PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Behixhe Ajdini

Title of PhD research: Omega-3 enriched insect (*Acheta domesticus*) as a novel and eco-sustainable food in Europe.

Name of PhD supervisor: Cristina Truzzi

Research lab name: Analytical Chemistry for Environment and Food lab

Cycle:

[ ] XXXVI
[X] XXXVII

PhD Curriculum:
[ ] Marine biology and ecology
[ ] Biomolecular Sciences
[X] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[X] Other. Please, indicate: Clean Room, Nutrinsect s.r.l

ABSTRACT (1000 characters, including spaces):
The house cricket (*Acheta domesticus*, AD) is considered one of the most promising farmed insect species for the novel food sector, thanks to its attractive nutritional profile and its great taste. However, being a terrestrial species, it lacks long-chain polyunsaturated fatty acids, such as the omega-3 eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3) acids, which are health-promoting nutrients for human consumption. My PhD project aims to study eco-sustainable rearing substrates to produce AD powder enriched in omega-3 and omega-6 fatty acids (FA) destined to human use. Among these, seaweed has been investigated as feed ingredients for insect rearing. During the 2nd year of this PhD, the evaluation of the nutritional profile (proximate composition, protein, lipid content, amino acid and fatty acid composition) of AD fed seaweed-enriched diets was carried out. Moreover, the chemical risk associated to AD fed seaweed-enriched diets was evaluated, to carry out an assessment of the risk of exposure to humans following the intake of AD as food.

Part 1. Scientific case of the PhD Research
BACKGROUND
In the near future, the increasing population will lead to a significant rise in the food demand. By 2050, the world population is expected to reach over 9 billion [1], and the demand for food is also expected to increase up to 70% above today's levels. Nowadays, traditional animal farming of e.g., cattle, swine, poultry, fish, is considered no longer sustainable due to the massive pressure on limited resources such as land, water and feed, and severe impacts on the environment from greenhouse gas emissions, significant loss of biodiversity, alteration of ecosystems, among other factors [2]. Recently, the consumption of edible insects is considered as an available alternative food being highly nutritious for humans [3], and at the same time, more sustainable than traditional animal farming when it comes to resource used and their overall ecological footprint [4-5].

SCIENTIFIC AIMS

The first aim of the 2nd year of this PhD was to investigate the nutritional profile of AD fed seaweed-enriched diets in terms of the proximate composition, protein, lipids, amino acid and fatty acids composition. The red algae *Palmaria palmata* (PP) and the brown algae *Ascophyllum nodosum* (AN) were added to the diet in different percentages. The second aim was the evaluation of the chemical risk of AD fed seaweed-enriched diets, with a focus on the potentially toxic elements (PTEs) contamination, to carry out an assessment of the risk of exposure to humans following the intake of AD as food.

Workplan 1.

Objective. First trial with the inclusion of the red algae PP in AD diet: determination of the proximate and amino acid composition of PP, PP-enriched diets and AD fed PP-enriched diets (fatty acid composition was determined during the first year of the PhD).

Methods. For the first experiment, PP was added to the traditional feed used at the insect company for the optimal cricket growth, in three percentages (5%, 10% and 20% of total feed) using three rearing crates for each feed. The experiment started at day 20 post hatching and ended at day 27 post hatching.

Seaweed biomass, experimental diets and the freeze-dried crickets were analysed for the proximate composition following AOAC (2016) procedures. Crude protein was determined as total nitrogen (N) through the Kjeldahl method. The analysis of the amino acid was determined by HPLC using Water AccQ-Tag column (3.9 x 150 mm) at a flow rate of 1 ml/min and Waters 2475 fluorometer detector set on 250 nm excitation and 395 nm emission.

