



Risk Factors of Asthma Associated a Mining and Phosphate Processing Factory Complex in Southern Tunisia: Cross Sectional and Computational Modeling Study

Olfa Jedli ¹, Hmed Ben Nasr ², Faten Brahmi ³, Arif J. Siddiqui ³, Tarek Rebai ¹, Riadh Badraoui ^{3,4*}

¹ Laboratory of Histo-Embryology & Cytogenetics, Medicine Faculty of Sfax. University of Sfax, Majida Boulila Street, 3029 Sfax- Tunisia

² Laboratory of Pharmacology, Medicine Faculty of Sfax, University of Sfax, Majida Boulila Street, 3029 Sfax- Tunisia

³ Laboratory of General Biology, Department of Biology, University of Ha'il, 854004 Ha'il, Saudi Arabia

⁴ Section of Histology – Cytology, Medicine Faculty of Tunis, University of Tunis El Manar , 1007 La Rabta-Tunis, Tunisia

(Received: 11 June 2024

Revised: 16 July 2024

Accepted: 10 August 2024)

KEYWORDS

Asthma, Mining factory, Epidemiology, Risk factors, STAT6, TNF- α , Molecular interactions.

ABSTRACT:

Introduction: Asthma is a common chronic health problem, which is etiologically complex with various risk factors.

Objectives: This study describes the epidemiology of asthma and its loco-regional variability in the southern-west of Tunisia. 3563 people, aged over 14 years, from 9 villages settled around Gafsa city (South-West Tunisia) fulfilled an adapted questionnaire, by direct interview.

Methods: All responders are resident for 10 years or more at the studied locations. The questionnaire concerned their socio-demographic status and asthma related signs such as the presence of physician-diagnosed asthma, wheeze, cough, phlegm, respiratory crisis, etc.). Statistical analyses were carried out using two dimensional contingency tables and Chi square tests.

Results: 26.89% of the general population is affected by asthma. The disease incidence was significantly associated to subjects' location. The highest prevalence was observed in R4 (47.48%) and R3 (36.86%) where the lowest values exist in Madjel and Tozeur. Wheeze and its severe forms occurred much frequently in these two regions when compared to others ($p < 0.001$). Furthermore, subjects living in couple and educated for less than 12 years were at higher risk to develop asthma. There was no significant correlation between regional distribution of the disease and the other considered risks of factors. These results were confirmed by the docking study regarding the binding and the interactions of sulfur dioxide (SD) and phosphate (PH) with Tumor Necrosis Factor (TNF- α) and Signal Transducer And Activator Of Transcription 6 (STAT6) proteins.

Conclusions: In conclusion, loco-regional disparities might be consequent to environmental conditions (e.g, dusts, SD and PH liberated from mining and PH treatment), which might enhance the hereditary predisposal to the asthma. The molecular interactions of SD and PH with TNF- α and STAT6 proteins supported the epidemiological findings.

1. Introduction

Asthma is a worldwide common chronic disease affecting particularly children and young adult populations. It still constitutes a major health problem

that causes disability, high morbidity and poor quality of life [1]. Global asthma survey estimated that asthma burden is projected to come close to 400 million people worldwide, by 2025 [2]. The disease manifests common



symptoms including wheeze, chest disorder, shortness of breath, phlegm production and cough. Patients might be at higher risk to develop anxiety, distress, pulmonary obstructive hypertension and cardiovascular events [1,3]. Asthma symptoms might also be exacerbated by COVID [4]. According to the WHO report, the disease is responsible of about 250 000 human deaths each year [5]. Its onset had been correlated to many risk factors including gender, genetics, ethnicity, obesity, socioeconomic status, occupational, environmental conditions, individual life history, culture and habits [6-9]. The global prevalence rates of diagnosed asthma and wheezing in adults varied as much as 21-fold amongst countries, suggesting the importance of geographic / regional location as a risk factor to instigate the disease [10,11]. The reason of these disparities is still unknown, but observations outlined that early life exposure and the length residence in a particular location would influence the exposure to environmental factors that contributes to asthma aetiology [1,12-15].

Mining and phosphate processing constitute the main industrial activity in the southern part of Tunisia. Accordingly, significant associations of health endpoints with mining activity and phosphate processing areas, such as the villages included in this study, were previously reported [16-20].

