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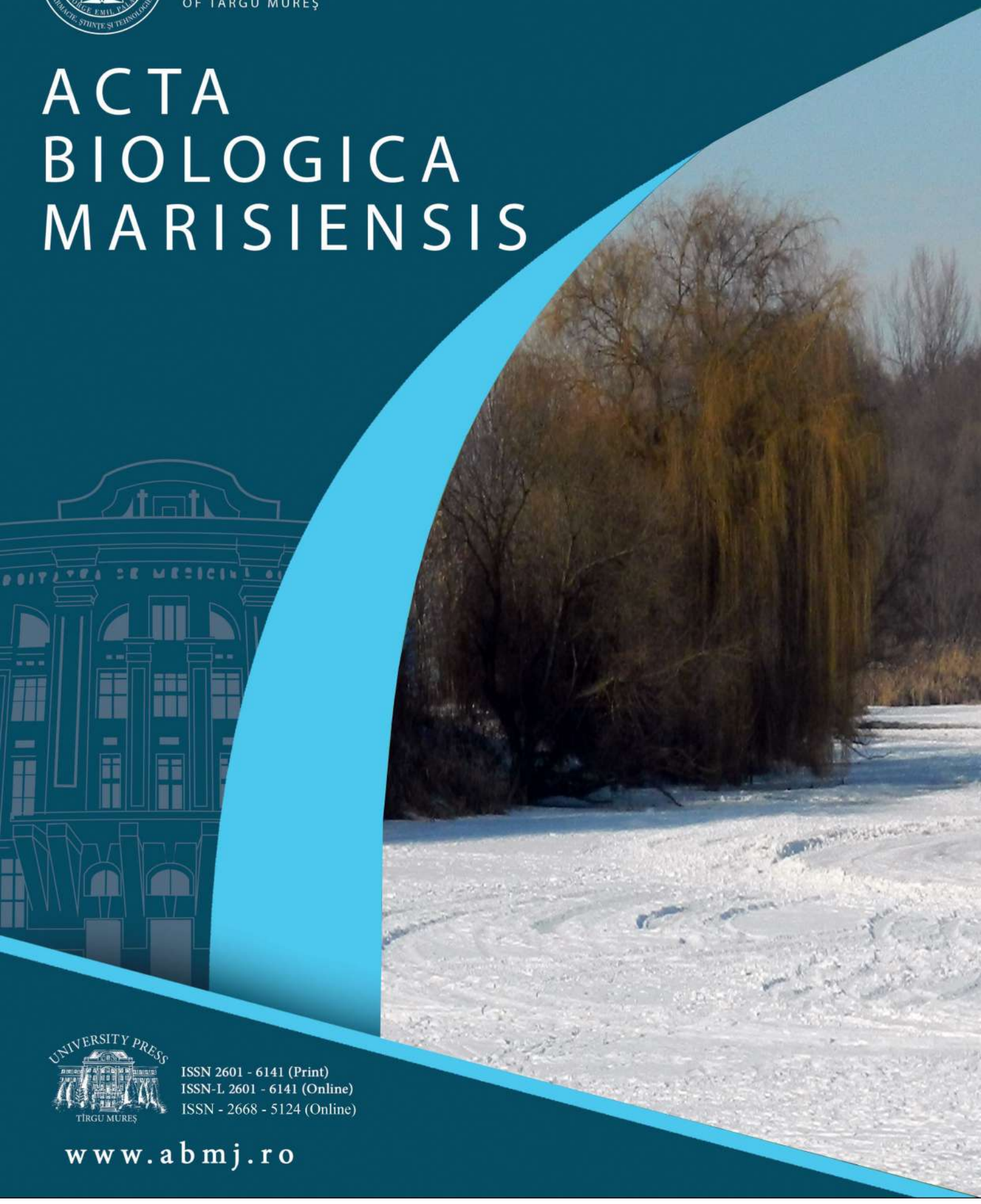

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BIOCHEMICAL CONTROVERSIES REGARDING THE USE OF VEGETAL PROTEINS IN PERFORMANCE ATHLETES

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Abstract: Consumption of animal proteins is increasingly contested by those who choose a vegetarian diet, but for athletes, protein quality is a key component in optimizing athletic performance. The purpose of this article is to provide a nutritional guide for the vegetarian athlete who does not have to give up nutritional preferences to achieve performance in sports, and well-informed counseling by respecting principles of biochemistry can overcome the already known deficiencies of vegetal proteins in certain amino acids. The second aim of this paper is to recommend methods to assess protein quality by consulting the recommendations of the world's most important regulatory agency in the field of nutrition and food quality: the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). In conclusion, even though vegetal proteins have a lower anabolic effect due to their low digestibility and a limited quantity of essential amino acids (especially leucine) and that amino acids of vegetal origin are more likely directed towards oxidative metabolism than towards anabolic processes, recent studies present strategies (consuming higher amounts of vegetal proteins, dietary supplements with amino acids) through which a well-planned vegetarian diet can have similar benefits to omnivorous diet regarding stimulating endogenous protein synthesis.

Keywords: leucine, protein synthesis, vegetal proteins, animal proteins.

1. Introduction

It is considered that in the Ancient Rome, gladiators were vegetarians, being called “hordearii (barley-eaters) - (Longo et al., 2008) and the first questions regarding the impact of diet on athletic performance have been asked since the 1980s (Nieman, 1988) when several literature studies reported enhanced muscular endurance in vegetarians over non-vegetarians, but these results have not been fully confirmed in subsequent studies.

An optimal diet with a balanced ratio between components (45-55% carbohydrates, 20% proteins, and 25-30% lipids) must ensure the energy substrates and nutritional principles adapted to the age and the intensity of physical activity. For example, it was demonstrated that both carbohydrates and BCAAs (branched chain amino acids) influence phosphorylation of mTOR (mammalian target of rapamycin), indicating that the ratio between nutrients can

have a huge impact on cellular processes (Solon-Biet et al., 2014).

It is well documented that diet and the quality of food components play a key role in the performance and health of athletes (Phillips and Van Loon, 2011). Regarding athletic performance, in terms of energy metabolism of striated muscle, the main energy substrates are carbohydrates and lipids (Bjorntorp, 1991). In order to favor the increase of muscle mass, a hypercaloric, high protein, normal or moderately low glycemic and normal lipidic diet is necessary. Proteins are macromolecules indispensable to the human body which, in addition to their structural role, also have a functional, physico-chemical and energetic role (Bytomski, 2018). Intense physical effort favors muscle hypertrophy correlated with the protein intake and a well-balanced amino acid composition (Phillips and Van Loon, 2011).

In the case of proteins, in addition to the quantitative requirement, there is the issue of their quality, the amino acid content being one of the most important criteria to define the quality of proteins (Wolfe et al., 2016). Of the total amino acids, 8 are considered essential for the body: leucine, isoleucine, methionine, valine, threonine, tryptophan, phenylalanine, and lysine. Proteins that contain all the essential amino acids in the required ratio are classified as proteins of high biological quality or complete proteins (Hoffman and Falvo, 2004). Performance athletes can choose between animal proteins such as milk casein, eggs, meat, fish, vegetal proteins such as soy, rice, peanuts, or a combination of them.

Recent studies confirm that plant proteins are inferior to animal proteins both quantitatively and qualitatively, and as they have an important role in the diet of athletes, serving as a substrate for sports performance, it is a subject that must be carefully regarded (Rogerson, 2017). Therefore, athletes with a vegetarian diet clearly consume a lower amount of protein than the omnivorous athletes (Berrazaga et al., 2019) and consequently, there is a lower anabolic effect of plant proteins also involving their incomplete content of essential amino acids.

There is also conclusive evidence that a vegetarian diet is associated with a multitude of health benefits for athletes (Appleby and Key, 2012; Marsh et al., 2012). Foods of vegetal origin are rich in antioxidants (polyphenols), micronutrients (vitamin C and E) and carbohydrates that can enhance sports performance, but have the disadvantage of a poor content of certain nutrients such as vitamin B12, vitamin D, omega-3 fatty acid docosahexaenoic acid, zinc, and iodine (Appleby and Key, 2012; Marsh et al., 2012; Clarys et al., 2014). On the other hand, the consumption of a high animal protein diet was associated with an increased risk of chronic metabolic diseases, especially cardiovascular diseases (Barnard et al., 2019).

Considering that vegetarian diets are more and more popular among athletes, because of ethical, religious, or ecological reasons, as well as for health purposes (Lynch et al., 2018), on the controversy regarding the quality of vegetal proteins but, also, that a well-planned vegetarian diet can fulfill nutritional needs of athletes, the purpose of this article is to assess the impact of the protein type (vegetable or animal) on sports performance. With this main purpose a review of the scientific literature using keywords: “vegetarian” AND “performance athletes” AND “proteins” on PubMed database was performed selecting 34 relevant articles for human nutrition.

2. Vegetal proteins vs. animal proteins for athletic performance

In order to improve athletic performance and ensure metabolic homeostasis, it is important to maintain the integrity of muscle mass. A net protein balance can be calculated

as a ratio between the amount of catabolized and the amount of synthesized proteins and this ratio depends a lot on physical activity, dietary intake of proteins and amino acids as well as on their quality and quantity. A high protein intake determined short time effects (post-prandial increase of protein synthesis) and longtime effects (increased muscle mass) (Berrazaga et al., 2019).

It is considered that both in strength sports (weightlifting, weight throwing, etc.) (Tinline-Goodfellow et al., 2020) and in endurance sports (marathon, cycling) (Kato et al., 2016; McKendry et al., 2019) protein intake should be higher than in a normal, healthy adult with moderate physical activity.

Most authors consider the optimal protein intake for adults to be 0.8 g/kg body weight (Campbell et al., 2007). Normally, in the case of a healthy adult, there must be a balanced nitrogen balance, the protein nitrogen ingested must be found entirely in the nitrogen eliminated in the form of urea. Athletes need higher protein content in the diet, the correct nitrogen balance ranges from 1.2 to 2.0 g/kg b.w. because the need of amino acids for endogenous protein synthesis is higher (Joy et al., 2013; Phillips and Van Loon, 2011).

Optimizing the protein intake for athletes to increase sports performance is a subject of continuous debate and requires a special evaluation of both the quantity and the quality of proteins (Berrazaga et al., 2019).

There are several methods to determine the quality of proteins. In 1989, the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Consultation on Protein Quality Evaluation recommended a series of parameters to evaluate the quality of proteins by assessing the nitrogen balance and considering to the degree of digestibility, net protein utilization and their biological importance (FAO/WHO, 2013).

2.1 Assessing the quality of proteins by protein digestibility-corrected amino acid score/digestible indispensable amino acid score

The Regarding digestibility, it is known that vegetal proteins are less digestible than animal proteins (FAO/WHO, 2013). This may be due to structural differences between the two types of proteins. The secondary structure of plant proteins consists of high beta chain content and a relatively low percentage of alpha-helix. It is assumed that in the gastrointestinal tract there is a resistance to proteolysis of vegetal proteins due to the high percentage of beta-sheet conformation, thus explaining the low digestibility of plant proteins (Carbonaro et al., 2012). Moreover, vegetal proteins contain non-starch polysaccharides or fibers that prevent the access of enzymes to the protein, thus leading to a decrease in digestibility (Duodua et al., 2003). The presence in plants of active compounds such as phytic acid or protease inhibitors can also influence the digestibility of proteins (Duodua et al., 2003). Vegetal proteins from processed foods present a higher digestibility rate (with 18%) compared to unprocessed foods (Wright et al., 2017).