Obtained results. *PP and PP-enriched diets.* The inclusion of PP in the diet for AD led to a significant decrease ($p<0.05$) of protein content compared to the Ctrl diet (27.23±0.04 g/100 g dry matter (DM) in PP20, compared to 28.68±0.23 g/100 g DM in the Ctrl). A similar trend was observed for total lipid content, with the highest values in the Ctrl diet (4.32±0.03 g/100 g of DM) and the lowest in PP20 (3.91±0.01 g/100 g DM). Moreover, the seaweed dietary inclusion also affected the amino acid profile of diets. Significant differences ($p<0.05$) between groups were evidenced only for Histidine, Alanine, Valine, and Methionine (Figure 1).

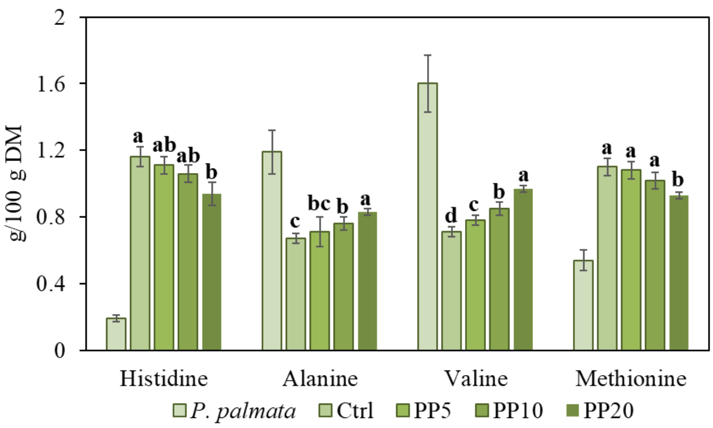

Figure 1. Histidine, Alanine, Valine and Methionine content (g/100 g DM) of PP and PP-enriched diets. PP, *Palmaria palmata*. Ctrl, control diet; PP5, PP10, and PP20, diets enriched with 5%, 10% and 20% of PP, respectively.

Acheta domesticus. All groups presented high protein levels, from 49.3 g/100 g DM to 54.2 g/100 g DM but increasing the percentage of PP in the diet led to a significant increase of protein content in AD fed PP10 and PP20 with respect to Ctrl group (Table 1). Moreover, the highest inclusion of PP (PP20) in the diet led to a significant decrease of lipid content in AD. Chitin was significantly higher in PP20 with respect to the other groups.

The amino acid profile of AD fed PP-enriched diets is reported in the Figure 2. Significant differences ($p<0.05$) were observed for Aspartic acid, Histidine, Arginine, Methionine, and Lysine. AD fed the Ctrl and PP20 diets showed a significant ($p<0.05$) decrease in the Aspartic acid content when compared to PP5 and PP10. Histidine resulted higher in PP20 compared to the Ctrl, PP5 and PP10, that did not present any differences between each other's. Arginine resulted significantly ($p<0.05$) higher in the Ctrl and PP20 with respect to PP5 and PP10. The Ctrl group also reported the lowest value of both Methionine and Lysine with respect to the other groups.

In all dietary groups, crickets consumed all the feed given at the end of the trial. The inclusion of PP in the crickets' diet did not affect crickets' growth parameters or survival (Figure 3).

Table 1. Proximate composition (g/100 g DM) of AD fed the test diets.

Proximate composition	**Ctrl**	**PP5**	**PP10**	**PP20**
Dry matter	93.45±0.21[a]	92.00±0.76[b]	91.89±0.36[b]	92.33±0.04[b]
Crude protein	51.3±1.1[b]	50.0±1.3[b]	53.9±0.5[a]	54.2±0.8[a]
Total lipids	16.4±2.6[a]	15.8±1.8[a]	16.6±0.3[a]	13.0±1.0[b]
Ash	5.9±0.7	6.1±0.8	6.5±0.2	6.7±0.4
Crude fiber	8.87±0.05	8.60±0.60	8.85±0.17	9.25±0.41
Carbohydrate*	17.5±2.8	19.5±3.0	14.1±0.6	16.9±1.4
Chitin	8.4±0.2[b]	8.6±1.3[b]	9.6±1.0[b]	10.1±1.5[a]
Gross Energy (MJ/kg)	21.3±0.5	22.1±1.2	21.7±0.3	20.6±0.3

Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD reared on diets enriched with 5%, 10% and 20% of PP, respectively. Within each raw, different letters denote significant differences among dietary treatments ($p<0.05$). Values are presented as mean±SD (n=3). Means within rows bearing different letters are significantly different ($p<0.05$).