2. Objectives

This work aimed to describe the loco-regional incidence of asthma in the southern Tunisian population. The possible intermolecular interactions of sulfur dioxide and phosphate, which are commonly involved in asthma pathogenesis, with tumor necrosis factor-alpha (TNF- α) and signal transducer and activator of transcription 6 (STAT6) proteins were also assessed by computational assay.

3. Methods

The study population

This cross-sectional study was conducted in order to describe the prevalence of asthma and its related risk factors, in a Southern- West Tunisian community. It was conducted in Gafsa city and the surrounding villages from March 2018 to March 2019. This circumscription inhabits about 350 000 people and has a mining and agriculture based economy. Nine villages were included in this study (Figure 1). People aged over 14 years (5479

persons) were asked to respond to an adapted questionnaire. Among 3814 persons who accepted to participate in this study, only 3563 responders gave full responses and were retained for the analysis. 10 years or more continual residing in the village was adopted as inclusion criterion.

The questionnaire

Participants answered a questionnaire concerning their socio-demographic status (age, gender, weight and height, smoking and alcohol intake habits, education, incomes, marital status and origin). The disease related questions were adopted from the European Community Respiratory Health Survey (ECRHS) (do you have asthma? Do you use inhaler medication? Do you have any of the following symptoms: wheeze, cough, nocturnal-cough, phlegm production, or crisis? How much frequent was the wheezing in the last month?). All of signs are reported for the last 12 months before the interview, except mentioned above. Hypersensitivity to any allergen was also registered (do you have hypersensitivity to any allergen?). Anthropometric parameters (weight and height) were measured for participant ignoring their current weight and height (if any). The questionnaire was full-filled by direct experimented staff interviewers, during Red Crescent association's campaigns holding in the included villages. Among all the included responders (3563) 98% were of low incomes, and smoking and alcohol intake were strictly masculine habits. When wheezing frequency was equal or superior to 4 times a month, it was considered as severe. All questions were carefully translated into the local language. If needed, further explanations were given to the study's participants.

Computational study

The tridimensional (3D) structures of ligands (sulfur dioxide and phosphate) were obtained from PubChem websites. The 3D crystal structures TNF- α and STAT6 were retrieved from PDB websites then targeted as previously described [1]. For TNF- α , the crystal structure of the extracellular domain of human BAFF (1KD7) was used. For STAT6, which is involved in classical immune responses linked to asthma and several diseases of the immune response, 5D39 macromolecule was used. the A molecular docking, based on the CHARMM force field was used to assess the binding abilities of sulfur dioxide and phosphate and their



interactions with the different target proteins as previously reported [21-24]. These proteins are involved in the pathogenesis and signaling pathways of asthma and several respiratory diseases [1,15,25].

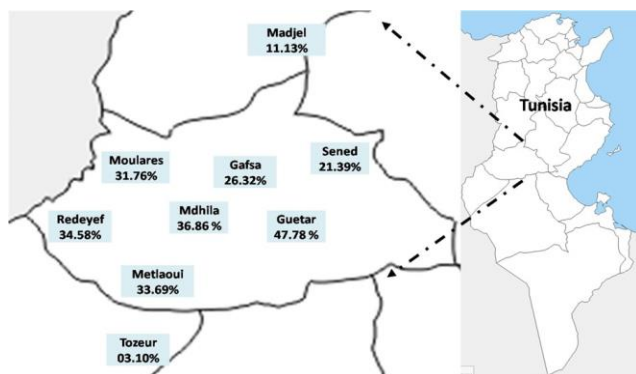


Figure 1. Illustration of the nine villages included in the study and asthma prevalence for each.

4. Results

As shown in Table 1, the overall incidence of asthma in the general population comes around 27% (CI95: 25.5-28.4), but it significantly varies within age. Old peoples (over 50 years old) present the highest incidence of the disease (41.9%, CI95: 35.9-46.2).

In this cross sectional survey, we also found that asthma was significantly associated to the subjects' marital status (OR = 1.66 [1.42-1.93], $p < 0.001$) and their educational levels (OR = 1.23, [1.06-1.42], $p = 0.004$), with higher risks for asthma development in married and less educated peoples. There were no relationships of asthma rate to gender, body mass index, smoking, alcohol intake or hypersensitivity.