The best parameter to characterize the quality of proteins in terms of digestibility is PDCAAS (Protein Digestibility-Corrected Amino Acid Score) and DIAAS (Digestible Indispensable Amino Acid Score), more recently introduced into practice. For a protein to contain all the essential amino acids PDCAAS should be no less than 1. As the use of PDCAAS has been increasingly criticized due to numerous limitations (Leser, 2013), DIAAS was introduced for quality assessment of proteins. Both scores prove the superiority of animal proteins over vegetal ones in terms of protein digestibility (Lynch et al., 2018; Leser, 2013).

However, these two parameters do not really show the anabolic potential of each type of protein, but rather a method to determine the minimum amino acids needed to prevent protein deficiency in the whole body. For example, the PDCAAS score for soybeans (0.91) is similar to that of beef protein (0.92). So theoretically both types of protein should stimulate approximately similar protein synthesis. However, a recent study by Philips et al. (2012) demonstrates the superior post-prandial anabolic effect of beef proteins compared to soybean. Further on, other studies compared soy protein isolates with milk proteins, as both have similar PDCAAS, but as presented in the previous study these two types of proteins have different anabolic abilities both during rest and post-exercise (Tang et al., 2009). Based on these data, it was recommended that vegetarian athletes should consume a higher amount of protein due to the poor digestibility of plant proteins (Kniskern and Johnston, 2011).

Literature data are controversial, a pilot study conducted over 8 weeks, of High-Intensity Functional Training, shows that both animal proteins (whey proteins) and vegetal (pea proteins) have similar effects upon body composition and muscle strength (Banaszek et al., 2019). Joy et al. (2013) and Mobay et al. (2017), in similar studies, using rice and whey proteins and whey protein and soy proteins, respectively, obtained no statistically significant difference between the groups (Joy et al., 2013; Mobley et al., 2017). Another clinical study conducted in 2015 on a larger number of participants compared to previous studies (n = 161) shows that the administration of vegetal protein supplements (peas) used in combination with resistance training could be just as effective as whey protein supplements in terms of increasing strength and muscle mass (Babault et al., 2015) while a recent review by Craddock et al. (2016) concludes that a vegetarian diet does not improve athletic performance but does not negatively influence it either (Craddock et al., 2016).

2.2. Assessing the quality of proteins by BCAAs content

For elite sports performance, a high-quality protein must contain all the essential amino acids and especially a proper amount of BCAAs. Most plant proteins do not contain all the essential amino acids or in the right amount, resulting in a decrease in their biological value.

BCAAs, leucine, valine and isoleucine, represent more than a third of muscle protein amino acids (Joy et al., 2013). It commonly accepted that essential amino acids and especially BCAAs induce an anabolic effect by stimulating protein synthesis, and their deficiency can limit the synthesis process (Wolfe, 2017; Joy et al., 2013). A study by Garlick et al state that BCAAs have the ability to stimulate protein synthesis in the same way as the other 8 essential amino acids (Garlick, 2005). High-doses of leucine can independently stimulate protein synthesis and inhibit degradation, this effect is potentiated by a small increase in insulinemia (Garlick, 2005; Wolfe, 2017). However, some studies recommend the use of leucine in the presence of other essential amino acids rather than alone (Wolfe, 2017), therefore it can be concluded that the optimal composition of proteins depends a lot on leucine and vice versa. Recent studies have shown that supplementation with 2-3 g of leucine or 0.05 g/kg b.w. increased protein synthesis (Norton and Wilson, 2009; Tipton et al., 1999). Above this threshold, there are no benefits in terms of maximizing protein synthesis. For example, consuming 40 g of egg protein (4 g leucine) did not show a higher increase in muscle protein synthesis than ingesting 20 g egg protein (2 g leucine) (Tang et al., 2009).

Plant-based proteins contain about 6-8% leucine, a reduced quantity compared to animal- proteins that contain about 8-11% leucine. There are also vegetal foods with a high content of BCAAs such as seeds, tree nuts and chickpeas, so the need for BCAAs could be met by consuming a variety of enriched proteins from plant foods (Rogerson, 2017). It was demonstrated that adding leucine to a plant-based diet (Norton et al., 2012) or consuming a larger amount of vegetal proteins increasing the percentage of leucine above the threshold level (Joy et al., 2013), the rate of protein synthesis is similar to a diet based on animal products. This hypothesis could support the increasing number of vegetarians among performance athletes.

A study by Yang et al. in 2012 reported a high rate of leucine oxidation after ingestion of 40g of soy protein, compared to the same amount of whey protein. So, it can be concluded that plant proteins are directed more towards oxidation processes than towards protein synthesis (Yang et al., 2012; van Vliet et al., 2015).

2.3 Other nutrients of vegetal origin and athletic performance

Although animal proteins are considered to have a better quality than those of vegetal origin, nowadays other aspects are also considered as vegetal food products (from quinoa, hemp seeds, etc.) also contain other nutrients responsible for improving sports performance among athletes.

A diet based on vegetal products decreases important parameters of lipid metabolism (hypercholesterolemia, hypertriglyceridemia, etc.), decreasing, therefore, the metabolic risk (Barnard et al., 2019) and, in the same time, increasing the maximum aerobic effort capacity by increasing VO_2 max (maximum rate of oxygen consumption) (Smith et al., 2015).

As vegetal products contain a high amount of carbohydrates, the major energy substrate during aerobic exercise, a vegetarian diet can increase sports endurance, both during sport events or on long term (Jacobs and Sherman, 1999).

Intense physical effort can generate free radicals that can then cause muscle weakness (Viña et al., 2000). In case of vegetarian athletes, a high antioxidant activity was observed due to the high amounts of vitamin C, E and beta-carotene in plants (Rauma et al., 1995), such as beets, garlic, onions, cherry juice (Barnard et al., 2019). Vegetarian diet also presents an anti-inflammatory effect. A 2017 meta-analysis, based on 18 previous studies, on vegetarian subjects, over two years, showed decreases in plasma C-reactive protein, as a marker of inflammation, thus suggesting that plant foods can reduce post-exercise inflammation and facilitate recovery (Haghighatdoost et al., 2017).

Conclusions

1. Literature data available so far emphasize that the "classic" nutritional principles don’t recommend a vegetarian diet for performance athletes as plant proteins have a lower quality than animal proteins, both in terms of content in essential amino acids and digestibility.

2. Recent studies show that vegetarian performance athletes only need larger amounts of vegetal proteins to provide the essential amino acids, especially leucine which is the biomarker of a protein’s involvement in endogenic anabolic processes.

3. Despite limited number of studies and study participants, literature data recommend a well-planned vegetarian diet, referring to certain nutritional strategies, in order to improve the quality of proteins (fortification of vegetal proteins with amino acids, consumption

of higher amounts of vegetal proteins) and, in the same time, highlight the presence of other compounds, specific for vegetal foods, beneficial in terms of sports performance. Still, this subject remains a controversial one.

4. This paper presents biochemical aspects of protein metabolism and tries to balance, as well as possible, the "pros" and "cons" of adopting a vegetarian diet in performance athletes, in whom the protein turn-over is high and protein malnutrition or deficiency in certain amino acids, especially leucine may represent an intrinsic risk factor for the athlete's health, along with other well-known factors: increased cardiovascular risk, hyperhomocysteinemia, low intake of omega 3 fatty acids, etc. The possibility of synergism between these risk factors remains a topic that can be further researched.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. *** (2013) FAO. Dietary Protein Evaluation in Human Nutrition: Report of an FAO Expert Consultation 2011; FAO Food and Nutrition Paper 92. http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf (accessed on June the 11th)
2. Appleby PN, Key TJ (2016) The long-term health of vegetarians and vegans. Proc Nutr Soc 75(3):287-293. https://doi.org/10.1017/S0029665115004334
3. Babault N, Païzis C, Deley G, Guérin-Deremaux L, Saniez MH, Lefranc-Millot C, Allaert FA (2015) Pea proteins oral supplementation promotes muscle thickness gains during resistance training: a double-blind, randomized, Placebo-controlled clinical trial vs. Whey protein. J Int Soc Sports Nutr 12(1):3. https://doi.org/10.1186/s12970-014-0064-5
4. Banaszek A, Townsend JR, Bender D, Vantrease WC, Marshall AC, Johnson KD (2019) The Effects of Whey vs. Pea Protein on Physical Adaptations Following 8-Weeks of High-Intensity Functional Training (HIFT): A Pilot Study. Sports (Basel) 7(1):12. https://doi.org/10.3390/sports7010012
5. Barnard ND, Goldman DM, Loomis JF, Kahleova H, Levin SM, Neabore S, Batts TC (2019) Plant-Based Diets for Cardiovascular Safety and Performance in Endurance Sports. Nutrients 11(1):130. DOI: 10.3390/nu11010130
6. Berrazaga I, Micard V, Gueugneau M, Walrand S (2019) The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. Nutrients 11(8):1825. DOI: 10.3390/nu11081825
7. Bjorntorp P (1991) Importance of fat as a support nutrient for energy: Metabolism of athletes. J Sports Sci 9(1): 71-76. https://doi.org/10.1080/02640419108729867
8. Bytomski JR (2018) Fueling for Performance, Sports Health 10(1):47-53. DOI: 10.1177/1941738117743913
9. Campbell B, Kreider RB, Ziegenfuss T (2007) International Society of Sports Nutrition position stand: protein and exercise, J Int Soc Sports Nutr 4:8. https://doi.org/10.1186/1550-2783-4-8
10. Carbonaro M, Maselli P, Nucara A (2012) Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic

study. Amino Acids 43(2):911-921. DOI: 10.1007/s00726-011-1151-4

11. Clarys P, Deliens T, Huybrechts I, Deriemaeker P, Vanaelst B, De Keyzer W, Hebbelinck M, Mullie P (2014) Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet. Nutrients 6(3):1318-1332. DOI: 10.3390/nu6031318
12. Craddock JC, Probst YC, Peoples GE (2016) Vegetarian and Omnivorous Nutrition - Comparing Physical Performance. Int J Sport Nutr Exerc Meta 26(3):212-220. DOI: 10.1123/ijsnem.2015-0231
13. Duodu KG, Taylor JRN, Belton PS, Hamaker BR (2003) Factors affecting sorghum protein digestibility, J Cereal Sci 38:117–131. https://doi.org/10.1016/S0733-5210(03)00016-X
14. Garlick PJ (2005) The role of leucine in the regulation of protein metabolism. J Nutr 135(6):1553S-6S. https://doi.org/10.1093/jn/135.6.1553S
15. Haghighatdoost F, Bellissimo N, Totosy de Zepetnek JO, Rouhani MH (2017) Association of vegetarian diet with inflammatory biomarkers: a systematic review and meta-analysis of observational studies. Public Health Nutr 20(15):2713-2721. DOI: 10.1017/S1368980017001768
16. Hoffman JR, Falvo MJ (2004) Protein - Which is Best?. J Sports Sci Med 3(3):118-130. PMCID: PMC3905294.
17. Jacobs KA, Sherman WM (1999) The efficacy of carbohydrate supplementation and chronic high- carbohydrate diets for improving endurance performance. Int J Sport Nutr 9(1):92-115. DOI: 10.1123/ijsn.9.1.92
18. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Mc Wilson S, Kalman DS, Dudeck JE, Jäger R (2013) The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. Nutr J 12:86. https://doi.org/10.1186/1475-2891-12-86
19. Kato H, Suzuki K, Bannai M, Moore DR (2016) Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. PLoS One 11(6):e0157406. DOI: 10.1371/journal.pone.0157406
20. Kniskern MA, Johnston CS (2011) Protein dietary reference intakes may be inadequate for vegetarians if low amounts of animal protein are consumed. Nutrition 27(6):727-730. DOI: 10.1016/j.nut.2010.08.024
21. Lemon PW, Yarasheski KE, Dolny DG (1984) The importance of protein for athletes. Sports Med 1(6) 474-484. DOI: 10.2165/00007256-198401060-00006
22. Leser S (2013) The 2013 FAO report on dietary protein quality evaluation in human nutrition: Recommendations and implications, Nutr Bull 38 (4): 421-428. https://doi.org/10.1111/nbu.12063
23. Longo UG, Spiezia F, Maffulli N, Denaro V (2008) The Best Athletes in Ancient Rome were Vegetarian!. J Sports Sci Med 7(4):565. PMID: 24137094; PMCID: PMC3761927.
24. Lynch H, Johnston C, Wharton C (2018) Plant-Based Diets: Considerations for Environmental Impact, Protein Quality, and Exercise Performance. Nutrients 10(12):1841. DOI: 10.3390/nu10121841
25. Marsh K, Zeuschner C, Saunders A (2012) Health Implications of a Vegetarian Diet: A Review, Am J Lifestyle Med 6(3):250–267. https://doi.org/10.1177/1559827611425762
26. McKendry J, Shad B, Smeuninx B, Oikawa SY, Wallis G, Greig C, Phillips S, Breen L (2019) Comparable Rates of Integrated Myofibrillar Protein Synthesis Between

Endurance-Trained Master Athletes and Untrained Older Individuals, Front Physiol 10:1084. https://doi.org/10.3389/fphys.2019.01084

27. Mobley CB, Haun CT, Roberson PA, Mumford PW, Romero MA, Kephart WC, Anderson RG, Vann CG, Osburn SC, Pledge CD, Martin JS, Young KC, Goodlett MD, Pascoe DD, Lockwood CM, Roberts MD (2017) Effects of Whey, Soy or Leucine Supplementation with 12 Weeks of Resistance Training on Strength, Body Composition, and Skeletal Muscle and Adipose Tissue Histological Attributes in College-Aged Males. Nutrients 9(9):972. doi: 10.3390/nu9090972.
28. Nieman DC (1988) Vegetarian dietary practices and endurance performance. Am J Clin Nutr 48(3):754-761. doi: 10.1093/ajcn/48.3.754.
29. Norton L, Wilson GJ (2009) Optimal protein intake to maximize muscle protein synthesis. Agro Food industry hi-tech 20:54–57
30. Norton LE, Wilson GJ, Layman DK, Moulton CJ, Garlick PJ (2012) Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. Nutr Metab (Lond) 9(1):67. https://doi.org/10.1186/1743-7075-9-67
31. Phillips SM, Van Loon LJC (2011) Dietary protein for athletes: From requirements to optimum adaptation, J Sports Sci 29(1):S29-S38. DOI: 10.1080/02640414.2011.619204
32. Phillips SM (2012) Nutrient-rich meat proteins in offsetting age-related muscle loss. Meat Sci 92(3):174-178. DOI: 10.1016/j.meatsci.2012.04.027
33. Rauma AL, Törrönen R, Hänninen O, Verhagen H, Mykkänen H (1995) Antioxidant status in long-term adherents to a strict uncooked vegan diet. The American Journal of Clinical Nutrition 62(6):1221-1227. DOI: 10.1093/ajcn/62.6.1221
34. Rogerson D (2017) Vegan diets: practical advice for athletes and exercisers. J Int Soc Sports Nutr 14:36. https://doi.org/10.1186/s12970-017-0192-9
35. Smith MM, Lucas AR, Hamlin RL, Devor ST (2015) Associations among hemorheological factors and maximal oxygen consumption. Is there a role for blood viscosity in explaining athletic performance?. Clin Hemorheol Microcirc 60(4):347-362. DOI: 10.3233/CH-131708
36. Solon-Biet SM, McMahon AC, Ballard JWO, Ruohonen K, Wu LE, Cogger VC, Warren A, Huang X, Pichaud N, Melvin RG, Gokarn R, Khalil M, Turner N, Cooney GJ, Sinclair DA, Raubenheimer D, Le Couteur DG, Simpson SJ (2014) The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice, Cell Metabolism 19(3): 418-430. doi: 10.1016/j.cmet.2014.02.009
37. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM (2009) Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 107(3):987-992. DOI: 10.1152/japplphysiol.00076.2009
38. Tinline-Goodfellow C, West D, Malowany J, Gillen J, Moore D (2020) An Acute Reduction in Habitual Protein Intake Attenuates Post Exercise Anabolism and May Bias Oxidation-Derived Protein Requirements in Resistance Trained Men, Front Nutr, 7:55. DOI: 10.3389/fnut.2020.00055
39. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR (1999) Post-exercise net protein synthesis in human muscle from

orally administered aminoacids, Am J Physiol 276:E628–E634.
DOI: 10.1152/ajpendo.1999.276.4.E628

40. van Vliet S, Burd NA, van Loon LJ (2015) The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. J Nutr 145(9):1981-1991. DOI: 10.3945/jn.114.204305
41. Viña J, Gomez-Cabrera MC, Lloret A, Marquez R, Miñana JB, Pallardó FV, Sastre J (2000) Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life 50(4-5):271-277. DOI: 10.1080/713803729
42. Wolfe RR, Rutherfurd SM, Kim IY, Moughan PJ (2016) Protein quality as determined by the Digestible Indispensable Amino Acid Score: evaluation of factors underlying the calculation. Nutr Rev 74(9):584-599.
DOI: 10.1093/nutrit/nuw022
43. Wolfe RR (2017) Branched-chain amino acids and muscle protein synthesis in humans: myth or reality?. J Int Soc Sports Nutr 14:30. https://doi.org/10.1186/s12970-017-0184-9
44. Wright CS, McMorrow AM, Weinheimer-Haus EM, Campbell WW (2017) Whey Protein Supplementation and Higher Total Protein Intake Do Not Influence Bone Quantity in Overweight and Obese Adults Following a 36-Week Exercise and Diet Intervention. J Nutr 147(2):179-186. doi: 10.3945/jn.116.240473
45. Yang Y, Churchward-Venne TA, Burd NA, Breen L, Tarnopolsky MA, Phillips SM (2012) Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. Nutr Metab (Lond) 9(1):57. DOI: 10.1186/1743-7075-9-57

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SAGA GIS FOR INFORMATION EXTRACTION ON PRESENCE AND CONDITIONS OF VEGETATION OF NORTHERN COAST OF ICELAND BASED ON THE LANDSAT TM

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Abstract: The paper aims to evaluate the presence and condition of vegetation by SAGA GIS. The study area covers northern coasts of Iceland including two fjords, the Eyjafjörður and the Skagafjörður, prosperous agricultural regions. The vegetation coverage in Iceland experience the impact of harsh climate, land use, livestock grazing, glacial ablation and volcanism. The data include the Landsat TM image. The methodology is based on computing raster bands for simulating Tassel Cap Transformation (wetness, greenness and brightness) and Enhanced Vegetation Index (EVI) sensitive to high biomass. The results include modelled three bands of brightness, greenness and wetness. Greenness variation shows the least values in ice-covered areas (-56.98 to -18.69). High values (-23.48 to 9.12) are in the valleys with dense vegetation, correlating with the geomorphology of the river network, the vegetation-free areas and ocean which corresponds to the peak of 30.87 to 41.19. The bell-shaped data distribution shows frequency 43.19–141.74 for vegetation indicating healthy state and canopy density. Maximal values are in ice-covered regions and glaciers (64°N-65°N). Very low values (0 to -20) show desertification and mountainous rocks. Moderate values (20-40) indicate healthy vegetation. The most frequent data: -28,17 to 11,8. The EVI shows data variations (-0.14 to 0.04). The study contributes both to the regional studies of Arctic Iceland and methodological approach of remote sensing data processing by SAGA GIS.

Keywords: Iceland, Landsat TM, SAGA GIS, cartography, vegetation index, machine learning, automatization, mapping.

1. Introduction

Geographically, the study area covers the northern coasts of Iceland including its two famous fjords, the Skagafjörður, a deep fjord with a valley (19.4°W-20.0°W), and the Eyjafjörður, (18.2°W-18.6°W) one of the longest fjords in Iceland (**Fig. 1**). The Skagafjörður is one of Iceland's most prosperous agricultural regions, with widespread dairy, sheep farming and horse breeding. Today the vegetation coverage in Iceland has gained more attention in landscape studies due to changes under the impact of various factors including impacts of harsh climate (Brombacher et al., 2020), specifics of land use, intensive livestock grazing, glacial ablation and geological processes. Active volcanism results in regional distribution of the highly erodible volcanic soils.

