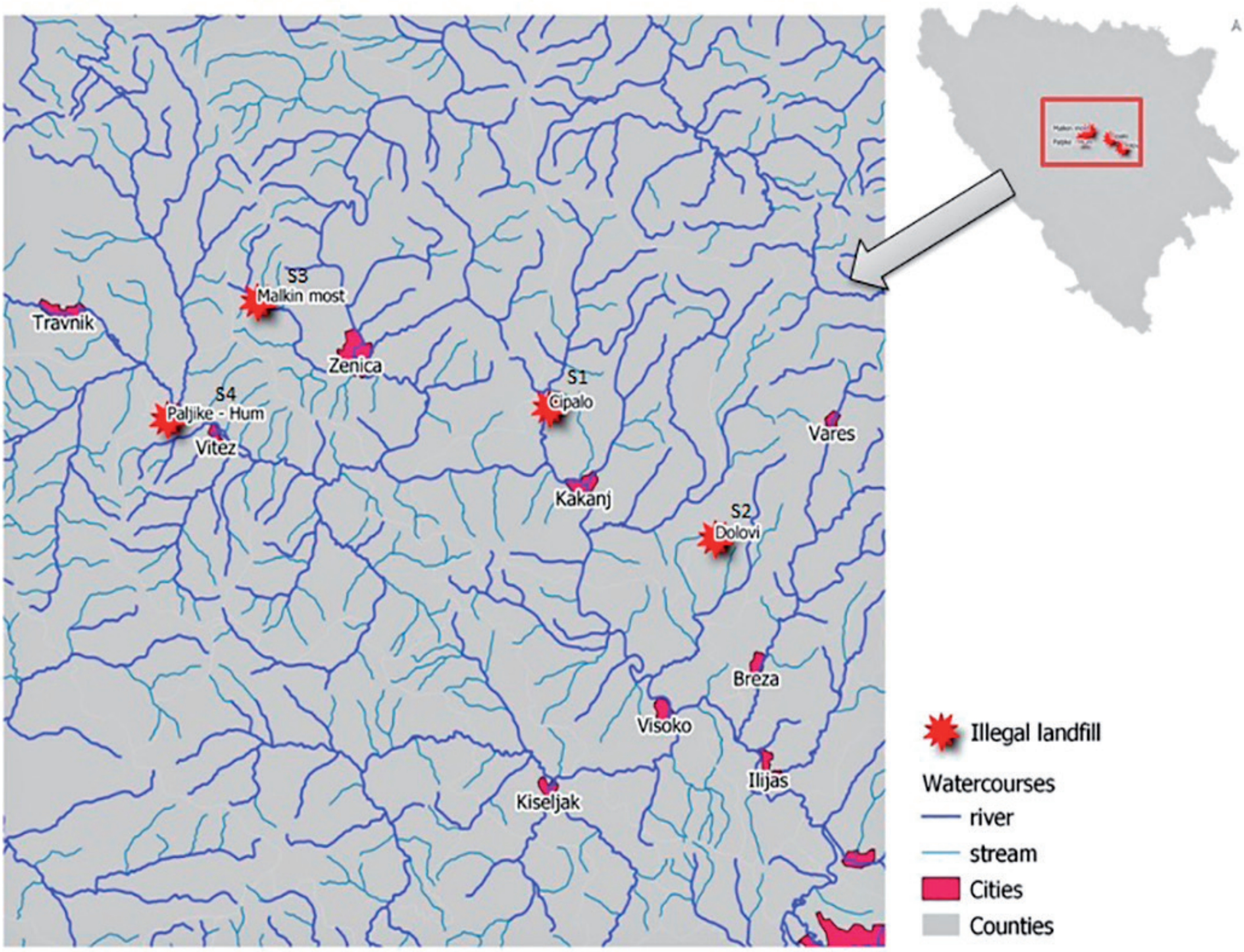


**Fig. 1** A map of the area studied: "Cipalo" (S1), "Dolovi" (S2), "Malkin Most" (S3) and "Paljike-Hum" (S4).

of heavy metals with background levels (Muller 1969; Atiemo et al. 2011). Geoaccumulation index was calculated using the equation:

$$I_{geo} = log_2\left(\frac{C_n}{1.5B_n}\right) \quad (1)$$

where $C_n$ is the measured concentration of the each element studied, and $B_n$ is the geochemical background value of the element in fossil clay sediment (shale). If the value of $I_{geo}$ is: < 0 = the soil is practically unpolluted, 0–1 = unpolluted to moderately polluted, 1–2 = moderately polluted, 2–3 = moderately to strongly polluted, 3–4 = strongly polluted, 4–5 = strongly to extremely polluted and > 5 = extremely polluted (Lu et al. 2009).

The enrichment factor (EF) of an element in soil samples are based on standardization of the measured element relative to the reference element. Mangan was used as a reference element in this study. The enrichment factor was calculated using the equation:

$$EF_x = \frac{(C_x/C_{ref})\ sample}{(B_x/B_{ref})\ Background} \quad (2)$$

where $C_x$ is the concentration of the element of interest and $C_{ref}$ represents the concentration of the reference element for normalization, $B_x$ is the concentration of the element in the crust and $B_{ref}$ is the concentration of the reference element used for normalization in the cortex (Ato et al. 2010). Based on enrichment, we distinguish five categories of contamination: $EF < 2$ = deficiency to minimal enrichment; $EF = 2–5$ = moderate enrichment; $EF = 5–20$ = significant enrichment; $EF = 20–40$ = very high enrichment; and $EF > 40$ = extremely high enrichment (Yongming et al. 2006).

The pollution index (PI) was defined as the ratio of the concentration of elements and the background content of the abundance of chemical elements in the continental crust. This index is often used to assess environmental pollution (dos Anjos et al. 2000). PLI is calculated as a geometric average of PI based on the following formula:

$$PLI = \sqrt[n]{PI_1 \times PI_2 \times PI_3 \times \ldots PI_n} \quad (3)$$

The pollution index for each heavy metal in this paper was rated as low ($PI \leq 1$), medium ($1 < PI \leq 3$) or high ($PI > 3$) according to Chen et al. (2005).

In this paper, for the assessment of soil pollution by Cd, Co, Pb and Cr we used a table in which are listed the maximum allowable concentrations of heavy metals in soil according to standard regulatory bodies such as World Health Organization, Food and Agricultural Organization and Standard guidelines in Europe.

### Microbiological analysis

Water sampling was carried out according to standard methods for the examination of water and wastewater (APHA 2005). Surface water samples were collected in pre-sterilized polypropylene bottles, each of 1 l capacity. After collection, samples were transported at 4 °C prior to analysis. The water was collected from three sites in the immediate surroundings below the waste dumps. The total numbers of aerobic mesophilic bacteria and heterotrophic bacteria were determined using the serial dilution method. The results were expressed in terms of colony-forming units, (cfu)/ml. Furthermore, water samples were analysed for total coliforms and fecal coliforms and intestinal enterococci using the membrane filtration (MF) technique and 0.45 mm pore-size membrane filters (Millipore Corp., Berdford, MA). The results were expressed in colony-forming units (cfu) per 100 ml.

### Statistical analysis

Experimental data were presented as mean values ± standard deviation (S.D.). To verify the statistical significance of the difference between various treatments, the data were analysed using a one-way analysis of variance and Tukey HSD post hoc test ($p < 0.05$). All statistical analyses were carried out using Statistica 10 software package (StatSoft. Inc).

## Results and Discussion

### Heavy metal concentration of soils at the different sites in the area studied

The mean concentrations of heavy metals (Pb, Cu, Cd and Co) recorded at landfill sites in Bosnia and Herzegovina are given in Table 1. Analysis of variance (ANOVA) revealed a significant ($p < 0.05$) variation in the concentrations of the four elements at the different sites (Table 1) and indicate the extent of metal pollution of the soils. The Tukey test revealed that the concentrations of lead were significantly ($p < 0.01$) higher at waste site (S4) than the other sites (Table 1).

The concentration of cadmium (Cd) ranged from 2.7 mg/kg at S2 to 4.96 mg/kg at S4, with a mean value of 4.04 mg/kg. The mean concentrations of Cd recorded in soil from S1, S3 and S4 sites were above the limit set by WHO/FAO (2001) of 3 mg/kg. However, the value for the soil from site S2 was 2.70 mg/kg, which is below the recommended limit. Concentrations of cadmium in the environment are constantly increasing due to continuous anthropogenic mobilization around the world, which is of great concern. According to Zheng et al. (2021) a large amount of Cd is discharged into the environment, greatly endangering the stability of the ecological environment and human health. Cd is an extremely toxic element that exhibits various toxic effects, including nephrotoxicity, carcinogenicity, teratogenicity and endocrine and reproductive toxicity (Wuana and Okieimen 2011; Genchi et al. 2020).

According to our results, high concentrations of lead (Pb) were recorded at all the sites. The concentrations

**Table 1** Summary of heavy metal mean concentration in soils at different sites in Bosnia and Herzegovina.

| Heavy metal (mg/kg) | Sample sites | | | | |
|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 |
| **Cadmium (Cd)** | 4.80 ± 0.30a | 2.70 ± 0.10b | 3.74 ± 0.05c | 4.96 ± 0.55a | 2.45 ± 0.07b |
| **Lead (Pb)** | 31.18 ± 0.68d | 42.48 ± 0.61c | 51.68 ± 0.58b | 206.97 ± 0.47a | 12.36 ± 0.55e |
| **Chromium (Cr)** | 31.97 ± 0.38c | 37.82 ± 0.73b | 52.62 ± 0.94a | 51.59 ± 1.42a | 12.99 ± 0.58d |
| **Cobalt (Co)** | 20.26 ± 1.21a | 27.19 ± 0.70b | 19.25 ± 0.43a | 15.46 ± 0.57c | 7.04 ± 0.22d |

Notes: Values are means (± SD) of three replicates, S1 = Cipolo, S2 = Dolovi, S3 = Malkin most, S4 = Paljike-Hum, S5 = control. Means followed by a different letter(s) in the same row differ significantly (P = 0.05) according to the Tukey's multiple range test.

ranged from 31.18 to 206.97 mg/kg, with a mean value of 83.07 mg/kg. The highest mean value was recorded at site (S4) and the lowest at S1. ANOVA revealed significant differences ($p < 0.05$) in the mean concentration of Pb among the sites. The mean concentration of Pb at S4 was above the WHO/FAO (2001) permissible limit for soils of 100.00 mg/kg. As the worldwide Pb concentration for surface soil averages 32 mg/kg and ranges from 10 to 67 mg/kg (Pendias and Pendias 2001; Kinuthia 2020) the levels recorded at the sites studied are above this limit.

Lead and cadmium are generally considered the most toxic to humans and animals. In addition to exposure to lead through the food chain, ingestion of soil and dust can also be an important source (Kinuthia 2020). The highest concentration of Pb recorded at site (S4) can be attributed to the burning of waste such as refrigerators, cables, car tyres, batteries, air-conditioners etc. This study revealed that the concentration of chromium (Cr) in the soil ranged from 31.97 mg/kg at S1 to 51.59 mg/kg at S4, with a mean value of 41.48 mg/kg. The high concentration of Cr recorded might also be a result of the waste including refrigerators, computers, cables, printers, photocopying machines, car tyres and batteries. In landfills in the USA, Cr is the third-largest pollutant and the second largest among the inorganic pollutants, after Pb (Vodyanitskii 2016). According to Kinuthia et al. (2020) hexavalent Chromium compounds, including chromates of Ca, Zn, Sr and Pb, are highly soluble in water, toxic and carcinogenic. Similarly, the concentration of cobalt ranged from 15.46 at S4, to 27.19 at S1, with a mean value of 20.54 mg/kg. However, waste site (S2) had the highest and S4 the lowest mean concentration of Co. Factors that affect cobalt speciation in soil and sediments include their nature, the concentration of chelating and complexing agents, pH and the redox potential.