As expected, we found that the disease prevalence significantly varied within the geographical distribution of the population. The highest rates were observed in Guetar and Mdhila (47.78%, CI95: 42.8-52.8, and 36.86%, CI95: 32.3-41.8 respectively) and the lowest in regions Madjel and Tozeur (11.13% and 03.10%, respectively). When analyzing symptoms that were developed by asthmatic, we found that wheezing significantly changes dependently to loco-regional distribution of peoples. Among 958 peoples with asthma, 59.5% manifest wheeze (Table 2).

Table 1. Asthma prevalence variation within socio-demographic, body mass index and hypersensitivity in the studied population

	N	I (%)	[CI95%]	χ^2 (p)	
Overall	3563	26.9	25.5-28.4		
Age	< 25 yr	1484	29.9	27.3-32.2	51.67 (***)
	25-50 yr	919	40.7	37.5-43.9	
	>50 yr	202	69.3	62.9-75.7	
Gender	Male	1905	26.6	24.7-28.6	0.156 (ns)
	Female	1658	27.2	25.1-29.4	
Marital status	Single	2342	23.4	21.7-25.2	42.31 (***)
	Married	1221	33.6	30.1-36.3	
BMI (kg/m ²)	< 25	2404	26.2	24.5-28.0	1.75 (ns)
	25-30	838	28.3	25.3-31.4	
	>30	321	28.4	23.7-33.5	
Education	≤ 12 yr	1608	29.1	26.9-31.4	8.93 (**)
	>12 yr	1954	25.1	23.2-27.1	
Smoking	Yes	1488	26.3	24.1-28.6	0.384 (ns)
	No	2075	27.5	25.6-29.5	
Alcohol	Yes	203	44.8	38.2-51.7	1.88 (ns)
	No	3360	26.6	25.1-28.1	
Hyper-sensitivity	Yes	1682	29.4	27.3-31.7	0.26 (ns)
	No	1881	26.5	24.5-28.6	

(**) and (***) respectively designate significant differences at $p < 0.01$ and $p < 0.001$. (ns) non-significant difference for $p > 0.05$

In particular, the rate of wheezing was significantly increased in Guetar (95%) and Mdhila (65%) in comparison to other studied origins where it did not surpass 51%. Patients having four or more wheezing events per month were classified as having severe form of asthma. As shown in Figure 2, the rate of severe forms significantly ($p < 0.001$) varies between patients' origin. The lowest (02.04%) and highest (73.77%) rates were respectively noticed in Madjel and Guetar. The occurrences of severe asthma in the other considered villages varied from 14% to 22%. The Chi square test revealed significant association of wheezing severity and sex (30.18% and 24.61%, for males and females respectively, $\chi^2 = 3.703$, $p = 0.032$), smoking (30.87% vs 25.27%, for smokers vs non smokers respectively, $\chi^2 = 3.641$, $p = 0.034$), and alcohol beverage (41.27% and 26.59%, for yes and no alcohol intake respectively, $\chi^2 = 6.352$, $p = 0.011$), in the overall population sample of asthmatic patients.



Table 1. Asthma signs variation within regional location of the studied population

Signs	N	Wheeze	Cough	Cough / night	Phlegm	Inhaler
Overall	958	59.5	50.0	61.9	56.8	82.8
Origin						
Madjel	49	40.8	44.9	61.2	42.9	00.0
Tozeur	13	46.2	38.5	46.2	46.1	100.0
Mdhila	146	65.8	48.6	58.9	59.6	88.4
Guetar	183	95.6	54.1	63.4	59.6	87.4
Moulares	121	50.4	56.2	67.8	57.0	88.4
Redayef	129	50.4	54.3	68.2	55.8	84.5
Metlaoui	127	45.7	40.9	59.8	52.0	82.7
Gafsa	110	44.6	49.1	52.7	58.2	92.7
Sened	80	50.0	47.5	63.7	61.2	85.0
χ^2		141.4 3 (***)	09.7 4 (ns)	10.3 2 (ns)	07.50 (ns)	255.07 (***)

(***) designate significant differences at $p < 0.001$. (ns) non-significant difference for $p > 0.05$.