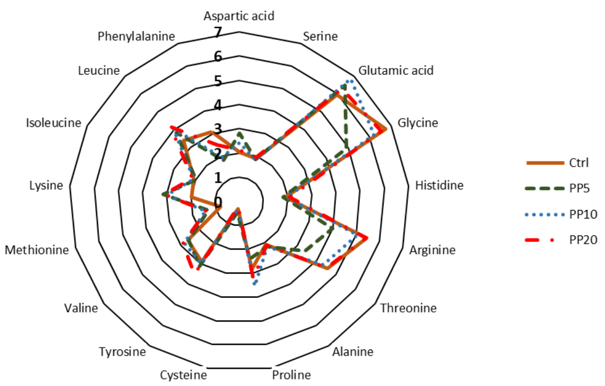

Figure 2. Amino acid profile (g/100 g DM) of AD fed seaweed-enriched diets. PP, *Palmaria palmata*. Ctrl, AD fed control diet; PP5, PP10, and PP20, AD fed diets enriched with 5%, 10% and 20% of PP, respectively.

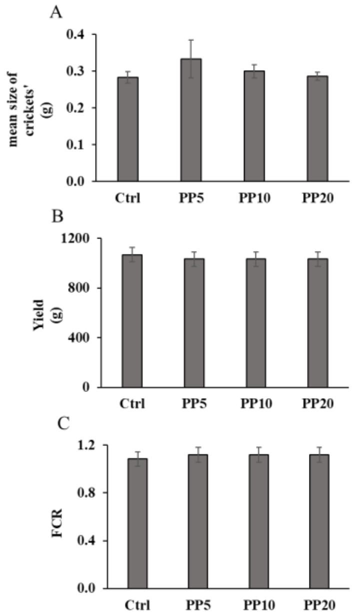

Figure 3. (A): final individual weight of crickets (wet weight); (B) cricket yield and (C) feed conversion ratio (FCR). Ctrl, AD reared on the control diet; PP5, PP10, and PP20, AD reared on diets enriched with 5%, 10% and 20% of PP, respectively.

To summarize, Seaweed-enriched diets were overall palatable for crickets and presented a high protein content, comparable to Ctrl diets, which led to good growth and welfare of the crickets. the addition of PP in the diet influenced the nutritional composition of AD in terms of both protein and lipid contents, but only to a minor extent. Based on the results from this study, we conclude that PP is a suitable substrate for cricket mass rearing up to 20 g/100 g of the diet. Regarding the fatty acid composition (reported in the 1st PhD report), we believe that the administration of PP-enriched diets to AD (7 days) was not long enough to generate a fast response in AD. Moreover, the use of other species of seaweed (containing more EPA) should be considered.

Workplan 2.

Objective: Second trial with the inclusion of the brown algae *Ascophyllum nodosum* (AN) in AD diet: determination of the fatty acid composition of AN-enriched diets and AD fed diets enriched with AN.

Methods. For the second experiment, *A. nodosum* was added to the control diet used at the insect company for the optimal cricket growth, in two percentages (20%, and 40% of total feed) using three crates for each diet. The experiment started at day 15 post hatching and ended at day 33 post hatching.

Fatty acid determination was performed by GC-MS after their derivatization to Fatty Acid Methyl Esters (FAMEs). FAMEs were determined using an Agilent-6890 GC equipped with a split-spitless injector and coupled to an Agilent-5973 N quadrupole Mass Selective Detector.