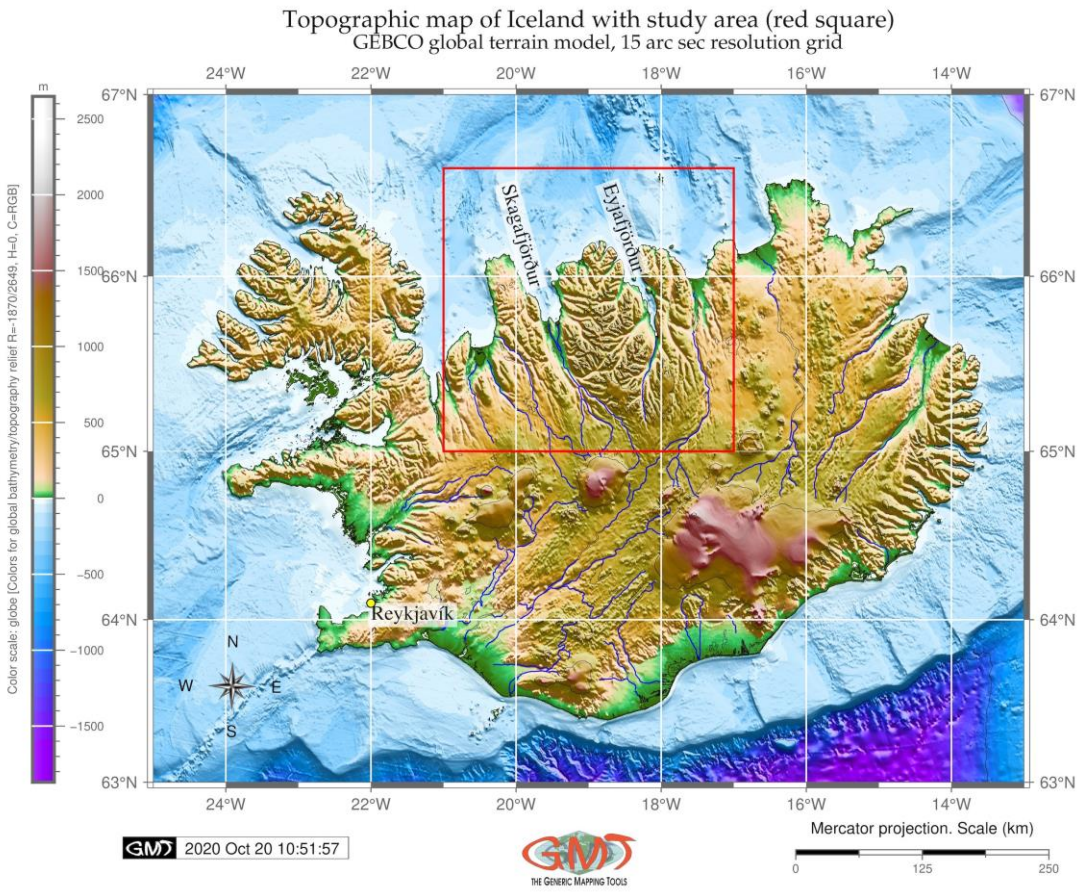

Fig. 1. Topographic map of the study area: Iceland, region of Skagafjörður and Eyjafjörður. Source: author.

According to the recent assessment (Eckert and Engesser, 2013), the country's surface experienced a severe soil erosion on about 40%. As a result, severe land degradation and desertification are considered the most severe environmental problems in Iceland (Eddudottir et al., 2020; Gísladottir, 2001). The examples of land cover changes in Iceland include soil erosion caused by grazing pressure in the processes of sheep farming (Arnalds and Barkarson, 2003), active aeolian processes causing the spread of the sandy areas which replace rich and vegetated ecosystems by lands with low fertility and water-holding capacity (Arnalds et al., 2001). This makes the assessment of the Arctic vegetation coverage by remote sensing methods as an important field at the cutting edge of geoscience.

Current environmental problems in Iceland include severe land degradation which is caused by the effects of both human and climate factors. Land degradation results in the deterioration of plant growth conditions, and the decline of land productivity in sensitive Arctic ecosystems. Land degradation is mirrored in soil deterioration, including its physical, chemical, and biological aspects. Besides, human impacts have been severe on Icelandic soils and vegetation which finally resulted in fragmentary desertification. In turn, vegetation degradation induced soil erosion which successively caused a decline in soil quality. Hence, in recent years soil erosion became an active negative process in Iceland which resulted in deterioration of the grazing areas in the central highlands of Iceland which are not suitable for grazing by sheep due to the

poor condition. Because the land is a natural complex and comprehensive system of geomorphic landforms, geologic factors (mineral rocks), climate settings, hydrologic conditions, and ecology (vegetation and fauna), land degradation and desertification can have worrying consequences for the fragile Arctic environment.

Analytical studies in the remote sensing of satellite images have unveiled the presence of indicators in plants that may be used to detect the healthiness of the canopy. For instance, chlorophyll as a health indicator of leaves absorbs visible light, and the leaf cell strongly reflects near-infrared light. Parameters of spectral reflectance of leaves are discussed (e.g. Kauth and Thomas, 1976; Broge and Leblanc, 2001; Kim et al., 2010; Lemenkova, 2011; Gao et al., 2020) and widely used for detecting vegetation coverage stage and monitoring their ecological stage. The bands constituting the Landsat TM image scene play an essential role in detecting vegetation health and quality assessed by such indicators as greenness, brightness or wetness. In this context, the use of cartographic methods applied for the satellite image processing, raster bands calculation and visualization have shown promising results in experiments carried out on Landsat TM imagery (Lemenkova, 2015a, 2015b, 2015c; Taufik et al., 2016; Ahmet and Akter, 2017; Zaitunah et al., 2018). The purpose of this study was to use advanced methods of the remote sensing data processing (SAGA GIS) in order to extract information on presence and conditions of vegetation and to receive models of the wetness, greenness and brightness using calculation of the selected raster bands, and to perform calculation of the enhanced vegetation index using embedded formulae in the SAGA GIS, and finally, to visualize data distribution using computed and presented histograms aimed at the environmental monitoring of the selected Arctic ecosystems in Iceland.

Benefits of the presented study include a contribution to the environmental monitoring of the selected regions of Iceland, which includes the two fjords, Skagafjörður and Eyjafjörður which can be used as information on the appropriate level (ecologists, environmentalists, authorities). Because the study is fully based on the open source software (SAGA GIS and occasional GMT) for mapping and data analysis, the benefits for broad public, scolars and students consists in the repeatability of the described methods, algorithms and advices for data capture and resources. Thus, this study presents a broad spectrum of remote sensing data processing and visualization by SAGA GIS, and the use of the standard suite of high-quality raster Landsat TM datasets, as demonstrated and described in this work. Generic Mapping Tools (GMT) was also used for topographic mapping (**Fig. 1**) aimed at the advanced cartographic visualization of Iceland using available mapping techniques (Lemenkova, 2020a, 2020b, 2020c). Therefore, this study can be effectively reused for analysis of similar landscapes in Arctic regions using presented workflow of the SAGA GIS and calculation of the Landsat TM raster bands for analysis of vegetation.

2. Materials and Methods

This study presents the use of SAGA GIS (Böhner et al., 2006) for processing the Landsat TM image (**Fig. 2**). The Landsat TM is the satellite imagery of Earth, a joint program of NASA/USGS launched on July 23, 1972 and constrantly being developed since then. Landsat 7 satellite images have eight spectral bands (channels) with spatial resolution ranging from 15 to 60 meters depending on the bands, but mostly 30-meters resolution. The temporal resolution of the Landsat TM is 16 days. Each Landsat scene covers a square approximately 185*185 km long and wide.

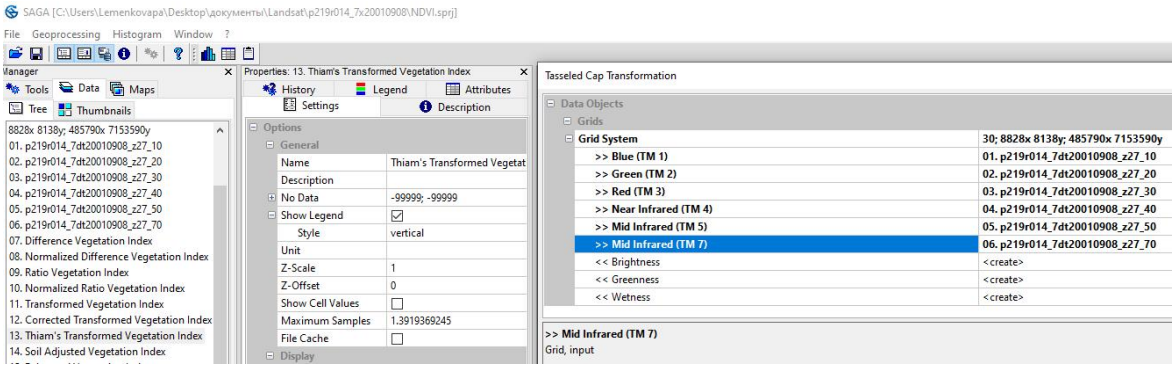

Fig. 2. Applying parameters in SAGA GIS menu. Source: author.

The official website of the Landsat TM is https://landsat.gsfc.nasa.gov/the-thematic-mapper/. However, the imagery can be freely downloaded from the GloVis website: https://glovis.usgs.gov/. The methods include two approaches of the image processing: 1) Tasseled cap transformation; 2) Enhanced Vegetation index.

2.1 Tasseled Cap Transformation

An algorithm for the Tasseled cap transformation was developed by Kauth and Thomas (1976) to transform the spectral information of the Landsat satellite data into indicators that turned to be useful for analysis of phenological stages of vegetation. In the menu of SAGA GIS it was applied using the following path: 'Geoprocessing>Imagery>Vegetation Indices>Tasseled Cap Transformation'. Using six of seven Landsat TM bands (except for the thermal channel 6) were used for the algorithm.

As a result, three types of information were extracted based on a weighted sum of the Landsat bands: 1) Tasseled Cap Band 1 showing brightness, which is a measurement value for the ground; 2) Tasseled Cap Band 2 showing greenness, which is a measured value for the vegetation; 3) Tasseled Cap Band 3 showing wetness, which is a measured value for interactions of soil and canopy moisture.

In the algorithm, each band (B) was multiplied by a certain coefficient and the three characteristics of the vegetation, brightness, greenness and wetness, were defined as follows (Crist and Cicone, 1984a; 1984b):

1. Brightness = 0.3037 (B1) + 0.2793 (B2) + 0.4743 (B3) + 0.5585 (B4) + 0.5082 (B5) + 0.1863 (B7)
2. Greenness = −0.2848 (B1) − 0.2435 (B2) − 0.5436 (B3) + 0.7243 (B4) + 0.0840 (B5) – 0.1800 (B7)
3. Wetness = 0.1509 (B1) + 0.1973 (B2) + 0.3279 (B3) + 0.3406 (B4) − 0.7112 (B5) − 0.4572 (B7)

2.2. Enhanced Vegetation Index

For The Enhanced Vegetation Index (EVI) was approached from the SAGA GIS menu using the following path: 'Geoprocessing>Imagery>Vegetation Indices>Enhanced Vegetation Index'. The computing of the EVI was addressed using an optimized numerical method, comparing to the traditional calculations of the vegetation indices, by enhancing the vegetation signal (Jiang et al., 2008).

The formula of the EVI is based on the following equation (Huete et al., 2002):

$$EVI = G \times (NIR - RED)/(NIR + C1 \times RED - C2 \times BLUE + L),$$

where NIR is a near-infrared band of the electromagnetic spectrum (wavelength at 750

to 2500 nm), RED is a red (wavelength at 625–740 nm) BLUE is blue band (wavelength et 450–485 nm). Specifically, the algorithm of EVI has been updated, by focusing on the higher biomass regions using the effects of the improved sensitivity in these specific areas, by de-coupling of the canopy background signal which eventually improved vegetation monitoring, and by a reduction in the atmosphere influences.

3. Results and discussions

The simulated Tasseled Cap transformations of Landsat TM data represent examples of linear combination features showing three characteristics of the vegetation: 1) wetness (**Fig. 3**); 2) greenness (**Fig. 4**); 3) brightness (**Fig. 5**). The results of the EVI model are presented in **Fig. 6**. Analysis of the moisture content in **Fig. 3** shows the maximal values concentrated in the ice-covered regions of the glaciers stretching an as long and narrow sheet of ice and two glaciers on the southern part of the region, 64°N-65°N (bright red spots in **Fig. 3**). Very low values of wetness (0 to -20) are notable for the areas of local desertification and mountainous rocks (dark grey areas in **Fig. 4**). Bright yellow colours correspond to moderate values (20-40) which indicates the healthy vegetation. According to the statistics (**Fig. 7**, upper right), the most frequent data of wetness vary from slightly negative values -28,17 to 11,8.