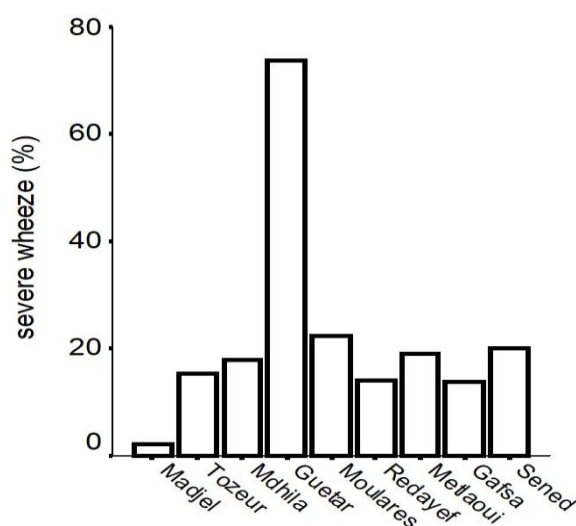


Figure 2. Distribution of patients with severe form of asthma (at least 4 wheezing events/month) in the different included villages

Both SD and PH exhibited at least three conventional hydrogen bonds with TNF- α and STAT6 associated several other interactions such as salt bridge-attractive charge and electrostatic attractive charge (Figure 3). While for TNF- α , both SD and PH interacted with THR143 as closest residues, for STAT6 the ligands showed different interactions. They were close to SER486 and GLN479, respectively. Overall, it was depicted that the binding affinities were all negative and ranging between -3.3 to -2.4 kcal \times mol $^{-1}$ (Table 3).

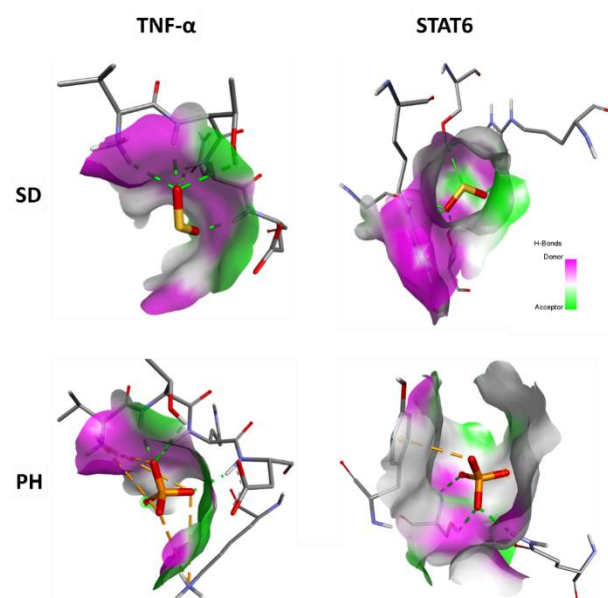


Figure 3. Receptor-Ligand 3D interactions between active site residues of TNF- α and STAT6 (receptors) and sulfur dioxide and phosphate (ligands).

5. Discussion

Asthma is a multi-factorial chronic disease that causes peoples disability and poor quality of life [1,15,26]. Its global rates put out of sight its geographical disparity that firmly depends in exposure risk to loco-regional environments. Previous surveys outlined that asthma prevalence, in Tunisia, reaches about (6.9 %) [27], but it geographically changes, and many authors presented variable rates of the disease [27,28]. In addition, it varies from 2.7 % to 4.5 % in a South-North trans-sectional cross study [29]. They estimated a rate of asthmatic peoples of 4.2 % in the same studied area. Our results showed that crude asthma prevalence is much higher than which have been previously reported in the above-



Table 3. Interactions of ligand (sulfur dioxide and phosphate) with TNF- α and STAT6 human receptors: assessment of binding affinity, number of conventional hydrogen bonds and distance to the closest interacting residue