High levels of these metals in the soil at the landfills studied may be associated with anthropogenic impact. The different types of waste disposed of in these landfills over the years are most likely the main sources of high concentrations of these metals. All of the concentrations of heavy metals except cadmium recorded at the sites studied differed significantly from those recorded in the control. This is in line with the results of similar studies (Ogundiran and Osibanjo 2008; Cortez and Ching 2014; Murtić et al. 2021).

The results of the $I_{geo}$ analysis indicate that S1, S2 and S3 were practically unpolluted with Pb, Cr and Co (Table 2). This may be attributed to the horizontality of the spread of metallic pollutants in these areas and the absence of e-waste. Moreover, these sites were strongly to extremely heavily contaminated with Cd. These findings corroborate the report of Pradhan and Kumar (2014) and Fossu-Mensah et al. (2017) that high concentrations of cadmium and other heavy metals may be due to e-waste. S4 was practically uncontaminated with Cr and Co, but extremely heavily contaminated with Cd and moderately to strongly contamination with Pb.

**Table 2** Recorded values of Geoaccumation index $I_{geo}$.

| Heavy metal | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| **Cd** | 5.029146 | 4.199071 | 4.670307 | 5.076452 |
| **Pb** | 0.055797 | 0.501821 | 0.784644 | 2.786387 |
| **Cr** | −0.7156 | −0.47317 | 0.003458 | −0.05347 |
| **Co** | 0.434099 | 0.858432 | 0.359896 | 0.043578 |

The enrichment Factor (EF) and pollution load index for all the heavy metals recorded in the soil at the different sites are presented in Table 3. Soil from S1 and S4 was extremely highly enriched with Cd, whereas S1 was minimally enriched with Pb, Cr and Co, and L4 significantly enriched with Pb and minimally so with Cr and Co.

Similarly, S2 and S3 were highly enriched with Cd, while S2 was moderately enriched with Co and minimally deficient in Pb and Cr. A comparison of the results for the different sites in terms of the EFs revealed that S1 and S4 were the most contaminated. However, the study also revealed that the concentration of cadmium (Cd) present in soil at all the sites was extremely high.

The PLI values recorded for the sites were in the high category, with PLI > 3. According to Chen et al. (2005) this means that the concentration of these heavy metals is high. The highest value of PLI was recorded for site 4, which was 5.84. These high values of PLI indicate the soils at the sites studied are heavily contaminated with heavy metals (Varol 2011).

**Table 3** Results recorded for the Enrichment factor and PLI.

| Heavy metal | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Cd | 41.5 | 23.48 | 32.67 | 42.89 |
| Pb | 1.32 | 1.8 | 2.19 | 8.76 |
| Cr | 0.77 | 0.91 | 1.27 | 1.22 |
| Co | 1.71 | 2.3 | 1.63 | 1.31 |
| PLI | 3.44 | 3.61 | 4.11 | 5.84 |

### Microbiological analysis

The results of microbiological examination of water samples from S1, S2 and S4 are in Table 4. The total number of aerobic mesophilic bacteria is expressed in terms of cfu/ml and this group includes opportunistic pathogens. Based on the maximum values, S3 stands out with 1168 cfu. Concentrations of heterotrophic bacteria, which usually correspond to organic matter contamination (Kamjunke et al. 2020) ranged from 34 cfu/ml at S2 to 4458 at S3.

Furthermore, results in Table 4 indicate that total coliforms varied between 28 cfu/100 ml at S2 to 3566 at S4. Total coliforms indicate the general sanitary condition of water, since this group includes bacteria of fecal origin resulting from the disposal of animal waste, sewage, land and urban waste and domestic wastewater. Mean values of fecal coliforms ranged from 6 cfu/100 ml at S1 to 2028 at S3 as indicated in Table 4. The numerous fecal coliforms recorded at S3 may be due to landfill runoff and solid waste containing fecal material of domestic animals and humans. Similar observations are recorded by Nartey et al. (2012) who determined the effect of solid waste dumpsites on surface water systems. In our study, intestinal enterococci in water varied from 3 cfu/100 ml at S1 to 266 at S3 and the greatest fecal pollution was recorded in the watercourse located near S3. However, the water sample for S1 was less contaminated. Numerous epidemiological studies in different aquatic environments have shown a relationship between coliform counts and incidence of infectious diseases in humans (Lugo et al. 2021). The results of our microbiological analyses are in line with the findings of Pepper et al. (2006) that municipal solid waste may contain a variety of pathogens.

## Conclusions

In conclusion, we found that soil samples from illegal landfills in Bosnia and Herzegovina contained heavy metals. Thus, there is an urgent need for banning illegal landfills in B&H in order to minimize environmental pollution as the results indicate that this waste contains significant levels of toxic metals and microorganisms that could pollute the environment. Future research should also concentrate on improving the efficiency of removing these pollutants by using environmentally efficient and cost-effective technologies such as bioremediation in order to reduce the potential adverse effect of this waste on the environment.

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**Table 4** The results of the microbiological analysis of water samples.

| Microorganisms | Sample sites | | |
|---|---|---|---|
| | S1 | S2 | S3 |
| Aerobic mesophilic bacteria | 18 ± 1.732b | 7.3 ± 1.1547c | 1168.33 ± 7.6376a |
| Aerobic heterotrophic bacteria | 169.333 ± 2.082b | 34.666 ± 2.309c | 4458 ± 7.000a |
| Total coliform bacteria | 349.333 ± 8.021b | 27.666 ± 1.528c | 3566.333 ± 5.508a |
| Fecal coliform bacteria | 6.333 ± 1.155b | 7.333 ± 1.155b | 2028.666 ± 3.215a |
| Intestinal enterococci | 2.333 ± 1.1547c | 24.000 ± 2.645b | 266.666 ± 2.516a |

Notes: Values are means (± SD) of three replicates, S1 = Cipolo, S2 = Dolovi, S3 = Malkin most. Means followed by different letter(s) in the same row differ significantly ($p = 0.05$) according to the Tukey’s multiple range test.

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# FEMALE CHOICE OF MATES IN THE APHIDOPHAGOUS LADYBIRD BEETLE, *HIPPODAMIA VARIEGATA* (COLEOPTERA: COCCINELLIDAE): THE EFFECT OF MALE AGE, SEXUAL STATUS AND FAMILIARITY

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**ABSTRACT**

Mating involves the transfer of sperm to females. Female reproductive investment is based on gamete numbers as well as gamete size. Females are more selective than males and this selection is based on numerous factors, like a male's age, sexual status and familiarity. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is an important natural enemy of many insect pests such as aphids, psyllids, whiteflies and mealybugs in many countries. We investigated female mate choice in *Hippodamia variegate* caging females with: (i) a young and a middle-aged male, (ii) a virgin and mated male and (iii) a novel and a familiar male. Time to and duration of copulation were recorded. Females preferred to mate with middle-aged and familiar males and did not differentiate between previously mated and virgin males. Females copulated quicker and for longer with young or familiar males.

**Keywords:** copulation duration; copulation latency; familiarity; female choice; individual recognition; mating experience

## Introduction

Sexual selection involves competition between members of one sex for access to mates or gametes of the opposite sex, which involves either mate choice or intrasexual competition (Darwin 1888; Andersson 1994). It is the main driving force for many evolutionary changes in organisms and the reasons for these changes has been studied in various species of animals (Thornhill and Alcock 1983; Eberhard 1996; Simmons 2001). The main function of mating is the transfer of sperm from males to females (Arnqvist and Nilsson 2000) and because of the larger investment in gametes of females (Bateman 1948), they are more selective than males (Trivers 1972). Mate choice helps females to choose among qualitatively different mates that could affect their reproductive output, provide better resources, genes and parental care (Kokko et al. 2003). Despite being costly in terms of energy and time invested in finding suitable mates (Johnstone et al. 1996; Watson et al. 1998), risk of injury and predation (Rowe 1994; Bonduriansky and Brooks 1998), of rejecting good mates (Real 1990) and choice of high-quality mates can greatly affect their fitness (Rosenthal 2017). Males have certain traits that benefit females (Holland and Rice 1998; Gavrilets et al. 2001) eventually leading to the co-evolution of certain mating preferences of males and females (Kirkpatrick and Ryan 1991; Jennions and Petrie 2000). Numerous abiotic (Gamble et al. 2003; Borg et al. 2006) and biotic (Mautz and Sakaluk 2008) factors influence mate choice. Studies on ladybirds have demonstrated the occurrence of age-based mate choice and its effect on parental reproduction and fitness of their progeny (Omkar and Pervez 2005; Omkar et al. 2006b; Avent et al. 2008; Omkar et al. 2010; Prathibha et al. 2011; Pandey and Omkar 2013), thus age is considered to be one of the most crucial factors.

Likewise, the mating status of males plays a crucial role (Wedell and Ritchie 2004). Females of the seed beetle, *Callosobruchus maculatus* (Fabricius 1775) (Coleoptera: Bruchidae) are more fecund when they mate with virgin than mated males (Savalli and Fox 1999). Though a single mating is sufficient for female insects to ensure fertilization and achieve reproductive success, multiple matings are reported for most species (Arnqvist and Nilsson 2000; Eberhard 2009; Parker and Birkhead 2013), including ladybirds (Haddrill et al. 2008). But in general, the costs of re-mating are high in terms of energy expenditure (Watson 1993), risk of predation or disease (Watson et al. 1998; Blanckenhorn et al. 2002), injury (Eberhard 1996) and reduction in life span (Maklakov et al. 2006). In addition, various benefits, such as genetic diversity (Arnqvist and Nilsson 2000) and ability of females to influence the outcome of sperm competition and offspring fitness (Eberhard 1996) are also reported.

Arthropods recognize each other (Liu et al. 2010) and in species that mate more than once, many females avoid copulating with familiar males (Ivy et al. 2005, Ödeen and Moray 2008) in order to ensure they gain superior genes for their offspring (Bateman 2004).

Mate choice is well studied in a range of insects (Reid 1991; Arnaud and Haubruge 1999; Birkinshaw and Smith 2001; Kobayashi and Ueda 2002; McNamara et al. 2004; Fedina and Lewis 2008; Maklakov and Arnqvist 2009; Suzuki 2009; Watson and Simmons 2010; Omkar and Afaq 2013; Pandey and Omkar 2013).