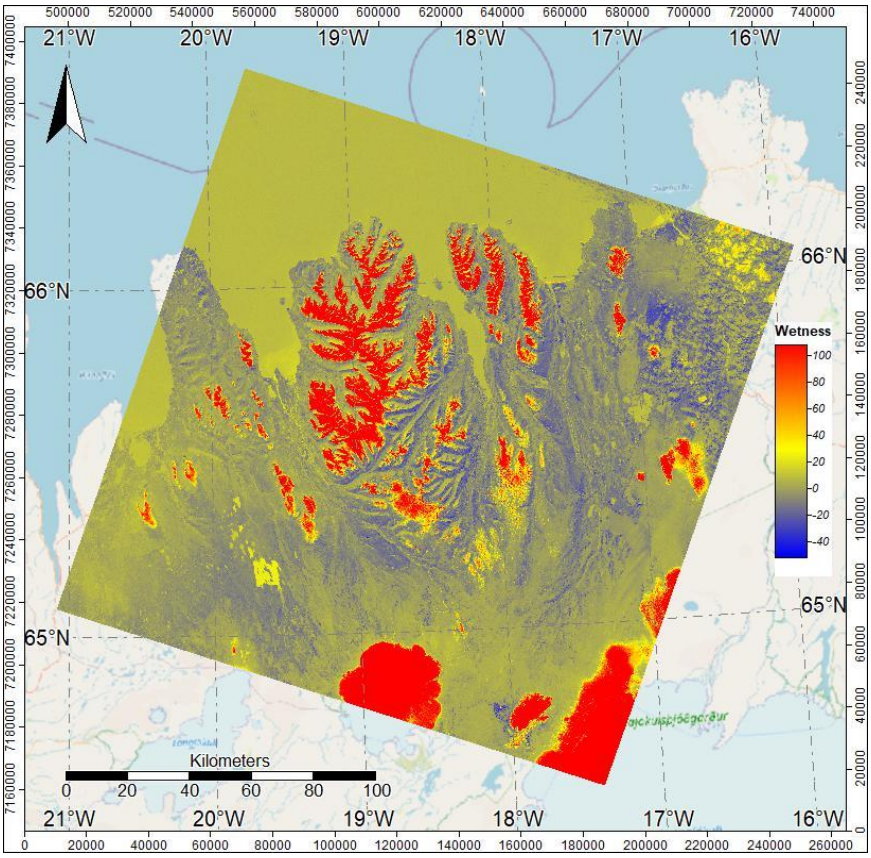

Fig. 3. Wetness of vegetation, modelled by SAGA GIS. Source: author.

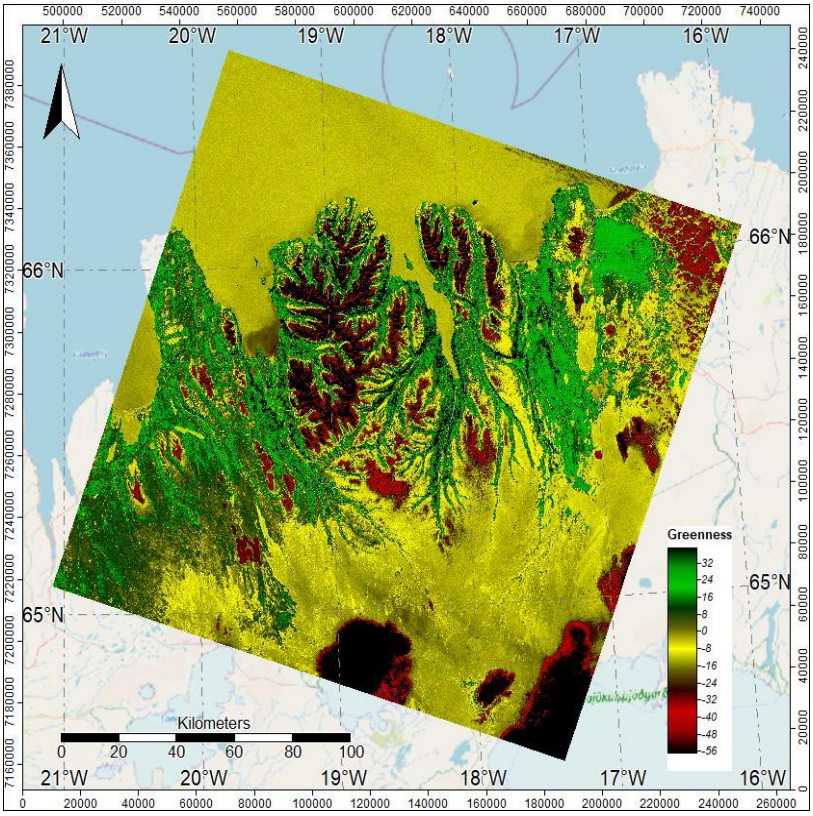

Fig. 4. Greenness of vegetation, modelled by SAGA GIS. Source: author.

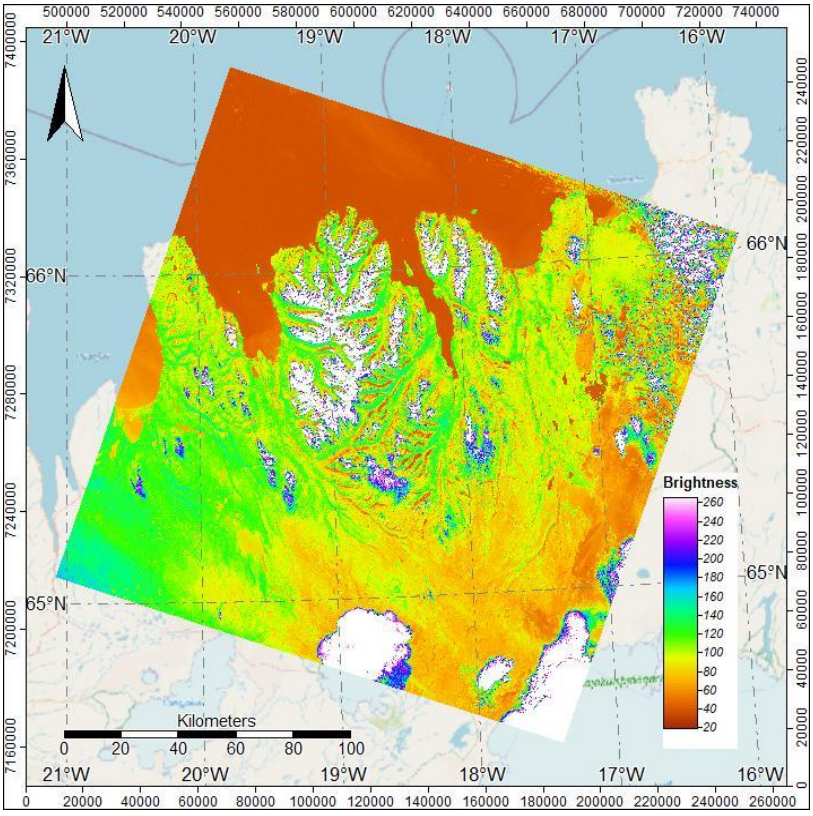

Fig. 5. Brightness of vegetation, modelled by SAGA GIS. Source: author.

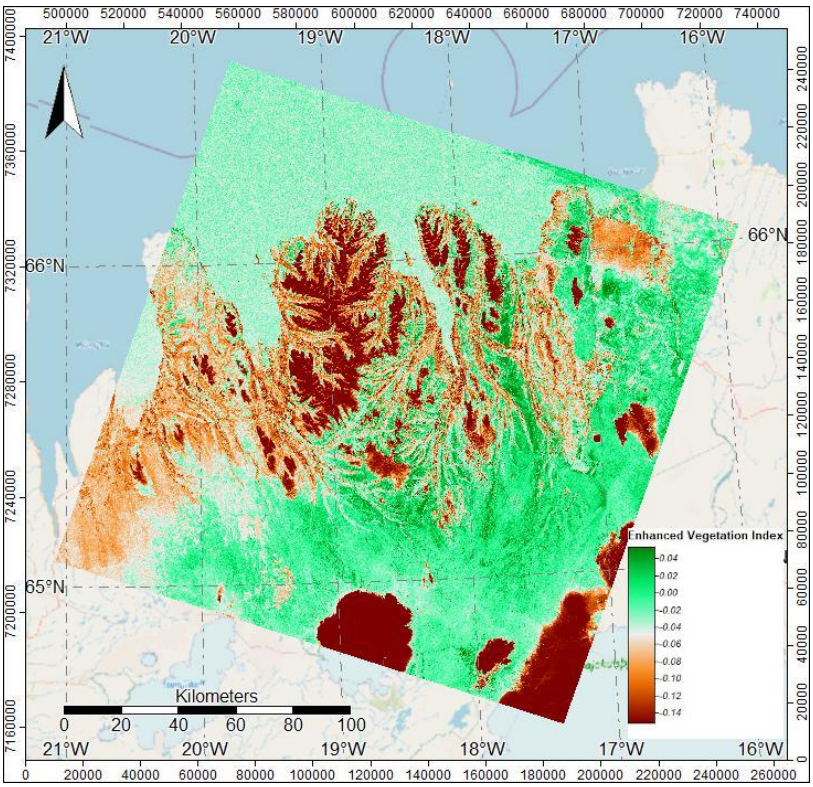

Fig. 6. Enhanced vegetation index, modelled by SAGA GIS. Source: author.

The analysis of greenness variations (**Fig. 4**) clearly shows that the lowest values of greenness correspond to the ice-covered areas (dark crimson brown colours in Fig. 4) which indicates the low values on the statistical histogram (**Fig. 7**) ranging from -56.98 to -18.69. On the contrary, high values correspond to the peak on the histogram with values between -23.48 to 9.12 (**Fig. 7**, lower left). High values of greenness are notable for the river valleys with dense vegetation coverage, clearly depicting the geomorphology of the river network. The absence or very low vegetation coverage can be interpreted from the mustard yellow colours with values 0 to -16. They are typical for the water areas and rocky terrain.

The analysis of brightness (**Fig. 5**) shows its variations in the range between 20 and 260 units of surface luminosity. In particular, the darkest areas (brown-coloured, **Fig. 5**) correspond to the vegetation-free areas and ocean, which corresponds to the peak of 30.87 to 41.19 in **Fig. 7** (upper left). In contrast, light green colours correlating with vegetation-covered areas (100-150) can be seen as depicting the river valleys (**Fig. 5**) and on the coastal areas. White regions show dominating ice-covered mountainous areas. The statistical analysis points at the bell-shaped distribution of the data lying in the range of 43.19 to 141.74 for the areas of vegetation with difference indicating its healthy state and density of canopy. The analysis of the EVI (**Fig. 6**) shows the variations of the data range between -0.14 to 0.04. Here the lowest values corresponding to the dark brown colours are notable for the ice-covered areas and glaciers, while vegetation in valleys and coastal areas is depicted by beige. The results showed SAGA GIS to be effective for analysis of the vegetation coverage by Landsat TM bands combination.