	TNF- α	STAT6	
Sulfur dioxide (SD)	Intermolecular interactions	Conventional hydrogen bond: (VAL142:HT)---(SD), (THR 143:HN)---(SD), (THR143:HG1)---(SD), (GLN144)---(SD) and (ASP145:HN)---(SD).	Conventional hydrogen bond: (SER486:HG)---(SD), (ARG495:HH22)---(SD), (ARG530:HH11)---(SD), (SER534:HG)---(SD) and (GLY464:O)---(SD).
	Affinity (kcal/mol)	-2.5	-3.1
	No. Conventional H-Bonds	5	5
	Closest interacting residue	THR143	SER486
	Distance to closest residue	1.913 Å	2.158 Å
Phosphate (PH)	Intermolecular interactions	Conventional hydrogen bond: (THR143:HN)---(PH), (THR143:HN)---(PH), (GLN144:HN)---(PH) and (ASP145:HN)---(PH); Salt bridge – Attractive charge : (VAL142:HT1)---(PH); Electrostatic attractive charge : 1 (VAL142:N)---(PH), 1 (VAL142:N)---(PH) and 2 (LYS283:NZ)---(PH).	Conventional hydrogen bond: (GLN479:HE21)---(PH), (SER543:HN)---(PH) and (SER543:HG)---(PH); Electrostatic Pi-anion (TYR546)---(PH).
	Affinity (kcal/mol)	-2.4	-3.3
	No. Conventional H-Bonds	4	3
	Closest interacting residue	THR143	GLN479
	Distance to closest residue	1.947 Å	2.266 Å

mentioned studies, and bring evidence for its changes within the residential areas.

The results of the current study may support findings of previous reports related to such polluted areas as assessed by biochemical and histological approaches [1,16,17,19]. Probably methodological and regional variability might explain differences between our findings and those previously reported by other authors. Mining and phosphate transforming are the main economic activities in the studied area. They turnover around 500 billion tons of rock each year, in order to extract the phosphate mineral. During such process huge amounts of dusts and chemicals like sulfurs, including sulfur dioxide, are emitted into the air and might disrupt its quality and expose the neighboring populations to develop respiratory stiffening and asthma [30-32]. In this study, SD and PH were assessed for their ability to bind to some receptors involved in asthma development.

TNF- α and STAT6 have been targeted for this assessment. For the different complexes, SD was found to establish five hydrogen bonds with both TNF- α and STAT6. Nevertheless, the affinity was better for STAT6. The best binding affinity was predicted for the STAT6–

PH complex, which reached $-3.3 \text{ kcal}\times\text{mol}^{-1}$. These interactions together with the negative binding energies may explain the potential effects of both SD and PH in inflammatory responses associated to this heterogeneous respiratory disease [1,26,33]. In this study, the PH showed the lowest binding energy and was tightly filled into the binding cavity of STAT6. This stable complex showed three strong hydrogen bonds with GN479 and SER543 amino acids associated one electrostatic Pi-anion with TYR546. This network of bonds is commonly reported to be associated with the stability of studied complexes: pollutants (ligand) and targeted receptors [21,23,24]. Our findings confirmed the STAT6–PH complex stability and its possible promotion of prostaglandins in asthma development [34]. It has been reported that TYR641 of STAT6 plays an important role in respiratory pathologies [35]. Our findings regarding binding results and intermolecular energies, mirror the previously reported epidemiological results and may explain the involvement of both TNF- α and STAT6 in the airway inflammatory diseases, particularly asthma pathogenesis [1,28]. These computational modelling results, as compared to others showed also that the effect



of SD and PH on TNF- α and STAT6 are thermodynamically possible [15,23,24].

The climate dryness and wind direction are sought as prominent agents in orienting the air-drained dusts and chemicals (particularly SD and PH), fact that explains the highest levels of asthma onset in some regions in comparison to other surrounding areas. Accordingly, epidemiological data highlighted the importance of environmental conditions and both birth and childhood country(ies) as risk factors for asthma onset [9,10,14,36-39]. Seemingly, residents of Guetar and Mdhila are the most exposed regions to mining and phosphate transforming, which lead to air-transported dusts and chemicals that explain the great occurrence of the asthma disease in these neighbouring villages.

Conclusion

Our study highlighted the important loco-geographical disparities of asthma prevalence by both epidemiological and computational assays. Such variability might probably be explained by the specific anthropological activities established in the area and its climatic conditions. Further epidemiological and analytical investigations might bring approval for these findings and illuminate the potential involved mechanisms, particularly TNF- α and STAT6 pathways.