Obtained results. *AN-enriched diets.* The most represented FA class in the AN-enriched diets was PUFAs (from 67±3 g/100 g FAs in the Ctrl diet to 50±2 g/100 g FAs in AN40). The increasing levels of AN in the diet led to a significant (p<0.05) increase of SFAs in AN20 (18±1 g/100 g FAs) and AN40 (23±1 g/100 g FAs), compared to the Ctrl diet (15±1 g/100 g FAs) and of MUFAs in AN40 (27±1 g/100 g FAs) compared to the Ctrl group (18±2 g/100 g FAs) and AN20 (21±2 g/100 g FAs). The essential fatty acid EPA was not present in the Ctrl diet but compared in the seaweed-enriched diets; its percentages increased when more seaweed was added in the diet (up to 2.47±0.08 g/100 g FAs in AN40). Also, DHA was introduced in the diet with the seaweed inclusion (0.022±0.002 g/100 g FAs in AN20, 0.21±0.01 g/100 g FAs in AN40).

Acheta domesticus. The inclusion of AN in the diets influenced the FA composition of AD. Regarding SFAs percentage, AD fed seaweed-enriched diets (AN20 and AN40) showed a significant (p<0.05) decrease compared to the Ctrl group (40±1 g/100 g FAs) (Figure 4). The inclusion of AN in the diets at 40% led in AD to a significant (p<0.05) increase of MUFAs, n3 and n9 with respect to the other groups. AN20 showed a statistically significant (p<0.05) increase of PUFAs and n6 with respect to the Ctrl and AN40. In terms of n6/n3 ratio, AN20 (16±1) showed a significant (p<0.05) increase with respect to AN40 (10±1). The essential FA, EPA, was not detected in the AD fed the Ctrl diet, but it increased significantly (p<0.05) in AD with increasing the level of AN in the diets, up to 0.472±0.015 g/100 g FAs in AD fed AN40. The DHA was detected only in AD fed the Ctrl and AN40 diets.

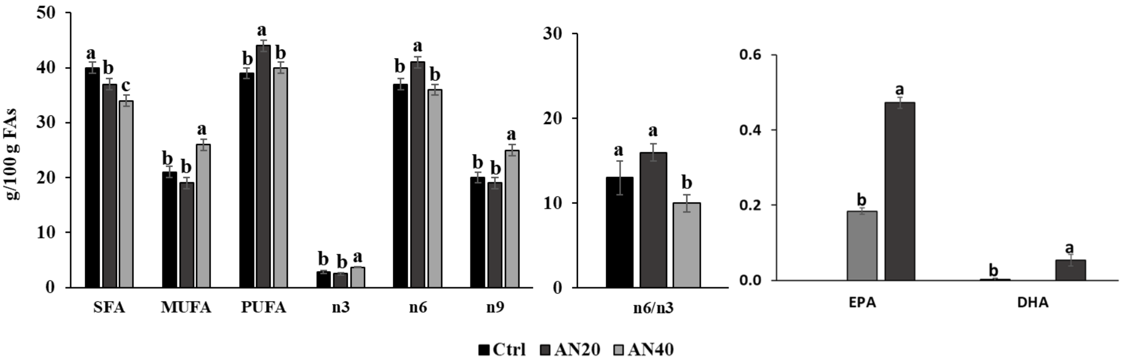

Figure 4. Fatty acid classes (g/100 g FAs) of AD fed the test diets. Data about diets palatability and AD growth, as well as data about proximate composition and amino acid profile, are in progress.

To conclude, the inclusion of AN in the diet led to a high increase of PUFAs content. Comparing AD fed diets with the same percentage of inclusion of seaweed (PP20 and AN20), AD fed with AN20 showed a 10-fold higher increase of EPA with respect to AD fed PP20.

Workplan 3.

Objective: Evaluation of the chemical risk associated to the use of AD fed diets enriched with PP (trial 1) and AN (trial 2) as food.