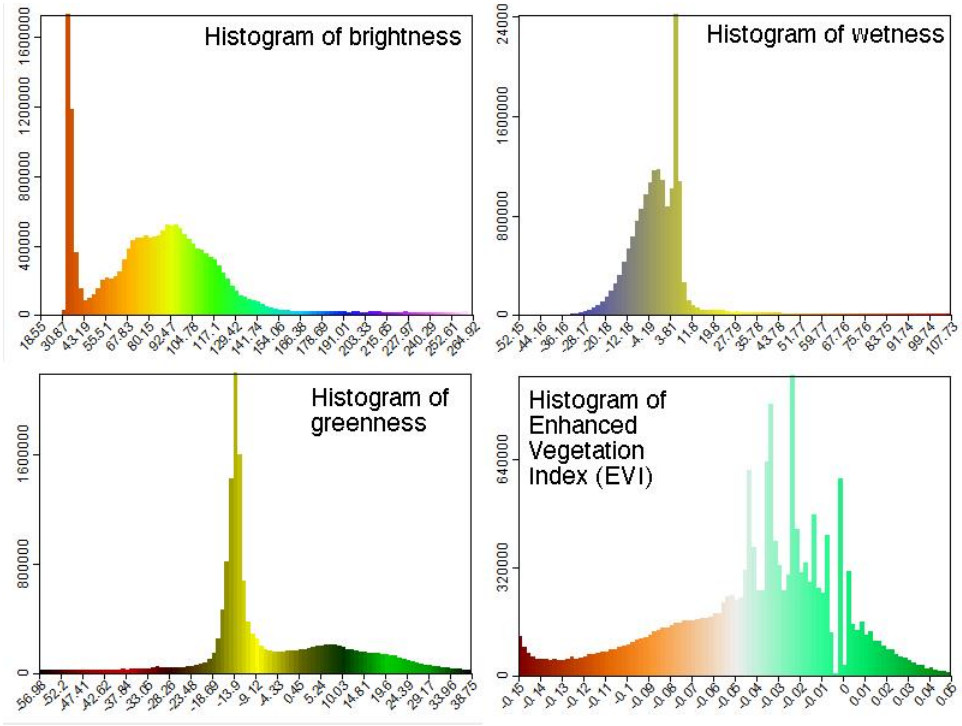

Fig. 7. Four statistical histograms of the data distribution: wetness, brightness, greenness, enhanced vegetation index, modelled by SAGA GIS. Source: author.

Current environmental problems of Iceland include changes in vegetation coverage and anthropogenic pressure, such as increased number of tourists which may cause some mechanical disturbances on fragile Polar landscapes (Ásgeirsdóttir and Karlsson, 2016; Óladóttir, 2019). A complex interaction between various factors affect land cover and vegetation in Iceland, for instance anthropogenic (Tverijonaite et al., 2018), climatic factors (Haraldsson and Ólafsdóttir, 2003). This can be illustrated by issues regarding the vulnerability of the Northern ecosystems, e.g. land use pressure and overgrazing. Since land degradation may affect land use sustainability in the future, these issues present the concern for the environmental monitoring in Iceland. According to previous studies (Bergþórsson et al., 1996), Iceland has experienced climatic variations over the past years which resulted in the changes in vegetation. Besides, due to the anthropogenic effects, over half of the Icelandic vegetation deteriorated from the time of Iceland's first settlement by vikings in ca. AD 830 (Hallsdóttir 1995) and over 90% of its forest cover (Þorsteinsson, 1972). In view of abovesaid, detailed studies of the selected landscapes of Iceland present a contribution to the monitoring of natural resources prevention of degradation of the Northern ecosystems.

Conclusions

Thanks to the advances in the remote sensing data processing by cartographic methods, the massive amount of Landsat TM images of 30-m resolution and high quality became available in agricultural studies, for instance for a crop or vegetation mapping. Reflectance curves for various land cover types on Earth, including healthy and unhealthy vegetation and its types (coniferous, broadleaf), have particular characteristics (Abburu and Golla, 2015; Knipling, 1970; Lemenkova, 2013). Remote sensing data analysis using spectral reflectance curves shown a trend in

land cover changes and variations in vegetation coverage of Earth (Lemenkova, 2013, 2015d).

The possibility of synergism between these risk factors remains a topic that can be further researched.

Other studies upscaled the question of the vegetation changes and focused on the quantification of landscape fragmentation by metrics approach for environmental sustainability (Klaučo et al., 2013b, 2014, 2017). Examples of the statistical analysis applied for geosciences provide more advanced methods of data visualization (Lindh, 2004; Klaučo et al. 2013a; Lemenkova, 2019a, 2019b, 2019c). Other ways of geodata processing include machine learning, GIS (Suetova et al., 2005a, 2005b; Schenke and Lemenkova, 2008; Lemenkova, 2014). This review focused on the effective methods of Enhanced Vegetation Index and Tasseled cap transformation by SAGA GIS applied for processing of the Landsat TM scene covering the region of northern Iceland.

The paper contributed both to the agricultural studies of vegetation coverage and the development of methods by presenting two-step methodology: computing raster bands for visualizing the Tassel Cap Transformation and EVI. The application of the Tasseled Cap Transformation and EVI in Arctic vegetation monitoring showed effective methods of data visualization. With a context of the presented case study of Iceland, notable for fragile ecosystems, these two methods demonstrated usefulness for Landsat TM scene analysis of the vegetation canopy, health status and land cover parameters by cartographic means of SAGA GIS.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abburu S, Golla SB (2015) Satellite Image Classification Methods and Techniques: A Review. International Journal of Computer Applications 119(8):20–25.
2. Ahmet KR, Akter S (2017) Analysis of landcover change in southwest Bengal delta due to floods by NDVI, NDWI and K-means cluster with Landsat multi-spectral surface reflectance satellite data. Remote Sensing Applications: Society and Environment 8:168–181.
3. Arnalds O, Barkarson BH (2003) Soil erosion and land use policy in Iceland in relation to sheep grazing and government subsidies. Environmental Science & Policy 6(1):105–113.
4. Arnalds O, Gisladottir F, Sigurjonsson H (2001) Sandy deserts of Iceland: an overview. J. Arid Environment 47:359–371.
5. Ásgeirsdóttir T, Karlsson T (2016) International visitors in Iceland – summer 2016. Icelandic Tourist Board. 405 p.
6. Bergþórsson P, Björnsson H, Dýrmundsson Ó, Guðmundsson B, Helgadóttir Á, Jónmundsson JV (1987) The effects on Climatic Variations on Agriculture in Iceland. In: Parry ML, Carter TR, Konijn NT (eds.). The Impact of Climatic Variations on Agriculture, 1. Assessment in Cool Temperate and Cool Regions, 387–444, IIASA and UNEP, Dordrecht.
7. Böhner J, McCloy KR, Strobl J (2006) SAGA – Analysis and Modelling Applications. Göttinger Geographische Abhandlungen 115, 130 pp.
8. Broge NH, Leblanc E (2001) Comparing prediction power and stability of broadband and hyperspectral vegetation indices for

estimation of green leaf area index and canopy chlorophyll density. Remote Sensing of the Environment 76:156–172.
9. Brombacher J, Reiche J, Dijksma R, Teuling AJ (2020) Near-daily discharge estimation in high latitudes from Sentinel-1 and 2: A case study for the Icelandic Þjórsá river. Remote Sensing of Environment 241:111684.
10. Crist EP, Cicone RC (1984a) Application of the Tasseled Cap concept to simulated Thematic Mapper data. Photogrammetric Engineering and Remote Sensing 50(3):343–352.
11. Crist EP, Cicone RC (1984b). A physically-based transformation of Thematic Mapper data – the TM Tasseled Cap. IEEE Transactions on Geoscience and Remote Sensing GE- 22(3):256–263.
12. Eckert S, Engesser M (2013) Assessing vegetation cover and biomass in restored erosion areas in Iceland using SPOT satellite data. Applied Geography 40:179–190.
13. Eddudóttir SD, Erlendsson E, Gísladottir G (2020) Landscape change in the Icelandic highland: A long-term record of the impacts of land use, climate and volcanism. Quaternary Science Reviews 240:106363.
14. Gao L, Wang X, Johnson BA, Tian Q, Wang Y, Verrelst J, Mu X, Gu X (2020) Remote sensing algorithms for estimation of fractional vegetation cover using pure vegetation index values: A review. ISPRS Journal of Photogrammetry and Remote Sensing 159:364–377.
15. Gísladottir G (2001) Ecological Disturbance and Soil Erosion on Grazing Land in Southwest Iceland, Land Degradation. Springer, 109–126.
16. Hallsdóttir M (1995) On the pre-settlement history of Icelandic vegetation. Icelandic Agricultural Science 9:17–29.
17. Haraldsson HV, Ólafsdóttir R (2003) Simulating vegetation cover dynamics with regards to long-term climatic variations in sub-arctic landscapes. Global and Planetary Change 38(3-4):313–325.
18. Huete A, Didan K, Miura T, Rodriguez EP, Gao X, Ferreira LG (2002) Overview of the radiometric and biophysical performance of the MODIS vegetation indices. Remote Sensing of Environment 83:195–213.
19. Jiang Z, Huete AR, Didan K, Miura T (2008) Development of a two-band Enhanced Vegetation Index without a blue band, Remote Sensing of Environment, 112(10):3833–3845.
20. Kim Y, Huete AR, Miura T, Jiang Z (2010) Spectral compatibility of vegetation indices across sensors: band decomposition analysis with Hyperion data. Journal of Applied Remote Sensing, 4(1):043520.
21. Kauth RJ, Thomas GS (1976) The Tasseled Cap – a graphic description of the spectral-temporal development of agricultural crops as seen by Landsat. Proceedings of the Symposium on Machine Processing of Remotely Sensed Data, Purdue University, West Lafayette, Indiana, 4B41-4B51.
22. Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2013a) Determination of ecological significance based on geostatistical assessment: a case study from the Slovak Natura 2000 protected area. Central European Journal of Geosciences, 5(1):28–42.
23. Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2013b) Interpretation of Landscape Values, Typology and Quality Using Methods of Spatial Metrics for Ecological Planning. 54th International Conference Environmental & Climate Technologies. Riga, Latvia.
24. Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2014)

Landscape metrics as indicator for ecological significance: assessment of Sitno Natura 2000 sites, Slovakia. Ecology and Environmental Protection. Proceedings of the International Conference. March 19–20, 2014. Minsk, Belarus, 85–90.