References

- Jedli O., Ben-Nasr H., Zammel N., Rebai T., Saoudi M., Elkahoui S., Jamal A., Siddiqui A.J., Sulieman A.E., Alreshidi M.M., Naili H., Badraoui R., 2022. Attenuation of ovalbumin-induced inflammation and lung oxidative injury in asthmatic rats by *Zingiber officinale* extract: combined in silico and in vivo study on antioxidant potential, STAT6 and TNF- α pathways. *3 Biotech*, 12 (9), 191. Doi: 10.1007/s13205-022-03249-5
- Masoli M., Fabian D., Holt S., Beasley R., 2004. Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary for the GINA dissemination committee report. *Allergy* 59(5), 469–478. Doi: 10.1111/j.1398-9995.2004.00526.x
- Ferguson S., Teodorescu M.C., Gangnon R.E., Peterson A.G., Consens F.B., Chervin R.D., Teodorescu M., 2014. Factors associated with systemic hypertension in asthma. *Lung*, 192 (5), 675–683. Doi: 10.1007/s00408-014-9600-y
- Ben-Nasr H., Badraoui R., 2022. Approach of utilizing Artemisia herbs to treat covid-19. *Braz J Pharmaceut Sci.* 58, e20345. Doi: 10.1590/s2175-97902022e20345
- D'Amato G., Vitale C., Molino A., Stanziola A., Sanduzzi A., Vatrella A., Mormile M., Lanza M., Calabrese G., Antonicelli L., 2016. Asthma-related deaths. *Multidiscip Respir Med.* 1:37. Doi: 10.1186/s40248-016-0073-0
- Caldeira R.D., Bettiol H., Barbeiri M.A., Terra-Filho J., Garcia C.A., Vianna E.O., 2006. Prevalence and risk factors for work related asthma in young adults. *Occup Environ Med.* 63, 694–699. Doi: 10.1136/ocem.2005.025916
- Camargo C.A., Weiss S.T., Zhang S., Willett W.C., Speizer F.E., 1999. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch Intern Med.* 159, 2582–2588. Doi:10.1001/archinte.159.21.2582
- Koinis-Mitchell D., McQuaid E.L., Jandasek B., Kopel S.J., Seifer R., Potter C., Fritz G.K., Identifying individual, cultural and asthma-related risk and protective factors associated with resilient asthma outcomes. *J Pediatric Psychol.* 2012, 37(4), 424–437. Doi: 10.1093/jpepsy/jss002
- Marks G.B., 2005. Environmental factors and gene-environment interactions in the aetiology of asthma. *Proceedings of the Australian Physiological Society* 36, 29–34. <http://www.aups.org.au/Proceedings/36/29>
- Holguin F., Mannino D.M., Anto J., Mott J., Ford E.S., Teague W.G., Redd S.C., Romieu I., 2005. Country of birth as a risk factor for asthma among Mexican Americans. *Am J Respir Crit Care Med.* 171,103–108. Doi: 10.1164/rccm.200402-143OC
- To T., Stanojevic S., Moores G., Gershon A., Bateman E.D., Cruz A.A., Boulet L.P., 2012. Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health*, 12, 204. Doi: 10.1186/1471-2458-12-204
- Singh G.K., Siahpush M., 2002. Ethnic-immigrant differentials in health behaviors, morbidity, and cause-specific mortality in the United States: an