In ladybirds mate choice is reported to be based on morph (Osawa and Nishida 1992; Mishra and Omkar 2014), age (Bista and Omkar 2015), body size (Dubey et al. 2016), mating status (Dubey et al. 2018) and familiarity (Saxena et al. 2018). *Hippodamia variegata* (Goeze)

Faghihi, M. R., Seiedy, M., Omkar: Female choice of mates in the aphidophagous ladybird beetle, *Hippodamia variegata* (Coleoptera: Coccinellidae): the effect of male age, sexual status and familiarity
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(Coleoptera: Coccinellidae) is an aphidophagous ladybird native to Asia and a potential biological control agent (Tan et al. 2014). Mainly only the effect of polyandry and male body size (Pervez and Navodita 2011; Pervez and Singh 2013) are studied in this ladybird and little is known about its sexual behaviour. The present study is on mate choice and mating parameters based on age, mating status and familiarity of males.

## Material and Methods

### Establishment of stock culture

A stock colony was established using *Hippodamia variegata* ladybirds from the College of Agriculture and Natural Resources field of University of Tehran in Karaj, Alborz Province, Iran (35°48′04.6″N, 50°57′39.6″E, at an altitude of 1315 m) in May 2019. Following the transfer of them to the laboratory, the colonies of *H. variegata* were fed *A. fabae* in plastic containers (18 × 15 × 5 cm). They were maintained in the laboratory conditions at 25 ± 1 °C, 55 ± 5% RH and under a 16L: 8D photoperiod. Eggs were laid on the inner surfaces of the containers or on leaves and were collected daily by transferring the beetles or the leaves on which they laid their eggs to new containers. All larvae were fed an *ad libitum* supply of *Aphis fabae* and kept in the same conditions as the adults. Adults were separated based on sex within a few hours of emergence from the pupae to prevent them from mating. All the experiments were carried out in the same containers and conditions.

### Experimental design

#### *The effect of age on female mate choice*

In this experiment, young and unmated females and unmated males of two different ages (young: 10–15 days post-emergence and middle aged: 30–40 days old) (Bista and Omkar 2015) of *H. variegata* were used. Each female was provided with two males (young and middle aged) in a container. Mate choice was recorded. Once the mating commenced, the other male was removed in order to avoid male-male competition. To discriminate between the males, one elytron of each male was marked blue or white (Dubey et al. 2018). Colours were changed to avoid colour biases. The pairs were given 3 h to mate (from 12:00 to 15:00), after which they were separated. The pairs that did not mate within 3 h were not included in the experiment. In addition, we recorded time to copulation (i.e., time to genital contact) and duration of copulation (i.e., time from the insertion of the male's aedeagus into the female's genitalia until its removal). There were 25 replicates of this experiment.

#### *The effect of mating status of males on female mate choice*

In this experiment, young and unmated females were paired with young males of different sexual status (virgin and mated). Each female was provided with two males (virgin and mated) in a container. The mated male had copulated several times previously and was isolated 24 h before the experiment. Mate choice was recorded as above. Once mating commenced the other male was removed. Time to and duration of copulation were also recorded. There were 25 replicates of this experiment.

#### *The effect of male familiarity on female mate choice*

In this experiment, young and unmated males and females of *H. variegata* were used. Initially, a female was placed in a container with a male. The pairs that successfully completed a first mating were used in the following experiment after 24 h. The females were then given an opportunity to select between two males: a 'familiar male' with which the female had previously copulated and a 'novel male' that the female had not encountered before, but had mated with another female and isolated 24 h before the experiment. Mate choice was recorded. In addition, time to and duration of copulation were recorded. There were 25 replicates of this experiment.

### Statistical analysis

All analyses were done using IBM SPSS Statistics V22.0 software. A binomial test was used to evaluate mating occurrence. Since the distributions of the results of these experiments was not normal, the Mann-Whitney U test was used to compare the means of the times to and duration of copulation.

## Results

### The effect of age of males on female mate choice

Females preferred to mate with middle-aged males rather than young males (two-tailed binomial test: $p = 0.043$) (Table 1). Out of 25 females, 18 (72%) mated with the middle-aged males and only 7 (28%) mated with young males. The time to copulation was shorter for young males ($U = 18.5$, $p = 0.006$) but there was no significant difference in the duration of copulation ($U = 55$, $p = 0.623$).

### The effect of sexual status of males on their choice of females

The choice of females of virgin and mated males did not differ (two-tailed binomial test: $p = 1.000$) (Table 2). Out of 25 females, 13 (52%) mated with virgin and 12 (48%) with mated males. The time to copulation was shorter ($U = 10.000$, $p = 0.0005$) and duration of copulation was longer for mated males ($U = 0.0005$, $p = 0.0005$).

### The effect of male familiarity on female mate choice

Females preferred to re-mate with familiar than unfamiliar males (two-tailed binomial test: $p = 0.015$) (Table 3). Out of the 25 females, 19 (76%) mated with familiar and only 6 (24%) with males they had not previously encountered. The time to copulation was shorter for fa-

**Table 1** The percentage of females of *Hippodamia variegata* that mated, mean (± SE) time to and duration of copulation when mated with a middle-aged or young male.

| Treatment | Mating Occurrence (%) | Time to mating (Seconds) | Duration of copulation (Seconds) |
|---|---|---|---|
| **Middle-age** | 72 | 438.06 ± 85.877 | 7019.00 ± 173.356 |
| **Young** | 28 | 15.14 ± 6.010 | 7225.14 ± 222.407 |
| **Test statistics** | Two-tailed binomial test | Mann-Whitney U<br>U = 18.5 | Mann-Whitney U<br>U = 55 |
| **P-values** | *p* = 0.043 | *p* = 0.006 | *p* = 0.623 |

**Table 2** The percentage of females of *Hippodamia variegata* that mated and mean (± SE) time to and duration of copulation when mated with a virgin or previously mated male.

| Treatment | Mating Occurrence (%) | Time to mating (Seconds) | Duration of copulation (Seconds) |
|---|---|---|---|
| **Unmated** | 52 | 1253.92 ± 364.129 | 5066.08 ± 153.916 |
| **Mated** | 48 | 315.08 ± 23.266 | 6742.58 ± 121.332 |
| **Test statistics** | Two-tailed binomial test | Mann-Whitney U<br>U = 10.000 | Mann-Whitney U<br>U = 0.0005 |
| **P-values** | *p* = 1.000 | *p* = 0.0005 | *p* = 0.0005 |

**Table 3** The percentage of females of *Hippodamia variegata* that mated and mean (± SE) time to and duration of copulation when mated with a familiar or unfamiliar male.

| Treatment | Mating Occurrence (%) | Time to mating (Seconds) | Duration of copulation (Seconds) |
|---|---|---|---|
| **Familiar** | 76 | 314.16 ± 34.502 | 6524.83 ± 398.288 |
| **Novel** | 24 | 568.67 ± 127.192 | 6671.74 ± 104.356 |
| **Test statistics** | Two-tailed binomial test | Mann-Whitney U<br>U = 24 | Mann-Whitney U<br>U = 45 |
| **P-values** | *p* = 0.015 | *p* = 0.035 | *p* = 0.442 |

miliar males (U = 24, $p$ = 0.035), but there was no difference in the duration of copulation (U = 45, $p$ = 0.442).

## Discussion

Mate choice is well studied in Coleoptera such as *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Arnaud and Haubruge 1999; Fedina and Lewis 2008), bark beetle, *Ips pini* (Say) (Coleoptera: Scolytidae) (Reid 1991), oak ambrosia beetle, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) (Kobayashi and Ueda 2002), grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Birkinshaw and Smith 2001), hide beetle, *Dermestes agittari* De Geer (Coleoptera, Dermestidae) (McNamara et al. 2004), burying beetle, *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae) (Suzuki 2009), seed beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) (Maklakov and Arnqvist 2009), dung beetle, *Onthophagus sagittarius* Fabricius (Coleoptera: Scarabaeidae) (Watson and Simmons 2010) and parthenium beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) (Omkar and Afaq 2013; Pandey and Omkar 2013), but there is little information on mate choice based on morph, age, body size, mating status and familiarity in ladybirds .

The results of the first experiment on *H. variegata* revealed that the females preferred middle-aged males as sexual partners. This is consistent with what Pervez et al. (2004) and Sahu and Omkar (2013), respectively, report for *Propylea dissecta* (Coleoptera: Coccinellidae) and *Anegleis cardoni* (Coleoptera: Coccinellidae) with a sigmoidal increase in willingness to mate with age. This could be due to prolonged mate deprivation as is documented for *Coccinella septempunctata* (Srivastava and Omkar 2004).

In many species, middle-aged males are known to transfer more sperm (Parker 1974; Ueno 1994; Bonduriansky and Brassil 2002; Jones and Elgar 2004) and have higher reproductive output than other aged individuals (Hansen and Price 1995; Beck and Powell 2000). This is in accordance previous studies on ladybirds (Pervez et al. 2004; Srivastava and Omkar 2004; Bista and Omkar 2015). However, numerous studies imply that females tend to choose older males as partners in order to gain indirect benefits, as older males are likely to be of superior genetic quality (Trivers 1972; Manning 1985; Andersson 1994; Hansen and Price 1995; Kokko and Lindström 1996). Since gamete production and fertilization are costly, a way of optimizing the allocation of gametes is to choose mates based on their sexual status (Trivers 1972; Savalli and Fox 1999; Koene et al. 2008; Simmons and Beveridge 2011). In this context, numerous authors imply that unmated males can better enhance a female's fecundity than previously mated males (Jiménez-Pérez and Wang 2004; McCartney and Heller

2008; Michaud et al. 2013; Jiaqin et al. 2014; Mirhosseini et al. 2014; Colares et al. 2015; McDonald and Pizzari 2016) and females tend not to copulate with recently mated males (Markow et al. 1978; Nakatsuru and Kramer 1982; Gerofotis et al. 2015).

The results of the second experiment did not reveal a significant difference in female's choice between virgin and mated males; although previously mated males had a shorter time to copulation. This may be due to experienced males being more competitive in mating (Milonas et al. 2011) than virgin males (Wiklund and Kaitala 1995). The results demonstrated that mating with experienced males continued for a longer, which could be attributed to repeated mating as many studies report that experienced males produce less ejaculate than virgin males (Kaitala and Wiklund 1994; Wedell and Ritchie 2004; Torres and Jennions 2005; Lauwers and Van Dyck 2006; McNamara et al. 2007; Dowling and Simmons 2012). As mentioned in (Omkar et al. 2006a), prolonged mating may increase sperm transfer and result in an increase in fecundity and fertility and assure paternity (Dubey et al. 2018). In contrast, the studies on *Coccinella septempunctata* (Omkar and Srivastava 2002) and *Anegleis cardoni* Weise (Coleoptera: Coccinellidae) (Omkar and Afaq 2013) indicate that the duration of copulation is longest for virgin males.

The results of the third experiment revealed that females of *H. variegata* preferred familiar males over unfamiliar males and preferred to re-mate quickly with them. These results are consistent with the studies on *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Jiaqin et al. 2014); *Tenuis valvaenotata* (Mulsant) (Coleoptera: Coccinellidae) (Túler et al. 2018) and cabbage beetle, *Colaphellus bowringi* (Baly) (Coleoptera: Chrysomelidae) (Liu et al. 2010), where females prefer to remate with familiar males.

Contrary to this, there are studies in which females prefer mating with unfamiliar males: hide beetles, *Dermestes maculatus* (Coleoptera: Dermestidae) (Archer and Elgar 1999), *Menochilus sexmaculatus* (Coleoptera: Coccinellidae) (Saxena et al. 2018) and see also Hosken et al. 2003; Bateman 2004; Ivy et al. 2005; Harris and Moore 2005 and Ödeen and Moray 2008. This may be in order to gain genetic benefits from different males, which ensures the genetic diversity of their progeny and avoids genetic incompatibility (Arnqvist and Nilsson 2000).