25. Klaučo M, Gregorová B, Stankov U, Marković V, Lemenkova P (2017) Land planning as a support for sustainable development based on tourism: A case study of Slovak Rural Region. Environmental Engineering and Management Journal, 2(16):449–458.
26. Knipling EB (1970) Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sensing of Environment 1:155-159.
27. Lemenkova P (2020a) GMT Based Comparative Geomorphological Analysis of the Vityaz and Vanuatu Trenches, Fiji Basin. Geodetski List, 74(1):19–39.
28. Lemenkova P (2020b) Variations in the bathymetry and bottom morphology of the Izu-Bonin Trench modelled by GMT. Bulletin of Geography. Physical Geography Series 18(1): 41–60.
29. Lemenkova P (2020c) GEBCO Gridded Bathymetric Datasets for Mapping Japan Trench Geomorphology by Means of GMT Scripting Toolset. Geodesy and Cartography 46(3):98–112.
30. Lemenkova P (2019a) Statistical Analysis of the Mariana Trench Geomorphology Using R Programming Language. Geodesy and Cartography 45(2):57–84.
31. Lemenkova P (2019b) Testing Linear Regressions by StatsModel Library of Python for Oceanological Data Interpretation. Aquatic Sciences and Engineering 34:51–60.
32. Lemenkova P (2019c) AWK and GNU Octave Programming Languages Integrated with Generic Mapping Tools for Geomorphological Analysis. GeoScience Engineering 65(4):1–22.
33. Lemenkova P. (2015a) Analysis of Landsat NDVI Time Series for Detecting Degradation of Vegetation. In: Geoecology and Sustainable Use of Mineral Resources. From Science to Practice, Belgorod, Russia, 11–13.
34. Lemenkova P (2015b) Innovations in the Geoscience Research: Classification of the Landsat TM Image Using ILWIS GIS for Geographic Studies. In: Prospects for the Higher School Development. Grodno, Belarus, May 28–29, 2015, 60–63.
35. Lemenkova P (2015c) To the Question of the Environmental Education: how Landsat TM, ETM+ and MSS Images can be Processed by GIS-Techniques for Geospatial Research. Trends and Perspectives in the Creating Regional Systems of the Additional Adults Education. Vitebsk, Belarus.
36. Lemenkova P (2015d) Processing Remote Sensing Data Using Erdas Imagine for Mapping Aegean Sea Region, Turkey. Informatics. Problems, Methodology, Technologies, 3, 11–15.
37. Lemenkova P (2014) Opportunities for Classes of Geography in the High School: the Use of 'CORINE' Project Data, Satellite Images and IDRISI GIS for Geovisualization. In: Perspectives for the Development of Higher Education. Belarus, Grodno, 284–286.
38. Lemenkova P (2013) Monitoring Changes in Agricultural Landscapes of Central Europe, Hungary: Application of ILWIS GIS for Image Processing. Geoinformatics: Theoretical and Applied Aspects. Ukraine, Kiev, 13–16 May, 2013.
39. Lemenkova P (2011) Seagrass Mapping and Monitoring Along the Coasts of Crete, Greece. M.Sc. Thesis. Netherlands: University of Twente. 158 pp.

40. Lindh P (2004) Compaction- and strength properties of stabilised and unstabilised fine-grained tills. PhD Thesis, Lund University, Lund.
41. Óladóttir OT (2019). Tourism in Iceland in Figures. Icelandic Tourist Board, 28 p.
42. Þorsteinsson, I. 1972. Gróðurvernd: byggð á hóflegri nýtingu og ræktun lands [Carrying capacity of Icelandic rangelands]. Rit landverndar, 2. Landvernd, Reykjavik, 128 pp.
43. Schenke HW, Lemenkova P (2008) Zur Frage der Meeresboden-Kartographie: Die Nutzung von AutoTrace Digitizer für die Vektorisierung der Bathymetrischen Daten in der Petschora-See. Hydrographische Nachrichten 81:16–21.
44. Suetova IA, Ushakova LA, Lemenkova P (2005a) Geoinformation mapping of the Barents and Pechora Seas. Geography and Natural Resources 4:138–142.
45. Suetova IA, Ushakova LA, Lemenkova P (2005b) Geoecological Mapping of the Barents Sea Using GIS. International Cartographic Conference.
46. Taufik A, Ahmad SSS, Ahmad A (2016) Classification of Landsat 8 satellite data using NDVI thresholds. Journal of Telecomunication Electronic and Computer Engineering 8(4):37–40.
47. Tverijonaite E, Ólafsdóttir R, Thorsteinsson T (2018). Accessibility of protected areas and visitor behaviour: A case study from Iceland. Journal of Outdoor Recreation and Toursim, 24:1–10.
48. Zaitunah A, Ahmad AG, Safitri RA (2018) Normalized difference vegetation index (ndvi) analysis for land cover type using landsat 8 oli in besitang watershed, Indonesia. IOP Conf. Series: Earth and Environmental Science 126:012112.

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STAPHYLOCOCCUS SP. STRAIN MY 83295F: A POTENTIAL *P,P'*-DDT-DEGRADING BACTERIUM ISOLATED FROM PESTICIDE CONTAMINATED SOIL

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Abstract: Although DDT has been on the ban list by the Stockholm Convention for its environmental degradation, still a wave of emerging shreds of evidence has proved its circulation in developing countries. The intensity of environmental degradation and human health problems posed by residual DDT and its metabolites become of serious ecological concern, warranting a search for novel strains with a capacity to biodegrade these environmental contaminants. A new strain of the genus *Staphylococcus* was isolated from pesticide-contaminated soil. The 16S rRNA and phylogenetic analyses were used to identify the isolate and the 16S rRNA partial gene sequence was deposited in the NCBI GenBank as *Staphylococcus* sp. strain MY 83295F. The isolate was capable of growing in up to 60 mg L^{-1} of *p,p'*-DDT as the sole carbon source at an optimum pH of 6.5 and optimum temperature of 30°C within 120 h. Zn^{2+} has demonstrated a stimulatory effect on the growth of the strain in *p,p'*-DDT containing medium. However, Fe, Cu, Pb, Hg, Ag, and Cr ions showed inhibitory effects on the strain's growth in the medium. The strain could be a handy tool for the bio-cleansing of residual *p,p'*-DDT in the contaminated environment.

Keywords: DDT biodegradation, *Staphylococcus*, 16S rRNA, pesticide contaminant, heavy metals.

1. Introduction

Among the most notorious and persistent agrochemical environmental contaminants, the dichlorodiphenyltrichloroethane (DDT) still occupies a prominent position in the ranking of the hazardous environmental pollutants. This arises due to its persistence and potential accumulation in both biotic and abiotic components of the ecosystem (Bussolaro et al., 2012; Devi, 2020). Although DDT and other Persistent Organic Pollutants (POPs) have been on the ban list by the Stockholm Convention for their environmental degradation capacity, DDT is, still in circulation in developing countries (Abdul Kader, 2019). A comprehensive report released by the United Nations Environmental Protection (UNEP) on the current status of DDT from 2015 to 2017 in developing countries indicated that India, Mozambique, South Africa, and Zimbabwe reported the use of DDT (UNEP, 2019). While Botswana, Eswatini, Ethiopia, Eritrea, Madagascar, Marshall Islands, Namibia, Uganda, Venezuela, and Zambia have refused to give any response on the status of the use of

DDT between 2015-2017, despite all the efforts by the UNEP. This perhaps signals the use of DDT for vector control in these countries (UNEP, 2019).

It was documented that in Nigeria several tons of chlorinated pesticides are used every year for both agriculture and disease-borne control (Asogwa and Dongo, 2009), and consequently, remnants of DDT were recently traced in domestic water samples (Ogbeide et al., 2015). Many studies across the world have reported the traces of DDT in ambient air, domestic water, and various food sources (Bussolaro et al., 2012; Mendes et al., 2019; Thompson et al., 2019). The levels of DDT reported in many food items exceeded the tolerable daily limits stated by the U.S. Environmental Protection Agency (Sheldon et al., 2019).

DDT and its metabolites such as dichlorodiphenyldichloroethylene (DDE) have been implicated in many health-related problems. They were reported to be linked to endocrine disruption (Mnif et al., 2011; Piazza and Urbanetz, 2019). Truong et al. (2019) reported an association between DDT metabolites and long-term impairments of muscle health. Many studies have established the probable involvement of DDT in cancer induction (Hadara et al., 2016; Cohn et al., 2019). DDT was also documented to induce cognitive decline and alteration in maternal metabolomes (Medehouenou et al., 2019; Hu et al., 2019). Yu et al. (2019) demonstrated that DDT is associated with some risks of triggering apoptosis of skin fibroblast in some aquatic animals.

From the beginning of the last decade to date, many bacterial strains have been identified to degrade DDT and its metabolites; DDD and DDE (Fang et al., 2010; Hug et al., 2013; Wang et al., 2017; Pan et al., 2017; Xie et al., 2018). The microbial ability to degrade DDT have strengthened the hope of employing them in the bioremediation of DDT contaminated sites.

Although DDT degradation was reported to be a multistep process (Fang *et al.*, 2010; Cutright and Erdem, 2012), it mostly occurs either through aerobic or anaerobic metabolism. With favourable conditions, suitable strains were shown to degrade DDT to 4-chlorobenzoic acid (4-CBA) aerobically or to 4,4-dichlorobenzophenone (4-DBP) under anaerobic conditions (Nadeau et al., 1998; Baczynski et al., 2010; Gao et al., 2011; Bao et al., 2012). *Alcaligenes* sp. and *Serratia marcaescens* DT-1P were reported to degrade DDT to 4-CBA aerobically via the DDE metabolic pathway in the presence of additional carbon sources (Bidlan and Manonmani, 2002). However, DDT was shown to be rapidly degraded anaerobically to 4-DBP through the DDD reductive dechlorination pathway (You et al., 1996; Baczynski et al., 2010; Fang et al., 2010).

The intensity of environmental degradation and human health problems posed by DDT and its metabolites, particularly their persistent nature in the environment, searching a microbial community for novel strains with a capacity to bio-clean the environment is pertinent. This work was focused on the isolation and characterization of *p,p'*-DDT-degrading bacterium from the tropical contaminated soil.

2. Materials and Methods

2.1 Sample collection

A soil sample was collected from irrigation sites located at Phase I, Kadawa Irrigation Site, Hadejia-Jama'are River Basin, Kano State with a history of continued agrochemical farming activities for more than three decades. The sample was collected at the surface of the soil to the depths of 15 cm. The sampling was focused on these horizons because a large

portion of microbial activity occurs in these horizons. The soil sample was mixed evenly and 20 g was carefully put into a sterile container and taken to the laboratory at 4°C for bacterial isolation.

2.2 Preparation of media for bacterial growth

The Luria-Bertani medium (LB) was used for the bacterial growth.

2.3 Preparation of *p,p'*-DDT-minimal salt enrichment medium (MSM)

The Minimal Salt Medium (MSM) had the following composition as described by Pant et al. (2013) with some modifications: per litre of distilled water, 0.1 g $CaCl_2.2H_2O$, 0.08 g $Ca(NO_3)_2$ $4H_2O$, 0.5 g $MgCl_2.6H_2O$, 1.0 g Na_2SO_4 and 1.0 g KH_2PO_4 were dissolved. Then before inoculation, MSM was enriched with *p,p'*-DDT (0.05 mg mL^{-1}). The *p,p'*-DDT-MSM contains *p,p'*-DDT as the only carbon source for the bacterial growth. Thus, growth in this media depends only on the strain's ability to metabolize the *p,p'*-DDT pesticide.