- analysis of two national data bases. *Hum Biol*, 74, 83–109. Doi: 10.1353/hub.2002.0011
13. Sundquist J., Winkleby M.A., 1999. Cardiovascular risk factors in Mexican American adults: a transcultural analysis of NHANES III, 1988–1994. *Am J Public Health*, 89,723–730. Doi: 10.2105/ajph.89.5.723
 14. Sundquist J., Winkleby M.A., 2000. Country of birth, acculturation status and abdominal obesity in a national sample of Mexican-American women and men. *Int J Epidemiol*. 29,470–477. Doi: 10.1093/ije/29.3.470
 15. Badraoui R., Adnan M., Bardakci F., Alreshidi M.M., 2021. Chloroquine and Hydroxychloroquine Interact Differently with ACE2 Domains Reported to Bind with the Coronavirus Spike Protein: Mediation by ACE2 Polymorphism. *Molecules*, 26(3), 673. Doi: 10.3390/molecules26030673
 16. Amri N., Hammouda A., Rahmouni F., Chokri M.A., Chaabane R., Selmi S., Rebai T., Badraoui R., 2016. Reproductive effects in hybrid sparrow from a polluted area in Tunisia: oxidative damage and altered testicular histomorphology. *Ecotoxicol Environ Safe*. 129, 164–170. Doi: 10.1016/j.ecoenv.2016.03.024
 17. Amri N., Rahmouni F., Chokri M.A., Rebai T., Badraoui R., 2017. Histological and biochemical biomarkers analysis reveal strong toxicological impacts of pollution in hybrid sparrow (*Passer domesticus* × *Passer hispaniolensis*) in southern Tunisia. *Environ Sci Poll Res*. 24(12), 17845–17852. Doi: 10.1007/s11356-017-9352-3
 18. Boyles A.L., Blain R.B., Rochester J.R., Avanasri R., Goldhaber S.B., McComb S., Holmgren S.D., Masten S.A., Thayer K.A., 2017. Systematic Review of Community Health Impacts of Mountaintop Removal Mining. *Environ Int*. 107, 163–172. Doi: 10.1016/j.envint.2017.07.002
 19. Oudi A., Chokri M.A., Hammouda A., Chaabane R., Badraoui R., Besnard A., Santos R., 2019. Physiological impacts of pollution exposure in seabird's progeny nesting Mediterranean contaminated area. *Mar Pollut Bull*. 142, 196–205. Doi: 10.1016/j.marpolbul.2019.02.056
 20. Sadeq M., Abouqal R., ElMarnissi A., 2015. Secular trends in consultations for asthma in early childhood, the 16 administrative regions of Morocco, 2004–2012. *BMC Public Health*, 15, 905. Doi: 10.1186/s12889-015-2262-8
 21. Akacha A., Badraoui R., Rebai T., Zourgui L., 2022. Effect of *Opuntia ficus indica* extract on methotrexate-induced testicular injury: a biochemical, docking and histological study. *J Biomol Struct Dyn*. 40(10) 40(10): 4341–4351. Doi: 10.1080/07391102.2020.1856187
 22. Hchicha K., Korb M., Badraoui R., Naïli H., 2021. A novel sulfate-bridged binuclear copper (II) complex: Structure, optical, ADMET and in vivo approach in a murine model of bone metastasis. *New J Chem*. 45, 13775–13784. Doi: 10.1039/D1NJ02388H
 23. Kraiem M., Ben Hamouda S., Eleroui M., Ajala M., Feki A., Dghim A., Boujhoud Z., Bouhamed M., Badraoui R., Pujo J.M., Essafi-Benkhadir K., Kallel H., Ben Amara I., 2024. Anti-Inflammatory and Immunomodulatory Properties of a Crude Polysaccharide Derived from Green Seaweed *Halimeda tuna*: Computational and Experimental Evidences. *Mar Drugs* 22 (2), 85. Doi: 10.3390/md22020085
 24. Ishak S., Allouche M., Alotaibi G.S., Alwtheyry N.S., Al-Subaie R.A, Al-Hoshani N., Plavan O-A., Selamoglu Z., Özdemir S., Plavan G., Badraoui R., Rudayni H.A., Boufahja F., 2024. Experimental and computational assessment of Antiparkinson Medication effects on meiofauna: Case study of Benserazide and Trihexyphenidyl. *Mar Pollut Bull*. 205, 116668. Doi: 10.1016/j.marpolbul.2024.116668
 25. Li X., Huang L., Wang N., Yi H., Wang H., 2018. Sulfur dioxide exposure enhances Th2 inflammatory responses via activating STAT6 pathway in asthmatic mice. *Toxicol Lett*. 285, 43–50. Doi: 10.1016/j.toxlet.2017.12.020
 26. Badraoui R., Alrashedi M.M., El-May M.V., Bardakci F., 2021. Acute respiratory distress syndrome: a life threatening associated complication of SARS-CoV-2 infection induced COVID-19. *J Biomol Struct Dyn*. 39(17), 6842–6851. Doi: 10.1080/07391102.2020.1803139
 27. Joobeur S., Mohamed S.C., Ben Saad A., Mribah H., Dekhil A., Rouabti N., El Kamel A., 2015. L'asthme allergique au centre tunisien. *Pan Afr*