In conclusion this study revealed previously unknown aspects of the mating behaviour of *H. variegata*. Females of *H. variegata* preferred to mate with familiar and middle- aged males and show no preference for mates based on mating status. Some research indicates that females of some insects do not prefer previously mated males (Archer and Elgar 1999; Bateman 2004) and of other species prefer to mate many times with mated familiar males (Liu et al. 2010). Further studies are needed to confirm these preferences by subjecting males and females to different combinations of the parameters. Also, the measure of the success in gaining access to mates is incomplete as competition also occurs in the reproductive tract of females, which could be better understood by studying post-copulatory sexual selection.

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### Authors' contributions

MS and MRF conceived the idea. MRF performed fieldwork and collected material. MRF, MS and O performed experiments and statistical analysis. MRF, MS and O contributed to writing the manuscript. All authors read and approved the final version of the manuscript.

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# PERCEPTION OF NATURAL ECOSYSTEMS AND URBAN GREENERY: ARE WE AFRAID OF NATURE?

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**ABSTRACT**

A set of pictures of natural vegetation in protected areas and urbanized ecosystems were shown to respondents. Protected areas were ranked as natural. Perceived naturalness of ecosystems was positively correlated with the sense of beauty and preference for recreational use, but negatively with the feeling of security. When the respondents rated ecosystems as natural, they also regarded them as more dangerous. A cumulative link mixed model supported the statement that perceived ecosystem naturalness decreased the feeling of safety; this relationship was comparatively weaker among people living in small villages and gardeners.

**Keywords:** natural ecosystems; protected areas; safety; urbanization; wilderness

## Introduction

Today, mankind is experiencing a dramatic shift towards urban life. Whereas in 1900 a mere 10 per cent of the global population were urban dwellers, that percentage now exceeds 50 per cent and will rise even more in the future (Grim et al. 2008). Vegetation may help to mitigate many aspects of urban development by moderating the climate, improving air and water quality, and by regulating run-off (Grim et al. 2008). Vegetation has also been shown to improve emotional states and relieve stress (Ulrich 1979; Kaplan 1995), enhance subjective well-being (Ulrich et al. 1991) and cognitive functions (Hartig et al. 2003), reduce pain in patients (Lechtzin et al. 2010) and bring other health- and wellness-related benefits (Velarde et al. 2007). Many studies focus on the role of city vegetation in comparison with non-vegetated urban areas, but less attention has been paid to comparing urban green spaces and natural vegetation. Urban greenery differs substantially from natural ecosystems in that it harbours larger proportions of non-native species and is usually artificially maintained (Grim et al. 2008), leading to landscape homogenization and reduction in biodiversity (McKinney 2002; Grim et al. 2008). In addition, urban vegetation usually requires more water and nutrients than local natural vegetation (McKinney 2002), which may affect ecosystem services provided by this type of landscape. In this study, we focus on differences in human perception of beauty and safety in natural ecosystems and urban green areas.

## Material and Methods

Prior to collecting data, we downloaded two hundred colour photographs of central-European landscapes from the internet. One hundred of these photographs depicted urban green spaces, parks and gardens, and the remaining hundred were pictures of protected areas. All photographs were landscape-oriented and taken in sunny weather during summer months. We then randomly selected eight pictures of urban green spaces and eight of protected areas, which were regarded as natural landscapes. The selected pictures reflected the heterogeneity of urban greenery and natural landscapes. The photographs of urban greenery included ornamental gardens, romantic gardens, aristocratic parks and city parks. Photographs of natural landscapes were classified as either forests, steppes or forest steppes, meadows or water ecosystems.

The group of respondents that participated in our study comprised 40 women and 27 men aged between 21 and 74, with an average age of 28.8 years, of whom 27 were from a large city (Prague), 35 from a medium-sized city (Pardubice) and 5 from villages with less than 200 inhabitants. They were asked to fill in questionnaires, which were used to collect the data.

The respondents were first asked to provide their socio-demographic information (age, gender, highest level of education, place of residence) and then indicate all their outdoor activities (sports, camping, gardening or other). In the next step, each respondent evaluated whether each of the photographs in the set of 16 photographs were of natural ecosystems or urban greenery. Finally, the respondents were asked to rate the beauty, naturalness and safety of each landscape depicted in the photographs using the four-point Likert scale.

The agreement between the researchers' and respondents' categorization of the photographs as natural landscapes or urban greenery was determined using a chi-square goodness of fit test. Correlations among the respondents' ratings of beauty, naturalness and safety and type of landscape were measured using Spearman's rank correlation coefficients. The perceived level of safety across the landscape categories was compared using one-way ANOVA. A cumulative link mixed model

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was used to evaluate the effects of the respondents' perception of landscape naturalness (independent variable) on their perception of safety (dependent variable), while controlling for the respondents' characteristics (covariates).

## Results

The match between the respondents' rating of landscape naturalness matched that of the researchers almost perfectly. Among the total of 1072 ratings (16 photographs per set, each rated by 67 respondents) there were only four mismatches. Consequently, we found no statistically significant difference between our classification of landscape naturalness and that of the respondents ($\chi^2$(DF) = 1071, p = 0.902).

The perceptions of beauty, security and naturalness were all positively and statistically significantly correlated with only one exception. The correlation between the perception of naturalness and security was statistically significant but negative (Table 1). Plotting the perception of safety against the perception of naturalness clearly showed that landscapes that were evaluated as natural were perceived also as less safe than those evaluated as urbanized (Fig. 1).

**Table 1** Spearman rank correlation coefficients (df = 1071) of the opinions of respondents to features of pictures of landscapes. All the correlations are significant at p < 0.01.

| | Beauty | Security | Naturalness |
|---|---|---|---|
| Security | 0.22 | | |
| Naturalness | 0.13 | –0.43 | |
| Recreation | 0.57 | 0.28 | 0.18 |

This simplified picture may have been affected by the fact that most of the evaluations were correlated, possibly due to autocorrelations introduced by the dependence of evaluations made by each respondent (note that each respondent rated all 16 photographs). To cope with the fact that evaluations made by the same respondents may be auto-correlated, we ran a cumulative link mixed model with the respondents' evaluations of landscape safety as the dependent variable and their evaluations of landscape naturalness and the respondents' characteristics as independent variables. This model also controlled for the dependence of evaluations made by the same respondents. Prior to fitting the data to the model, we converted the ratings of naturalness into binary format by collapsing the first and second, and third and fourth category of naturalness (cf. Fig. 1).

Results provided by the cumulative link mixed model are consistent with our previous result showing a significant negative effect of the perception of naturalness on the perception of safety (Table 2). This effectively means that landscapes which are perceived as natural are also perceived as dangerous. Other than that, the tendency to rate natural landscapes as dangerous was weaker among people who live in the country and among people who practise gardening as a hobby.

**Table 2** Results of a cumulative link mixed model in which respondents' evaluation of the dangerousness of different landscapes is the dependent variable, and their evaluation of naturalness of the same landscapes and the respondents' characteristics are independent variables. Ratings of naturalness were collapsed into two categories (natural vs. urban by pooling ratings 1 and 2 from Fig. 1 as natural, and 3 and 4 as urban). Gender, size of the home municipality and the preferred type of recreation were used as explanatory variables. Only significant variables and significant interactions are shown. *** Significant at p < 0.001, * significant at p < 0.05.

| Model parameters | Estimate | Significance |
|---|---|---|
| Naturalness (binary) | 1.9114 | *** |
| *Interactions* | | |
| Naturalness *villagers | –2.842 | *** |
| Naturalness *gardeners | –0.5818 | * |
| Variance of random effects (respondent) | 0.37 | |
| AIC | 2278 | |

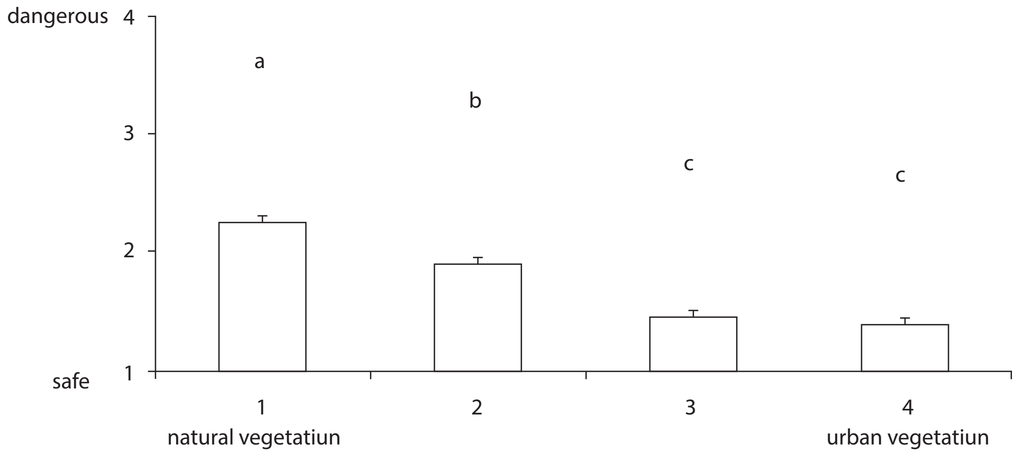


**Fig. 1** Respondents' perception of safety on a scale of from 1 (safe) to 4 (dangerous) in relation to the perception of naturalness of vegetation rated from completely natural (1) to strongly urban (4). Bars represent SD; statistically similar columns are indicated by the same letter (LSD post hoc test, p < 0.05).

## Discussion

Our results indicate that people perceive natural landscapes as dangerous. This finding may seem to contrast with observations made by others (Ulrich 1979; Parson 1991; Kaplan 1995; Van den Berg et al. 2003), who report an increase in positive emotions and a healthy, restorative effect of vegetation and natural habitats in comparison with urban man-made environments dominated by buildings. Lee et al. (2008), for instance, show that neighbourhood satisfaction increases with the amount of vegetation surrounding houses. However, contrary to previous studies, we did not compare vegetated vs. non-vegetated urban areas. Instead, our study focused on vegetated landscapes that differ only in whether they are natural or man-made. We revealed that the way vegetation is organized in the landscape (natural vs. urban) can affect the perception of the safety it provides. Interestingly, Jorgensen et al. (2002) also conclude that it is the spatial arrangement rather than the amount of vegetation that affects people perception of safety.

Our questionnaire did not enquire about the characteristics of the risks perceived by respondents in relation to natural landscapes. Note, however, that the average level of perceived risk recorded in our study was rather low even in natural landscapes (being roughly equal to the bottom third of the risk perception scale). This means that the level of risk perceived is probably not related to any life-threatening danger. Rather, we may think of it as perhaps caused by fear of minor injury from a wrong step on an *uneven surface, getting stung by* stinging nettles, being bitten by an insect or a tick. Fear of animals that are actually harmless, such as various spiders or frogs, may also be responsible for this increased perception of risk. Other possible components of perceived risk are virtual, evolutionary or atavistic fears related to threats such as dangerous wild animals, once posed to humans. Interestingly, the evolutionary fear of "wild beasts" is strongly persistent in human culture, as evidenced by traditional stories, fairy tales and religious texts (Cronon 1996).