Methods:

1) For the determination of PTEs such as Cd, Pb, Ni, As and Al, the lyophilized samples were digested in a Microwave Accelerated Reaction System, MARS-X, 1500 W (CEM, Mathews, NC, USA) before the analysis, performed with an Agilent DUO 240FS atomic absorption spectrometer (Agilent, Santa Clara, CA 95051, USA) equipped with graphite furnace (GTA120 Graphite Tube Atomizer). The total mercury (Hg) content was quantified by a Direct Mercury Analyzer (FKV, Milestone, Italy).
2) For the risk assessment, the Hazard Index (HI) was calculated as the sum of the Health Hazard Estimation (HQ) of each PTE in AD fed seaweed-enriched diets.

Obtained results. *seaweed-enriched diets.* Concentrations of all PTEs, except for Ni, Cr and Al (as no legal limit are reported for these elements in animal feed) in seaweed-enriched diets for both the trial 1 and the trial 2 did not exceed any of the current legal limits referring to EC limit (2002/32/EC) and amendments on undesirable substances in animal feed, then diets based on PP and AN inclusion are considered safe for animal consumption.

Acheta domesticus. AD bioaccumulated Hg and As (data not shown), however concentrations of PTEs (Table 2) were well below the legal limit proposed by EU No 1881/2006 of 19 December 2006, which set maximum levels for certain contaminants in foodstuffs, and amendments (Regulation No. 420/2011 of 19 April 2011 and No 1006/2015 of 25 June 2015). Ni, Cr and Al are not regulated, but since they were present in significant concentrations both in the diets and AD, and considering the toxicity of these elements, it should be important setting legal limits for these elements.

Table 2. PTEs content (mg/kg DM) in AD fed diets enriched with PP and AN.

Sample*	Cd	As	Pb	Hg	Ni	Cr	Al
Trial 1							
Ctrl	0.121±0.005[b]	0.09±0.02[ab]	0.07±0.01[a]	0.0070±0.0002[b]	1.20±0.10[b]	0.57±0.01[b]	61±1[c]
PP5	0.118±0.003[b]	0.09±0.02[a]	0.06±0.01[a]	0.0065±0.0002[a]	0.74±0.02[a]	0.31±0.02[a]	40±4[a]
PP10	0.106±0.006[a]	0.11±0.01[ab]	0.10±0.01[b]	0.0072±0.0001[b]	0.79±0.03[a]	0.29±0.02[a]	55±2[b]
PP20	0.127±0.002[b]	0.12±0.01[b]	0.12±0.01[c]	0.0080±0.0003[c]	0.85±0.06[a]	0.25±0.01[a]	53±2[b]
Trial 2							
Ctrl	0.069±0.005[a]	0.08±0.01[a]	0.05±0.01[a]	0.0066±0.0002[a]	0.64±0.06[a]	0.58±0.01[b]	17±2[a]
AN20	0.084±0.005[b]	0.17±0.01[b]	0.07±0.01[a]	0.0104±0.0001[b]	0.76±0.06[a]	0.22±0.01[a]	18±1[a]
AN40	0.13±0.02[c]	0.36±0.03[c]	0.07±0.01[a]	0.0141±0.0010[c]	0.80±0.09[a]	0.16±0.02[a]	24±1[b]

*AD fed standard diet with 0% of *P. palmata* (Ctrl) or 0% of *A. nodosum* (Ctrl); AD fed diets enriched with 5% of PP (PP5), 10% (PP10) and 20% (PP20); AD fed diets enriched with 20% AN (AN20) and 40% AN (AN40). Different letters in the column indicate statistically significant differences between experimental groups ($p<0.05$).

Table 3 reports the average HQ (Hazard Quotient) value for each element, and Figure 5 shows the contribution (in percentage) of each PTE to the average HQ value calculated in AD fed experimental diets. Since all HQ values were well below 1, the ingestion of AD fed experimental diets pose a low chemical risk to human health.

Table 3. Average value of HQ for each element.