- Med J. 20, 133. Doi: 10.11604/pamj. 2015.20. 133. 5642
28. Nafti S., Taright S., El Ftouh M., Yassine N., Benkheder A., Bouacha H., Fakhfakh H., Ali-Khoudja M., Texier N., El-Hasnaoui A., 2009. Prevalence of asthma in North Africa: the asthma insights and reality in the Maghreb (AIRmAG) study. *Respir Med.* 103, (S2)-S11. Doi:10.1016/s0954-6111(09)70022-8
29. Habibeche S., Toujani S., Mjid M., Snen H., Ben Salah N., Ouahchi Y., Louzir B., Mhiri N., Cherif J., Beji M., 2015. Asthma et tabagisme actif: étude épidémiologique sur un échantillon représentatif du Grand Tunis. 19e Congrès de pneumologie de langue française - Lille, 30 janvier-1er février.2015. Doi : 10.1016/j.rmr.2014.10.450
30. El-Afifi E.M., Hilel M.A., Attallah M.F., El-Reefy S.A., 2009. Characterization of phosphogypsum wastes associated with phosphoric acid and fertilizers production. *J Environ Radioact.* 100, 407–412. Doi:10.1016/j.jenvrad.2009.01.005
31. Fluegge K., Fluegge K., 2017. Air pollution and risk of hospitalization for epilepsy: the role of farm use of nitrogen fertilizers and emissions of the agricultural air pollutant, nitrous oxide. *Arq Neuropsiquiatr.* 75(9), 614–619. Doi: 10.1590/0004-282X20170107
32. Perez-Lopez R., Alvarez-Valero A.M., Vieto J.M., 2007. Changes in mobility of toxic elements during the production of phosphoric acid in the fertilizer industry of Huelva (SW Spain) and environmental impact of phosphogypsum wastes. *J Hazard Mater.* 148, 745-750. Doi: 10.1016/j.jhazmat.2007.06.068
33. Valladao A.C., Frevert C.W., Koch L.K., Campbell D.J., Ziegler S.F., 2016. STAT6 regulates the development of eosinophilic versus neutrophilic asthma in response to *Alternaria alternate*. *J Immunol.* 197(12), 4541-4551. Doi: 10.4049/jimmunol.1600007
34. Wu J., Wang Y., Zhou Y., Wang Y., Sun X., Zhao Y., Guan Y., Zhang Y., Wang W., 2020. PPAR γ as an E3 Ubiquitin-Ligase Impedes Phosphate-Stat6 Stability and Promotes Prostaglandins E2-Mediated Inhibition of IgE Production in Asthma. *Front Immunol.* 11, 1224. Doi: 10.3389/fimmu.2020.01224
35. Mandal P.K., Morlacchi P., Knight J.M., Link T.M., Lee G.R., IV, Nurieva R., Singh D., Dhanik A., Kaviraki L., Corry D.B., Ladbury J.E., McMurray J.S., 2015. Targeting the Src Homology 2 (SH2) Domain of Signal Transducer and Activator of Transcription 6 (STAT6) with Cell-Permeable, Phosphatase-Stable Phosphopeptide Mimics Potently Inhibits Tyr641 Phosphorylation and Transcriptional Activity. *J Med Chem.* 58(22), 8970–8984. Doi: 10.1021/acs.jmedchem.5b01321
36. Arlian L.G., Morgan M.S., Neal J.S., 2002. Dust mite allergens: ecology and distribution. *Curr Allergy Asthma Rep* 2(5), 401–411. Doi: 10.1007/s11882-002-0074-2
37. Metsala J., Kilkkinen A., Kaila M., Tapanainen H., Klaukka T., Gissler M., Virtanen S.M., 2008. Perinatal factors and the risk of asthma in childhood- a population based register study in Finland. *Am J Epidemiol.* 168(2), 170–178. Doi: 10.1093/aje/kwn105
38. Molter A., Simpson A., Berdel D., Brunekreef B., Custovic A., Cyrys J., De Jongste J., De Vocht F., Fuertes E., Gehring U., Gruzieva O., Heinrich J., Hoek G., Hoffman B., Klumper C., Korek M., Kuhlbusch T.A.J., Lindley S., Postma D., Tischer C., Wijga A., Pershagen G., Agius R., 2015. A multicentre study of air pollution exposure and childhood asthma prevalence: the ESCAPE project. *Eur Respir J.* 45, 589–591. Doi: 10.1183/09031936.00008415
39. Subbarao P., Mandhane P.J., Sears M.R., 2009. Asthma: epidemiology; etiology and risk factors. *CMAJ* 181(9), E181-E190. Doi: 10.1503/cmaj.080612