Kaplan (1987) points out that humans prefer habitats offering some aspect of safety, namely refuge from an approaching enemy. This is also the explanation offered for the fact that people prefer savanna-like landscapes (Balling and Falk 1982). Indeed, the perceived dangerousness of natural landscapes may also be related to higher vegetation density and fear of criminals lurking in thick vegetation, in spite of the fact that in reality crime rates are lower in more vegetated environments (Kuo and Sullivan 2001).

More generally, the lack of safety associated with natural landscapes might be related to how unpredictable some natural landscapes are. By contrast, man-made environments are created for a purpose, so they must be more predictable by definition.

Our results indicate that natural habitats are perceived as less dangerous by people who live in villages or are gardeners. This observation is, in fact, consistent with fears of relatively benign dangers such as ticks, nettles or ankle-spraining holes. After all, one is unlikely to live close to natural habitats or look after a garden if he or she is afraid of natural habitats.

Evaluation of natural and urbanized landscapes in the sense of habitats affected either by natural forces or by human activity may seem a bit fuzzy in the context of European landscapes because most of them are affected by humans to some extent. The distinctiveness of protected areas is apparent also in our study, which found a surprisingly high ability of laypersons to differentiate between urban and natural landscapes (i.e. driven mainly by natural processes). This fact implies that a set marker must exist that allows even lay people to distinguish between natural landscapes formed to a large degree by natural processes and man-made urban greenery where natural forces play a lesser role.

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# REGRESSION ANALYSIS OF THE LENGTH-WEIGHT RELATIONSHIPS FOR 17 COMMON EUROPEAN FISH IN RIVERS IN THE CZECH REPUBLIC

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**ABSTRACT**

Length-weight relationships (LWRs) are useful for calculating weight based on measurements of length. Here we provide LWRs for 17 species of fish from the rivers Vltava and Elbe in the Czech Republic, Central Europe. The samples were collected by electrofishing from May 2016 to August 2019. There are far fewer LWRs for riverine than lotic fish. All LWRs were significant with $r^2$ values ranging from 0.99 for the European barbel (*Barbus barbus*) to 0.95 for European bullhead (*Cottus gobio*) and with estimated *b* values ranging from 2.93 in common dace (*Leuciscus leuciscus*) to 3.26 in non-native round goby (*Neogobius melanostomus*). These results increase the data on LWRs for fish in riverine environments and provides a good tool for managing fisheries and future studies.

**Keywords:** European fish; freshwater fish; length-weight relations; LWR; riverine environment

## Introduction

Length-weight relationships provide a quantitative description of the relation between length and weight of individuals in fish populations, which may be used to determine biomass and further indices – e.g., condition based on length distribution data (Ricker et al. 1975; Froese et al. 2011; Verreycken et al. 2011). An obvious advantage of LWR is that just by having length measurements it is possible to estimate weight, thus avoiding further laboratory work and not having to kill the specimens. The relationships provided can also be used to estimate biomass, when only lengths of fishes are available, e.g. in both recreational and commercial fishing. Growth of fish stocks depends on various factors, such as species, sex, age and season (Le Cren 1951; Bagenal and Tesch 1978; Khristenko and Kotovska 2017), but it also differs depending on habitats and nutritional status of fish in different environments. LWR equation is $W = aL^b$, where $W$ is the weight (g), $L$ is the standard length (cm), $a$ is the intercept and $b$ the slope of regression and an allometric coefficient (Le Cren 1951; Froese 2006). Despite the many LWR studies on European freshwater fish, these are mostly for lakes and hence may differ in terms of the parameter $b$ derived from the data for the lotic environment. The aim of this study is to provide LWRs for common European riverine fishes, for which there are far fewer LWRs (Tsionki et al. 2021). LWR were estimated for 17 species of riverine fish species collected in the two largest rivers in the Czech Republic, Vltava and Elbe Rivers.

## Material and Methods

Fish were collected by electric fishing powered by a Honda engine and a LENA generator the output voltage of which was 300–600 V (50 Hz) (Bednář, Czech Republic; https://www.r-bednar.cz/). Fish of a range of different sizes were sampled from the entire community by wading upstream along the shoreline for about 100 metres. These surveys were done from May 2016 to August 2019.

In total, 1385 individuals belonging to 17 species were measured and the samples were kept frozen until processed in the laboratory. All fish were identified to species according to Kottelat and Freyhoff (2007) and their length ($L$; cm; nearest to 0.1 cm), wet weight ($W$; g; nearest to 0.1 g) measured. The LWRs were calculated using the least square regression method and $r^2$ (coefficient of determination) used as an indication of the robustness of the relationships (Le Cren 1951; Froese 2006). The coefficient of allometry $b$ (i.e. the slope) describes how the weight of fish (g) scales with body length (cm). The regression equation for the LWRs is $W = aL^b$, the logarithmic form of which is:

$$\log_{10}(W) = \log_{10}(a) + b \log_{10}(L)$$

Curvilinear plots of the length and weight data were generated and used to check for outliers in the dataset (Froese 2006). The significance of the regression analyses was tested using an ANOVA. All statistical analyses were performed in the software R 4.0.5 (R Core Team 2015).

## Results

LWRs were calculated for 17 species of fish, see Table 1 for detailed information on sample size, ranges in length (cm) and body weight (g), LWR parameters with 95% CI of $a$ and $b$, and coefficient of determination ($r^2$) for each species.

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**Table 1** Length-weight relationships for 17 species of fish collected from the Vltava and Elbe Rivers, Czech Republic, from May 2016 to August 2020 (*a* and *b* are parameters of the length-weight relationship; CI confidence interval; *N* sample size; SE standard error (*b*); $r^2$ coefficient of determination; *L* total length; *W* body weight).

| | | Length (cm) | | Weight (g) | | Regression parameters | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **N** | **Min** | **Max** | **Min** | **Max** | **a** | **95% CI** | **b** | **95% CI** | **$r^2$** |
| *Squalius cephalus* | 261 | 4.7 | 41.2 | 1.02 | 1000 | 0.113 | 0.1179–0.1274 | 3.121 | 3.0864–3.1551 | 0.992 |
| *Rutilus rutilus* | 292 | 3 | 28.1 | 0.22 | 264.12 | 0.106 | 0.1023–0.1103 | 3.234 | 3.1977–3.2706 | 0.991 |
| *Alburnus alburnus* | 124 | 2.5 | 16.7 | 0.13 | 47.31 | 0.101 | 0.0942–0.1083 | 3.158 | 3.0898–3.2264 | 0.986 |
| *Barbus barbus* | 72 | 2.4 | 52.7 | 0.13 | 1220 | 0.101 | 0.1218–0.1314 | 3.044 | 3.0112–3.07664 | 0.998 |
| *Gobio gobio* | 136 | 2.7 | 17 | 0.16 | 50.21 | 0.126 | 0.1214–0.1298 | 3.029 | 2.9924–3.0654 | 0.995 |
| *Leuciscus leuciscus* | 120 | 2.4 | 25.2 | 0.22 | 154.74 | 0.143 | 0.1344–0.1522 | 2.930 | 2.8657–2.9936 | 0.986 |
| *Chondrostoma nasus* | 63 | 3.4 | 45.1 | 0.32 | 1160 | 0.112 | 0.1019–0.1227 | 3.143 | 3.0760–3.2099 | 0.993 |
| *Perca fluviatilis* | 54 | 5.3 | 29 | 1.58 | 277.93 | 0.144 | 0.1196–0.1738 | 3.007 | 2.8252–3.1884 | 0.955 |
| *Leuciscus idus* | 32 | 4.4 | 44 | 0.65 | 980 | 0.111 | 0.0928–0.1333 | 3.221 | 3.0582–3.3845 | 0.982 |
| *Cottus gobio* | 51 | 5.1 | 10.3 | 1.72 | 15.88 | 0.138 | 0.1150–0.1661 | 3.119 | 2.9095–3.3275 | 0.948 |
| *Gymnocephalus cernua* | 20 | 0.12 | 5.2 | 12.5 | 22.66 | 0.123 | 0.1077–0.1567 | 3.116 | 2.9130–3.3186 | 0.983 |
| *Neogobius melanostomus* | 89 | 0.12 | 5.1 | 1.62 | 17.68 | 0.128 | 0.1165–0.1402 | 3.255 | 3.1473–3.3623 | 0.976 |
| *Rhodeus amarus* | 26 | 2.3 | 6.9 | 0.12 | 4.28 | 0.137 | 0.1249–0.1495 | 3.108 | 2.9769–3.2398 | 0.989 |
| *Abramis brama* | 11 | 0.14 | 11.1 | 11.36 | 600 | 0.138 | 0.0972–0.1957 | 2.971 | 2.6976–3.2433 | 0.985 |
| *Blicca bjoerkna* | 10 | 9.5 | 385 | 10.83 | 860 | 0.134 | 0.1803–0.1340 | 3.114 | 2.8734–3.3539 | 0.993 |
| *Barbatula barbatula* | 10 | 0.14 | 5.9 | 11.3 | 10.87 | 0.122 | 0.0935–0.1591 | 2.988 | 2.6991–3.2771 | 0.986 |
| *Pseudorasbora parva* | 14 | 0.12 | 3.8 | 7.8 | 4.79 | 0.120 | 0.1017–0.1407 | 3.100 | 2.9392–3.3883 | 0.987 |

## Discussion

The LWRs presented in this study are for common European fish within their usual size ranges, except for round goby (*Neogobius melanostomus*), which is a non-native species recently reported in the Czech Republic. The coefficient of allometry (*b*), reported in this study varied from 2.92 to 3.25; the latter being the upper limit for all the species of fish evaluated in this study.

Most of the studies on LWRs have been conducted in the lakes, whereas studies on riverine species are far less common. Our results can be compared to a few of such studies. More specifically, European chub (*Squalius cephalus*), common roach (*Rutilus rutilus*) and European barbel (*Barbus barbus*) in rivers across Europe, for which there are similar *b* coefficients (Prokeš et al. 2006; Verreycken et al. 2011). Although invasive species in Western Europe (Spain, Portugal, and the Middle East), the Common bleak (*Alburnus alburnus)* is only occasionally from lakes (Kleanthidis et al. 1999). The only recent data on this species in a riverine environment comes from the tributaries of the Ebro River in Spain, where the values of $b = 2.84$ and $b = 3.05$ (Leunda et al. 2006) are lower than those recorded in our study. We increased the data on the LWR for gudgeon (*Gobio gobio)*, for which Verrycken et al. (2011) report the value *b* as 3.18, which is higher than our result for this species ($b = 3.03$). We also recorded the LWR for common nase (*Chondrostoma nasus*), for which there is only a single record for $b = 3.04$ from the Skadar Lake (Milosević and Mrdak 2016). Although there are several studies on the LWRs of European perch (*Perca fluviatilis*), most are for lakes and very few for riverine habitats (Rajkova-Petrova 2001). Data on the LWR for European bullhead (*Cottus gobio*) can only be compared with results of a study on this species in the Tiber River, Italy, which reports a *b* value of 3.304 (Bevagna et al. 1990).