PTEs	HQ (mean±SD)
Cd	0.008±0.002
As	0.005±0.004
Pb	0.0016±0.0005
Hg	0.0010±0.0006
Ni	0.0031±0.0007
Cr	0.008±0.005
Al	0.014±0.007

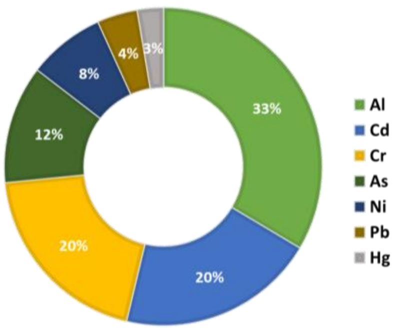

Figure 5. HQ percentage of each PTE for AD fed diets.

The average value of HI obtained by summing the HQ calculated for each experimental group, was 0.04±0.01 (ranging from a minimum value of 0.026 to a maximum value of 0.056), confirming that AD fed seaweed-enriched diets does not present a significant risk for human health, and can be used safely for human consumption as food.

Future perspectives:

-Data elaboration of the proximate and amino acid composition of AN, AN-enriched diets and AD fed AN-enriched diets.
-Studying an innovative, circular and environmentally friendly production of AD through the inclusion in the diet of microalgae grown on by-products of AD rearing, for novel eco-sustainable functional foods formulations.
-Enhance the efficiency of insect breeding for the development of innovative food products, focusing on both production and nutritional aspects.

- Developing a system to distinguish males and females crickets, to study new rearing substrates to enhance the fertility of crickets themselves.

- REFERENCES

[1] World Health Organization (WHO) 2019.

[2] Guiné, R.P.F., Correia, P., Coelho, C., Costa, C.A., 2021. Open Agriculture.

[3] Orkusz, A., 2021. Nutrients, 13(4): 1207.

[4] van Huis, A., Oonincx, D.G.A.B., 2017. Agronomy for Sustainable Development, 37: 1-14.

[5] Patel, S., Suleria, H.A.R. and Rauf, A., 2019. Trends in Food Science & Technology, 86: 352-359.

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools.

1. Formazione specifica salute e sicurezza sul lavoro Rischio Medio (24/01/2023)

2. A shot of science: Toxicological effects of cigarette butts for marine organisms (20/12/2022)

5. Tips and tricks, CASS-Scifinder-n (07/02/2023)

6. Lezioni di informatica, Corso getting started with R: environmental computing

7. Progetto scienziato per un giorno: centri estivi sportivi JUMP (03/09/2023)

8. Computer science: Theory and application of complex networks (Maria Grazia Ortore)

9. Ecotoxicology under global changes: impacts in marine wildlife (27/04/2023).

List of periods spent abroad.

None

List of conferences/workshops attended and of contributions eventually presented.

1. **5th MS Envy Day**, Milan. Fatty acids as potential biomarkers in marine suspended particulate matter across the Southern Ocean: extraction and quantification by gas chromatography-mass spectrometry C. Truzzi, F. Girolametti, A. Annibaldi, M. Fanelli, **B. Ajdini**, S. Illuminati.
2. **Bioanalitica 2023**, Florence. Chemical characterization of a deep Ionian Sea sediment core M. Fanelli, S. Zampatti, F. Girolametti, **B. Ajdini**, A.Annibaldi, C. Truzzi, S. Illuminati, F. Spagnoli.
3. **Bioanalitica 2023**, Florence. Chemical composition of European tea leaves: elemental content and health risk assessment for consumers F.Girolametti, A. Annibaldi, S. Illuminati, **B. Ajdini**, M. Fanelli, E. Damiani, E. Giorgini, P. Carloni, C. Truzzi.
4. **TUMA 2023**, Francavilla al Mare. Up-take of potentially toxic elements in the novel food *Acheta domesticus* grown of seaweed-enriched media: risk assessment for human health **B. Ajdini**, I. Biancarosa, S. Illuminati, A. Annibaldi, F. Girolametti, M. Fanelli, C. Truzzi. (**Oral presentation by B. Ajdini**).
5. **TUMA 2023**, Francavilla al Mare. Mercury (Hg) and other potentially toxic elements (Cd, Pb, As) in honey from the Marche Region (Central Italy). Risk assessment for human health F. Girolametti, **B. Ajdini**, A. Mezzaluna, A. Annibaldi, S. Illuminati, M. Fanelli, C. Truzzi.
6. **Science for the Planet**, Campobasso. Studio delle deposizioni atmosferiche nell’area portuale di Ancona M. Fanelli, F. Girolametti, **B. Ajdini**, M. Lorenzo, A.Falgiani, G.M. Quero, M. Basili, P. Penna, E. Frapiccini, A. Annibaldi, G.M. Luna, C. Truzzi, S. Illuminati.