Despite being an invasive species in Europe and North America, there is little data on the LWRs of round goby (*Neogobius melanostomus*). MacInnis and Corkum (2000) report the LWR for this species in rivers in the USA, for which $b = 3.0$. Finally, our results for the LWR of silver bream (*Blicca bjoerkna*) can be compared with an older study in the Berounka River, Czech Republic (Hanel 1991), for which $b = 3.27$.

For lakes, the average value for ruffe (*Gymnocephalus cernua)* based on 11 studies 1 is $b = 3.17$ (Ogle and Winfield 2009), which is higher than that recorded in this study ($b = 3.12$). The value recorded for *Abramis brama* in this study is $b = 2.97$, which is lower than that recorded in other studies such as in the Marmara region in Turkey $b = 3.25$ (Tarkan et al. 2006), or in the Danube Delta in Romania, where the average value is $b = 3.20$ (Cernisencu and Staras 1992). Older studies on common bleak *Alburnus alburnus* in 6 lakes in Greece report an average $b = 3.34$ (Kleanthidis et al. 1999), which is higher than the $b = 3.16$ recorded in this study. That is, higher *b* values are reported for lentic environments than for rivers.

Data for European dace (*Leuciscus leuciscus*) $b = 3.19$, Ide (*Leuciscus idus*) $b = 3.26$, ruffe (*Gymnocephalus cernua*) $b = 3.04$, stone loach (*Barbatula barbatula*) $b = 3.14$ and barbel (*Barbus barbus*) $b = 3.10$, are reported by Verreycken et al. (2011) for Flanders (Belgium) and for European chub (*Squalius cephalus*) by Koç et al. (2007).

Comparison of the parameters *b* of the above species studied by Verreycken et al. (2011) indicates that dace (*Leuciscus leuciscus*), stone loach (*Barbatula barbatula*) and barbel (*Barbus barbus*) have low values and ide (*Leuciscus idus*), ruffe (*Gymnocephalus cernua*) and European chub (*Squalius cephalus*; Koç et al. 2007) have high values.

This study aims to provide data for fisheries regulation and management of rivers (Kottelat and Freyhof 2007; Lyach and Čech 2018). The LWRs presented increase the accuracy of fish biomass estimates for rivers and hence can serve as a primary source for fisheries and/or future scientific studies focused on riverine fish communities.

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# EFFECT OF CLIMATE CHANGE ON THE SPATIO-TEMPORAL DISTRIBUTION OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* WIEDEMANN (1824) IN ALGERIA

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**ABSTRACT**

This study was carried out in order to determine the initial distribution of Mediterranean fruit fly *Ceratitis capitata* Wiedemann (1824) (Diptera, TephridIdae) in Algeria, the area now occupied and assess the role of climate change in determining its current distribution. The various surveys and trapping of adults indicate that in addition to the coastal, sub-coastal and Argan regions, this fly is also present in oases. It is reported in all the traditional oases where fruit trees are intercropped with date palm. From the 2000s, the area occupied by this fruit fly increased and spread into provinces where it was not previously reported. Gradually, it increased in abundance and became an important pest. The climate data recorded in the province of Batna (Aurès Mountains, Saharan Atlas) indicate there has been a change in climate. The annual and monthly average temperatures and relative humidity for the period 2000–2018, in this province became more favourable for this fruit fly than in the period 1913–1937, in particular, during the months of March, April, October and November. The results also indicate that in orchards where there are several types of fruit, the numbers of this fly are higher than where mainly apples are grown.

**Keywords:** climate change; initial area; recently colonized area; Tephritidae

## Introduction

The original distribution of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (1824) (Diptera, Tephrididae), has always been a subject of controversy. However, the studies of Malacrida et al. (1992) and Baruffi et al. (1995), using molecular biology techniques, confirmed that it is native to sub-Saharan Africa. Its appearance in the Mediterranean basin is fairly well documented and dated. It was reported in Spain shortly after 1840, around Algiers in 1858, Italy in 1860 and Tunisia in 1885 (Piguet 1960). Between 1920–1930, it colonized almost all of its current distribution (Piguet 1960). This fruit fly is present in many countries in Africa, Central and South America, the Mediterranean Basin and Australia (Quilici 1997). In North Africa, it occurs along the coast and in sub-coastal areas from Tunisia to Morocco (Balachowsky and Mesnil 1935; IAEA 1995). Based on its extreme polyphagia, it is considered to be one of the most economically important pests in the world. In fact, its host range includes more than 360 species of plants (Liquido et al. 1991). In the Mediterranean basin, it is considered to be the most harmful pest, especially in Maghreb countries (Algeria, Libya, Morocco and Tunisia) (IAEA 1995). The financial loss due to this pest in these countries is estimated to be US $60–90 million per year, of which US $7–10 million per year is spent on insecticides (IAEA 1995). In addition, a survey conducted in Morocco, evaluated the annual losses caused by this fly on the main fruit and *Citrus* crops at 53 422 200 DH (Aboussaid et al. 2009). In Tunisia, Boulahia-Kheder and Jerraya (2009) also consider this pest as the most important of several types of fruit, in particular, *Citrus*, peaches, figs and prickly pears and Jerraya (2003), estimates that the losses would be 90% of production in the absence of chemical treatments.

In Algeria, this fly was reported for the first time in 1858 (Balachowsky and Mesnil 1935) and is a major problem for the production and export of fruit (Oukil et al. 2002). In northern Algeria, the presence of *C. capitata* has been confirmed in all the coastal provinces, in particular, in Algiers (Oukil et al. 2002), Blida (Dridi 1990; Oukil et al. 2002), Tizi Ouzou (Sadoud Ali-Ahmed et al. 2011; Bachi and Sadoud Ali-Ahmed 2017), Jijel (INPV 2019), Annaba (Boudjelida and Soltani 2011), Oran (Oukil et al. 2002) and Tlemcen (Settaoui et al. 2017). In southern Algeria (Sahara), Oukil et al. (2002) report collecting specimens of this fly from apricots and oranges grown in the oases Djanet and Ghardaia. According to Laamari et al. (2015), this fly is always associated with fruit in coastal regions. In the interior provinces, with a semi-arid climate (cold winter and hot and dry summer), this pest is unknown despite the presence of its favourite hosts. Laamari et al. (2015) add that due to the effect of climate change in Algeria, especially from the 2000s, the distribution of this pest has increased. In addition to apricots, peaches, figs and pomegranates, very significant losses are reported for apples grown mainly in the mountainous areas of Batna and Khenchela (Aurès Mountains, northeast of Algeria). The objective of this study is to determine the distribution of this fly in Algeria before and after the increase by means of

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surveys and trapping of adults. In addition, a comparison of climatic data from the province of Batna during 1913–1937 and 2000–2018 was carried out, in order to determine the reasons for the increase in the distribution of this fly.

## Material and Methods

### Initial distribution area

In order to delimit the initial distribution of this fly in Algeria, the collection of fruit and trapping of adults was used in arid provinces where previously reported (Balachowsky and Mesnil 1935; Oukil et al. 2002) and in provinces with a semi-arid climate, where previously reported before the 2000s (Laamari et al. 2015).

The first part of this study was carried out during 1995–1997. During this period, fruit of *Prunus armeniaca* L., *P. domestica* L., *P. persica* L., *Ficus carica* L., *Pyrus communis* L., *Citrus sinensis* (L.), *Citrus reticulata* Blanco, *Citrus aurantium* L., *Punica granatum* L., *Eriobotrya japonica* (Thunb.), *Opuntia ficus-indica* L., *Malus communis* L., *Cydonia oblonga* Mill. and *Juglans regia* L., were collected and kept in cages until the adults emerged. This involved fruit collected from the oasis region (provinces of Biskra, El Oued, Ouargla and Ghardaïa) and provinces with a semi-arid climate (Tébessa, Oum El Bouaghi, Khenchela, Batna, M'sila, Laghouat and Djelfa).

In addition to collecting infested fruit, pheromone traps were set in two provinces. Four traps in two oases located at Guerara (province of Ghardaïa, Sahara) (32°49´09.68″N, 4°31´39.05″E, altitude: 296 m a.s.l.) during 1995–1997. These oases are characterized by very high catches. In addition to date palm, *Citrus*, fruit trees and vegetable crops, are grown there. During the same period, four traps were set in two apple orchards located at Ichemoul (province of Batna, Aurès Mountains, Saharan Atlas) (35°16´09.39″N, 6°26´41.05″E, altitude: 1203 m a.s.l.). The traps used are of type: AA-TRAP and the pheromone capsule was renewed every four weeks. The traps were checked once a week.

### Recently colonized area

In order to delimit the area colonized by the Mediterranean fruit fly in Algeria in the 2000s, infested fruit was collected in the provinces where this fly did not previously occur. These are the provinces of Tébessa, Oum El Bouaghi, Khenchela, Batna, M'sila, Laghouat and Djelfa, located throughout the Saharan Atlas and characterized by a semi-arid climate. In addition, pheromone traps were set in two orchards in the province of Batna during 2014–2018. The first orchard was located at T'kout (35°7´44.30″N, 6°16´55.99″E, altitude: 934 m a.s.l.) and in which fig, apricot, pomegranate, peach, pear trees and prickly pear were cultivated. The second was located at Ichemoul (35°16´09.39″N, 6°26´41.05″E, altitude: 1203 m a.s.l.) in which there was mainly apple trees.

### Effect of climate change

Among climatic factors, temperature and relative humidity are very important for the survival of the Mediterranean fly (Messenger and Flitters 1958; Shoukry and Hafez 1979; Delrio et al. 1986). Based on the requirements of the Mediterranean fruit fly, Bodenheimer (1951) delimits 4 zones: optimal (temperature 15–30 °C, relative humidity 75–85%), favourable (temperature 10–35 °C, relative humidity 60–90%), tolerance zone (temperature 2.5–40 °C, relative humidity 40–100%) and unfavourable (other values). Dajoz (1975) presents these zones graphically in the form of an eco-climagram for *C. capitata* (Fig. 1). If the annual or monthly average values of these climatic factors for a region is known then using an eco-climagram it is possible to determine the level of development of this fly. In order to assess the effect of climate change on changes in the distribution of *C. capitata* in Algeria, the data re-

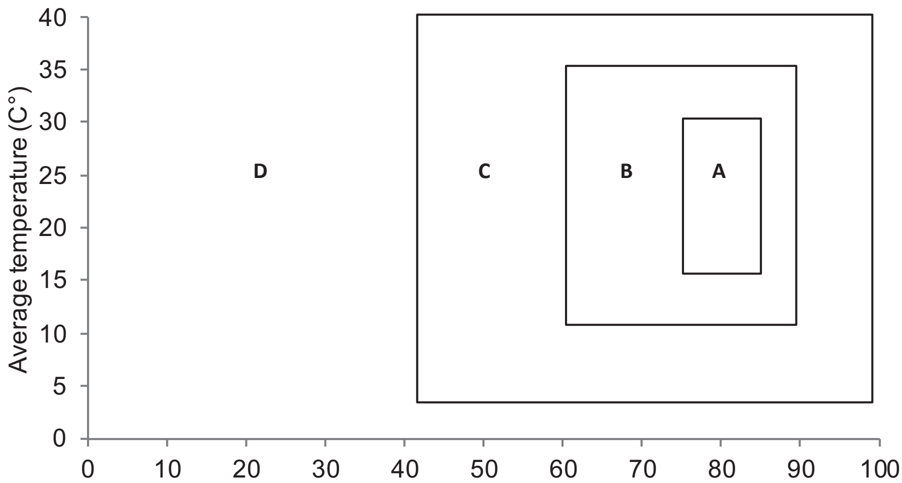


**Fig. 1** Eco-climagram of *C. capitata* based on average temperature and relative humidity. A: Optimal zone, B: Favorable zone, C: Tolerable zone, D: Unfavorable zone (Dajoz, 1975).