7. **IV International Ross Sea Conference 2023**, Naples. From New Zealand to Antarctica (Ross Sea): the fatty acid composition of marine suspended particulate matter F. Girolametti, A. Annibaldi. S. Illuminati, M. Fanelli, **B. Ajdini**, A. Ardini, C. Truzzi.
8. **Analitica 2023**, Vasto. Nutritional value of house cricket (*Acheta domesticus*) fed diets supplemented with different levels of the seaweed *Palmaria palmata* in the feeding media **B. Ajdini**, I. Biancarosa, G. Cardinaletti, S. Illuminati, A. Annibaldi, F. Girolametti, M. Fanelli, G. Pascon, M. Martinoli, C. Truzzi. (**Oral presentation by B. Ajdini**).
9. **XX National Congress of the Division of Environmental Chemistry and Cultural Heritage**, Ischia. Chemical characterisation in arctic marine sediments in Kongsfjorden M. Fanelli, L. Massi, F. Girolametti, **B. Ajdini**, A. Annibaldi, C. Truzzi, Ø. Mikkelsen, S. Illuminati.
10. **XX National Congress of the Division of Environmental Chemistry and Cultural Heritage**, Ischia. Trace elements and nutrients evaluation in the caostal area of Ancona subjected to different anthropogenic pressures M. Fanelli, A. Pelella, F. Girolametti, **B. Ajdini**, C. Truzzi, A. Annibaldi, S. Illuminati.

Part 3. PhD student information on publications

1. In preparation: **B. Ajdini**[1], I. Biancarosa[2], G. Cardinaletti[3], S. Illuminati[1], A. Annibaldi[1], F. Girolametti[1], M. Fanelli[1], G. Pascon[3], M. Martinoli[4], F. Tulli[3], T. Pinto[2], C. Truzzi[1]. Modulation of the nutritional profile of *Acheta domesticus* by dietary enrichment with graded levels of the brown seaweed *Ascophyllum nodosum.*
2. In preparation: Behixhe Ajdini[1], Irene Biancarosa[2], Silvia Illuminati[1], Anna Annibaldi[1], Federico Girolametti[1], Matteo Fanelli[1], Cristina Truzzi[1]. Up-take of Potentially Toxic Elements from the novel food *Acheta domesticus* grown of seaweed-enriched diets: risk assessment for human health.

List of publications on international journals

1. Girolametti, F., Illuminati, S., Annibaldi, A., **Ajdini, B**., Fanelli, M., & Truzzi, C. "Mercury in honey from the Marche region (central Italy). Risk assessment from human consumption and its use as bioindicator of environmental pollution." Heliyon, 2023, 9(10), e20502, DOI: https://doi.org/10.1016/j.heliyon.2023.e20502
2. **B. Ajdini[1]**, I. Biancarosa[2], G. Cardinaletti[3], S. Illuminati[1], A. Annibaldi[1], F. Girolametti[1], M. Fanelli[1], G. Pascon[3], M. Martinoli[4], F. Tulli[3], T. Pinto[2], C. Truzzi[1]. The use of seaweed as feed ingredient in the diet of the house cricket (*Acheta domesticus*): investigating growth performance and nutritional composition. Journal of Insects as Food and Feed. (submitted August 2023).

List of publications on conference proceedings

None

List of other publications (books, book chapters, patents)

-Co-Supervisor of master thesis of Beatrice Papa (February 2023) and Alice Tamborra (July 2023)

[Date 13/11/2023

Student signature

Behixhe Ajdini

Supervisor signature