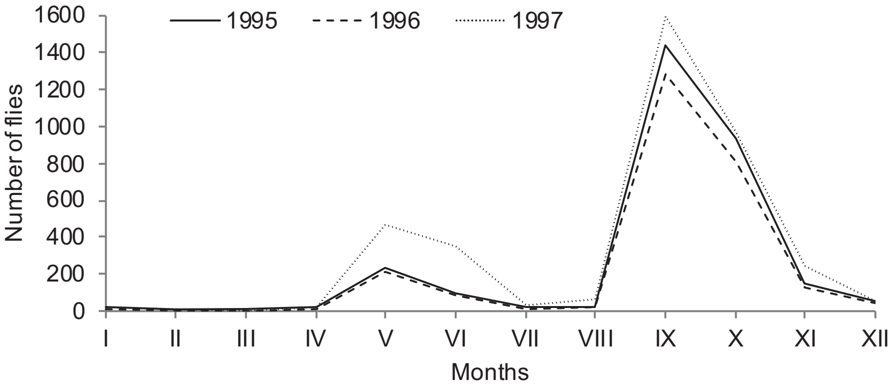


**Fig. 2** Evolution of the average monthly number of *C. capitata* flies in the traps installed in the locality of Guerara (Provience of Ghardaia) during 1995–1997 (average per trap).


**Fig. 3** Initial distribution of *C. capitata* in Algeria. Administrative code of the province: 07 Biskra, 09 Bilda, 13 Tlemcen, 15 Tizi Ouzou, 16 Algiers, 18 Jijel, 23 Annaba, 30 Ouargla, 31 Oran, 37 Tindouf, 39 El Oued, 47 Ghardaia, 51 Ouled Djellal, 55 Touggourt, 56 Djanet, 57 El M'ghaier, 58 El Meniaa.

corded in the province of Batna during 1913–1937 and 2000–2018, were compared. The first period is the earliest for which climatic data is available for Algeria (Seltzer 1946). The second period coincides with the occurrence of this fruit fly in this province. It should be noted that Batna is known as one of the coldest provinces in Algeria. It is part of the mountainous region of Aurès, where the cultivation of fruit trees is very old and most orchards are located at altitudes above 1000 m a.s.l.

## Results

### Initial distribution

The results obtained in 1995–1997 revealed that the Mediterranean fruit fly was present in all oases, where polyculture was practiced (Table 1). In addition to fruit trees and *Citrus*, melon was severely infested, but no other Cucurbitaceae or Solanaceae. In the provinces located throughout the Saharan Atlas, this pest was completely

**Table 1** Crops infested with *C. capitata* during 1995–1997 in the regions of oases and the Saharan Atlas.

| Regions | Oases | Saharan Atlas |
|---|---|---|
| Provinces | Ghardaïa, El Oued, Biskra, Ouargla, Djanet, Ouled Djellal, Touggourt, El M'Ghaier, El Meniaa | Tébessa, Oum El Bouaghi, Khenchela, Batna, M'sila, Laghouat and Djelfa |
| Infested plants | *Eriobotrya japonica* (Thunb.), *Prunus armeniaca* L., *Ficus carica* L., *Pyrus communis* L., *Citrus sinensis* (L.), *Citrus reticulata* Blanco, *Citrus aurantium* L., *Punica granatum* L., *Cucumis melo* L. | No infestation |
| Uninfested plants | *Phoenix dactylifera* L. | |

**Table 2** Annual average number of *C. capitata* in pheromone traps installed in the provinces of Ghardaïa and Batna during 1995–1997.

| Regions | Oases | | | Saharan Atlas | | |
|---|---|---|---|---|---|---|
| Provinces | Ghardaïa | | | Batna | | |
| Localities | Guerara | | | Ichemoul | | |
| Years | 1995 | 1996 | 1997 | 1995 | 1996 | 1997 |
| Average number of flies per trap | 3003 | 2630 | 3840 | 0 | 0 | 0 |

absent. The pheromone traps set during 1995–1997 at Guerara (province of Ghardaïa), confirmed the presence of this fly in the Algerian oases and an annual average of 3840 flies / trap was recorded in 1997 (Table 2). It should be noted that this fly is present in this oasis throughout the year and is most active during the months of September (1596 flies) and October (965 flies) (Fig. 2). However, the pheromone traps set during the same period, at Ichemoul (province of Batna), caught no fruit flies (Tables 1 and 2). On the basis of these results and those already obtained by other authors in coastal, sub-coastal regions and natural stands of *A. spinosa*, the initial distribution area of the Mediterranean fruit fly in Algeria was delimited (Fig. 3).

## Recently colonized area

In the 2000s, the first appearance of the Mediterranean fruit fly is reported in the provinces located throughout the Saharan Atlas. The infested fruit was apricot, peach, fig, pear, pomegranate, prickly pear and apple (Golden Delicious) (Table 3). The pheromone traps set in the province of Batna during 2014–2018, confirmed the presence there of this pest. Despite annual variations, the numbers caught are considered to be very large, especially in the orchard at T'kout. At this locality 9479 flies / trap was recorded in 2015 (Table 4). A maximum of 4450 flies / trap was recorded in August, or about 1113 flies per week or 36 flies per day (Fig. 4). In the apple orchard at Ichemoul, this fly was less abundant than that recorded in the previous locality. The overall number did not exceed 354 flies per trap in 2018 (Table 4). About 85% of this number was caught in September (123 flies) and October (179 flies), which coincides with the maturity of the apples (Golden delicious) (Fig. 5). The results obtained in the 2000s were used to determine the area recently colonized by the Mediterranean fruit fly in Algeria (Fig. 6).

## Effect of climate change

Comparison of the climatic data from the Batna meteorological station for the periods 1913–1937 and 2000–2018, revealed marked differences (Figs 4, 5 and 6). The average annual temperature was 1.5 °C higher in the second period, while precipitation decreased by 80 mm. Relative humidity also increased by 10.5%. Based on the annual average temperature and relative humidity, the eco-climagram of this fly in province of Batna changed from a zone of tolerance (1913–1937) towards

**Table 3** Crops infested with *C. capitata* in the provinces located throughout the Saharan Atlas from the 2000s.

| Region | Saharan Atlas |
|---|---|
| Provinces | Tébessa, Oum El Bouaghi, Khenchela, Batna, M'sila, Laghouat et Djelfa |
| Infested plants | *Prunus armeniaca* L., *Prunus persica* L., *Ficus carica* L., *Pyrus communis* L., *Punica granatum* L., *Opuntia ficus-indica* L., *Malus communis* L. variety Golden Delicious and occasionally the variety Royal Gala |
| Uninfested plants | *Malus communis* (L.) variété Starkrimson, *Cydonia oblonga* Mill., *Prunus domestica* L., *Juglans regia* L. |

**Table 4** Number of *C. capitata* in pheromone traps installed in the province of Batna during the period 2014–2018.

| Region | Saharan Atlas | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Province | Batna | | | | | | | | | |
| Localities | T'kout | | | | | Ichemoul | | | | |
| Years | 2014 | 2015 | 2016 | 2017 | 2018 | 2014 | 2015 | 2016 | 2017 | 2018 |
| Average number of flies per trap | 8435 | 9479 | 5894 | 6509 | 7750 | 302 | 274 | 240 | 261 | 354 |

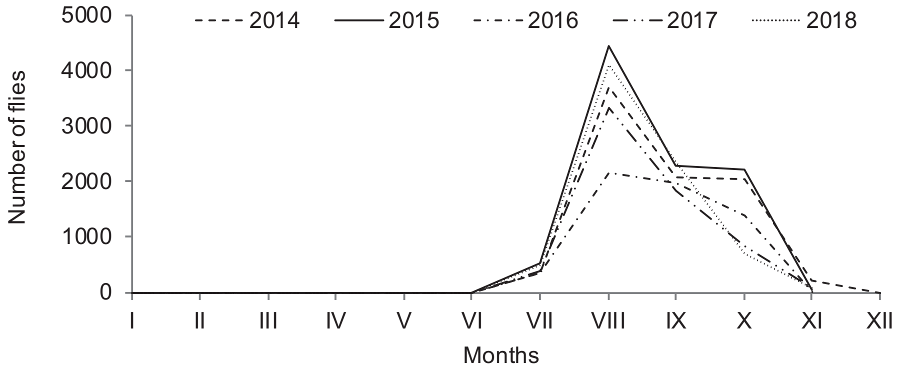


**Fig. 4** Evolution of the average monthly of *C. capitata* in the traps installed in the locality of T'kout (Province of Batna) during the period 2014–2018 (average per trap).

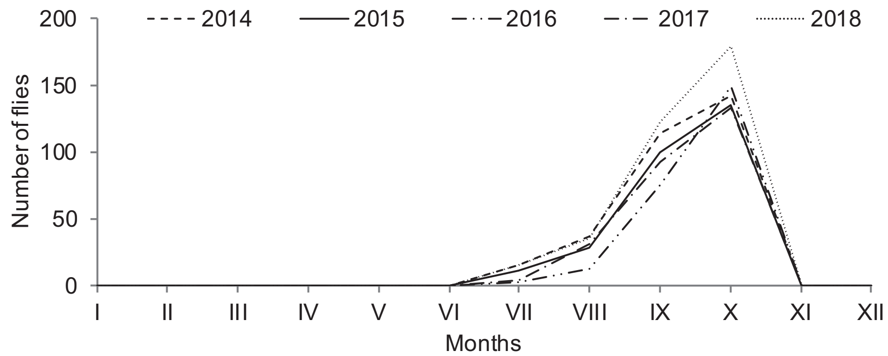


**Fig. 5** Evolution of the average monthly numbers of *C. capitata* in the traps installed in the locality of Ichemoul (Province of Batna) during the period 2014–2018 (average per trap).

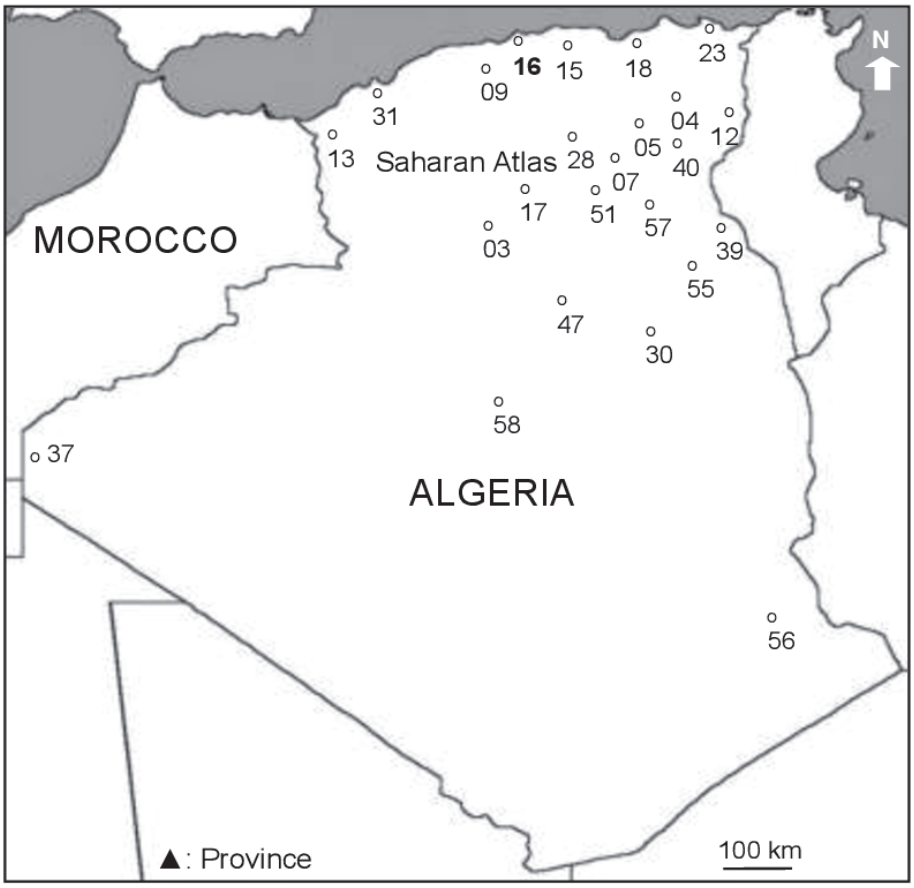


**Fig. 6** New distribution area of *C. capitata* in Algeria. Administrative code of the province: 03 Laghouat, 04 Oum El Bouaghi, 05 Batna, 07 Biskra, 09 Bilda, 12 Tébessa, 13 Tlemcen, 15 Tizi Ouzou, 16 Algiers, 17 Djelfa, 18 Jijel, 23 Annaba, 28 Msila, 30 Ouargla, 31 Oran, 37 Tindouf, 39 El Oued, 40 Khenchela, 47 Ghardaia, 51 Ouled Djellal, 55 Touggourt, 56 Djanet, 57 El M'ghaier, 58 El Meniaa.

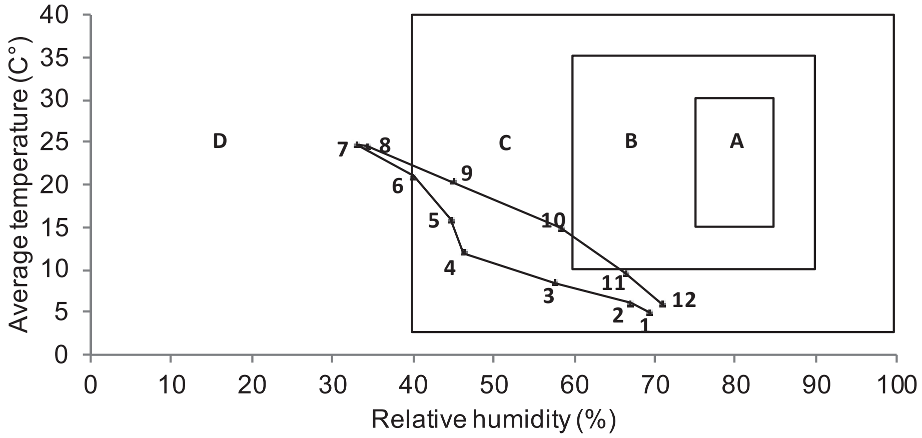


**Fig. 7** Location of monthly average temperature and relative humidity values for Batna during 1913–1937 in the eco-climagram of *C. capitata*. A: Optimal zone, B: Favorable zone, C: Tolerable zone, D: Unfavorable zone, 1, 2, 3 … months.

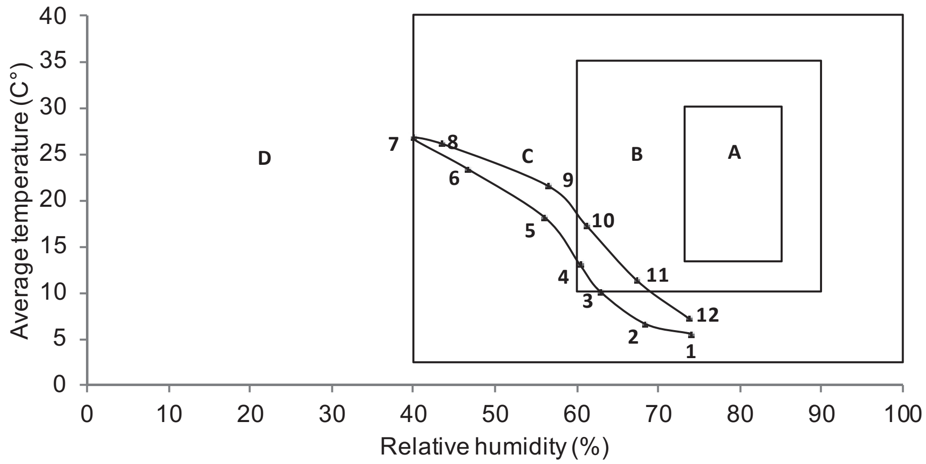


**Fig. 8** Location of monthly average temperature and relative humidity values for Batna during 2000–2018 in the eco-climagram of *C. capitata*. A: Optimal zone, B: Favorable zone, C: Tolerable zone, D: Unfavorable zone, 1, 2, 3 … months.

favourable (2000–2018), especially in March, April, October and November (Figs 7 and 8).

## Discussion

According to Balachowsky and Mesnil (1935); Oukil (1995); Sadoud-Ali Ahmed et al. (2011) and Settaoui et al. (2017), the Mediterranean fruit fly occurs in the coastal and sub-coastal region in Algeria, where the climatic and nutritional conditions are favourable for its proliferation. In these regions, with a Mediterranean climate, it attacks mainly oranges, mandarins, peaches, apricots, figs and medlars and it can complete up to 6 generations per year (Balachowsky and Mesnil 1935). In the Sahara, this fly is also present at the Algerian-Moroccan border where it infests the Argan tree (*Argania spinosa* L.), which is its natural host (Zhar et al. 2013). In Tunisia, this fly is reported occurring in oases by Ben Chaaban et al. (2018) and in Algeria for the first time. Its damage to 9 crops was reported in different provinces surveyed during 1995–1997. Very large numbers were also caught by pheromone traps in 1997 in a traditional oasis at Guerara (Ghardaïa). Despite the hot and dry climate of the Sahara, this pest occurs in these oases, where the microclimate is favourable for its development. It is even reported in the province of El Oued on *Capsicum annuum* cultivated in glasshouses (Mostefaoui et al. 2020). In addition, the different crops in these oases mature at different times, which enables this pest to reproduce continuously. Date palm, in particular, the variety Deglet Nour the main variety grown in these regions was not attacked. During the same period, this pest was not recorded during surveys and trapping in the provinces throughout the Saharan Atlas. Apparently, the semi-arid climate, characterized by a cold winter and a hot and dry summer in these provinces was unfavourable for this fly at that time.

From the 2000s, the first cases of fruit infestations by this pest were reported in provinces in the Saharan Atlas and have become increasingly important. Surveys and trapping, confirmed that this pest had spread into provinces in the Saharan Atlas and was infesting 7 types of fruit in all the provinces surveyed. The infestation can affect the whole production if it is harvested when the fruit is mature. In the Batna and Khenchela provinces, if the apples (Golden Delicious) are not stored in cold rooms, this pest can destroy the entire harvest. Laamari et al. (2015) report that attacks by this fly in recently infested provinces (Batna and Khenchela) have reached alarming levels of an average of 10 larvae per fruit. During mild years, this fly can infest orchards located at 1600m. On the other varieties of apple attacks are very rare (Royal Gala) or not recorded (Starkrimson). Pomegranate and fig are the most affected. It is very rare to find an uninfected pomegranate in the market. All preserved fruit take one to two days to mummify. Pomegranate and fig are the most affected. It is very rare to find uninfected pomegranates in the market as their mummification takes a few days. In addition, the numbers of this fly caught in traps placed in the province of Batna were very high, in particular at T'kout. In this orchard there are several types of fruit. The number of individuals per trap in this orchard exceeded those reported by Sadoud-Ali Ahmed et al. (2011) (4590 flies) and Settaoui et al. (2017) (781 flies) in the coastal regions and in the traditional oasis at Guerara (3838 flies). In addition to suitable hosts that mature at different times, the presence of *Opuntia* at the edges of this orchard have enabled this fly to thrive. The fruit of *Opuntia* can be exploited by this fly (reservoir host) when other suitable fruit is not available. In Tunisia, Jerraya (2003) where *Opuntia* is abundant this fly reproduces continuously. In the orchard at Ichemoul, where there are only apple trees, the numbers of this fly caught by traps were lower. Apparently, at this locality, the absence of hosts that can harbour this fly during the period prior to the maturity of the apples is responsible for the lower abundance of this pest there than at the first orchard. These results are used to define the distribution of this fly in Algeria in the 2000s.

In order to understand the reasons for the change in the behaviour of this fly, the climatic data for the province of Batna was analysed. Comparison of the old (1913–1937) and recent (2000–2018) climatic data revealed a marked increase in the temperature and decrease in precipitation in this province. Despite this, the relative humidity has increased. It may be that the increase in cultivated and irrigated areas, construction of new dams and storage of water in basins has contributed to the increase in humidity in summer and autumn over the last few years. Based on the eco-climagram proposed by Dajoz (1975) the annual average values of temperature and relative humidity for the years 1913–1937, indicate that the province of Batna was a tolerance zone for development of this fly. The monthly data for these two climatic parameters for the months of June, July and August were in the unfavourable zone. Conversely, the data for the years 2000–2018 placed the province of Batna in the favourable zone especially in the months of March, April, October and November. If during the first two months, the fly resumes activity after winter diapause, the other two months coincide with the maturity of late varieties of apple, fig and all varieties of pomegranate. In addition to the increase in the area reserved for arboriculture, it is clear that the change in climate that has occurred in these provinces in the Saharan Atlas has favoured the spread of this pest. In the provinces recently infested with this pest, farmers cannot control it. The oviposition by this pest generally goes unnoticed and it is only after harvesting that its presence becomes apparent. It continues to seriously threaten fruit production in these regions.

## Conclusion

The rearing of this pest from infested fruit and the sexual trapping of adults, started in 1995 in Algeria and initially confirmed the presence of *C. capitata* in the oasis region and absence in the provinces in the interior, located in the Saharan Atlas. In addition, the second part of the study started in the 2000s, just after the reports of the first cases of infestation of fruit in other provinces, confirmed that the distribution of this fly in Algeria had changed. It spread into the Saharan Atlas region, where the climate is semi-arid. The pheromone traps set in the province of Batna during the years 2014–2018 highlighted the abundance there of this fly, especially in orchards where there several types of fruit were grown rather than a single species (apple tree). The meteorological data for this province revealed that the climate was warmer and drier in 2000–2018. In order to assess the effect of this change in climate, the annual and monthly average values of temperatures and relative humidity were plotted on the eco-climagram for this fly. The results revealed that compared with the old data (1913–1937) this province became more favourable for the development of this fly in 2000–2018, in particular, during the months of March, April, October and November.

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