2.4 Isolation of *p,p'*-DDT-degrading bacterium from soil samples

Isolation of strain MY 83295F was carried out using a modified isolation procedure described by Pant *et al.* (2013). Air-dried soil (0.5 g) was suspended in 20 mL of the prepared LB medium. The suspension was kept for 48 h at 30°C on a shaker. After the incubation, the LB medium was allowed to settle down for 2 h. An aliquot (150 µL) from the cleared LB supernatant was used to inoculate 6 mL of *p,p'*-DDT enrichment MSM. The culture was then incubated for 1 week at 30°C on a rotary shaker at 100 rpm. After incubation, 100 µL of the bacterial suspension was transferred into 4 mL of fresh *p,p'*-DDT enriched MSM and the incubation step was repeated. After four sequential cultivations, the isolate was inoculated on to MSM agar plates enriched with 0.05 mg mL^{-1} of *p,p'*-DDT and incubated for 72 h at 30°C and the isolate formed was preserved. This ensures adequate exposure of the isolate to the *p,p'*-DDT as a sole carbon source.

2.5 Extraction of genomic DNA

A single loop of the isolate was used to inoculate 8 mL of LB medium. Followed by incubation at 37°C and 200 rpm for 24 h. The bacterial suspension (OD_{600nm} = 0.6) formed was centrifuged for 5 min at 10,000 rpm. Then the bacterial DNA was extracted following the protocol stated by Schmidt et al. (1991).

2.6 16S ribosomal RNA gene (16S rRNA) amplification

To amplify ~1.5 Kb gene from the isolated genomic DNA, 16S rRNA gene primers (BAC27F and BAC1492R) [16SRNA BAC27F: 5'-AGA GTT TGA TCC TGG CTC AAG-3' and 16SRNA BAC1492R: 5'- GGT TAC CTT GTT ACG ACT T-3'] purchased from Sigma-Aldrich, United Kingdom, were used (Sangwan et al., 2005). The PCR was carried out using TC-E-48FA Gene Touch Thermocycler, Hangzhou Bioer Technology, (China). The total reaction volume was 15 µL, in which the reaction mix comprises of 1 µL of the genomic DNA, 1.5 µL of 10X TaqA buffer, 0.5 µL of each of 10 µM forward and reverse primers, 0.75 µL of 1.25 mM of $MgCl_2$, 0.15 µL of 0.25 mM of dNTP and 0.12 µL of Taq DNA polymerase in ddH_2O. The PCR protocol was set as follows: the initial melting temperature was 95°C for 5 min, 35 cycles each at melting temperature of 94°C for 0.5 min. The annealing temperature was 52°C for 0.5 min and extension at 72°C for 1 min. The final elongation was set for 10 min at 72°C.

After the final elongation cycle, the size of the DNA fragment was compared with the Hyper Ladder-1K marker Bioline (Lot No: H4-

111B). Then 3 of the µL PCR product was mixed with 5XDNA loading buffer blue (1.5 µL) Bioline (Lot No: hLBB-415704) and loaded onto 1.5% agarose gel electrophoresis that has been stained with ethidium bromide. The electrophoresis was run for 35 min under 120V and 300mA current. The product was then visualized with the Syngene Gel Documentation System of Ingenius, England (IG31459). The presence of a product of the expected size was considered to be a positive result.

2.7 Agarose gel purification and sequencing of amplified 16S rRNA gene

The gel was purified using the PrepEase gel purification kit (Affymetrix inc., USA) by following the manufacturer's protocol. The gel-purified product was sequenced using the protocols described by Sanger et al. (1977). Then, DNA sequence alignment was carried out using the ClustalW 2.0.12 version (http://www.clustal.org/). The sequence was then blasted in the National Center for Biotechnology Information (NCBI) nucleotide databases to identify the organism. The sequence was deposited in the NCBI GenBank under the accession number MN812290.

2.8 Phylogenetic analysis of the isolates

Phylogeny and evolutionary history of strain MY 83295F were constructed using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2013). The phylogenetic tree was constructed using MEGA version 6 software program.

2.9 Characterization of the isolate in minimal salt-*p,p'*-DDT enrichment medium

During the characterization of the strain MY 83295F in the *p'p*-DDT enrichment medium, we have determined the optimum *p,p'*-DDT concentration (as a sole carbon source), pH, temperature and incubation time. The characterization was performed by modifications of the methods described by Mwangi et al. (2010) and Pant et al. (2013). The isolate's capacity to grow in the *p,p'*-DDT enrichment medium was determined *in vitro* after adjusting the OD_{600nm} of cell density of the inoculum source to 0.6. The inoculum (150 µL) was then inoculated into 4 mL of MSM media containing varying concentrations *p,p'*-DDT (10, 20, 30. 40 50, 60 and 70 mgL^{-1}) at various pH values (5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) and incubated at different temperatures (20, 25, 30, 35, 40 and 45°C) under shaking (150 rpm) at different incubation periods of 24, 48, 72, 96, 120, 144 and 168 h. The experiments were conducted one factor at a time in triplicate.

2.10 Effects of heavy metals on *p,p'*-DDT utilization capacity of the isolates

The effect of each heavy metals (Fe, Zn, Cu, Pb, Hg, Ag and Cr) on *p,p'*-DDT degrading capacity of the strain MY 83295F was determined *in vitro* after adjusting the OD_{600nm} of cell density of the inoculum source to 0.6, the cells (100 µL) were then inoculated into MSM-DDT enrichment media containing varying metal concentrations (0.2, 0,4, 0.6, 0.8 and 1.0 mgL^{-1}) and incubated on a rotary shaker (150 rpm) at 30 °C and pH 6.5 for 168 h. This protocol is a modified version of the procedure described by Sandrin and Maier, (2003).

3. Results and discussions

In In this study, minimal salt-*p,p'*-DDT enrichment medium was used for the isolation and screening of *p,p'*-DDT bio-degrader bacterial strain that used *p,p'*-DDT as the sole carbon and energy source obtained from pesticide-contaminated agricultural soil. The biodegradation capacity of *p,p'*-DDT by the isolate was indicated by the formation of

turbidity as the index of the biomass formed in the minimal salt-*p,p'*-DDT enrichment medium (Mwangi et al., 2010; Pant et al., 2013).

Staphylococcus sp. strain MY 83295F was found to be a Gram-positive, non-spore-forming, non-motile, catalase-positive, urease, and cytochrome oxidase negative cocci. However, the strain was found to ferment D-glucose, starch and D-mannitol while negative with xylose and indole (data not presented here). A substantial literature on the phenotypic and biochemical characteristics of the genus *Staphylococcus* showed clear similarities with the above-mentioned characteristics of strain MY 83295F (Bascomb and Manafi, 1998; Khattak et al., 2015; Karmaker et al., 2016).

The 16S rRNA gene sequencing was found to maintain reasonable accuracy and reliability for bacterial identification (Roy et al., 2013; Hong and Farrence, 2015). Thus, for the molecular identification of this isolate to confirm the preceding phenotypic and biochemical identifications, the 16S rRNA gene was amplified. The amplification product for the isolate revealed about 1500 bp upon running on 1.5% agarose gel electrophoresis (**Fig. 1.**). Many researchers have earlier reported similar ranges of 16S rRNA gene amplification products between the ranges of 1200-1500 bp for the genera *Staphylococcus* depending on the species, segment amplified or the type of primers used (Saruta et al., 1997; Jill and Clarridge, 2004; Mitra and Roy, 2010).

The 16S rRNA gene amplicon was successfully sequenced, revealing 1057 bp as the partial gene sequence for the strain MY 83295F. The sequence was blasted in the NCBI GenBank that revealed the genus of the strain as *Staphylococcus.* The 16S RNA gene partial sequence was deposited in public databases of the National Center for Biotechnology Information (NCBI) GenBank as *Staphylococcus* sp. strain MY 83295F under the universal accession number MN812290.

The BLAST search on strain MY 83295F showed about 80% of the first hundred representatives were of the genus *Staphylococcus*, revealing the highest sequence similarity of 99.53% with *Staphylococcus hominis* subsp. *novobiosepticus* strain GTC 1228, followed by *Staphylococcus hominis* strain DM 122 with 99.15%.

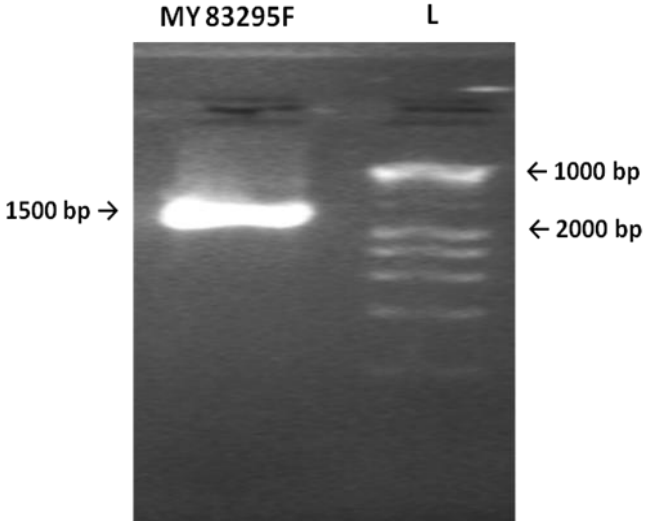

Fig. 1. Agarose gel electrophoretic image of the 16S rRNA amplicon of MY 83295F strain. The gene was amplified using BAC27F and BAC1492R as forward and reverse primers respectively. L represents Hyper Ladder-1K marker Bioline (Lot No: H4-q111B)

The least % sequence similarity of 95.27% was found in *Staphylococcus agenesis* strain M4S-6 among the representatives of the genus *Staphylococcus*. The phylogeny and evolutionary analysis revealed a single clade of *Staphylococcus hominis* strain DM 122 and a cluster of strain MY 83295F and *Staphylococcus hominis* subsp. *novobiosepticus* strain GTC 1228 with a bootstrap value of 85%, indicating a closer relatedness of these organisms. However, the cluster of strain MY 83295F and *Staphylococcus hominis* subsp. *novobiosepticus* strain GTC 1228 indicated a bootsrap value of 59% (**Fig. 2.**). This value is very low to state with certainty that strain MY 83295F belongs to the same subspecies with *Staphylococcus hominis* subsp. *novobiosepticus* strain GTC 1228. Perhaps, suggesting a new subspecies within the group of *Staphylococcus hominis*. Therefore, strain MY 83295F was tentatively designated as *Staphylococcus* sp. strain MY 83295F, where the sp. indicates an unclassified species of this strain, subject to in-depth taxonomical approach. Several studies on the identification and taxonomy of the genus *Staphylococcus* have reported a similar approach in the identification and grouping of *Staphylococcal* species (Stackebrandt and Goebel, 1994; Takahashi et al., 1999; Ghebremedhin et al., 2008; Naushad et al., 2016).

Microorganisms are vital tools for the removal of various toxic contaminants including the persistent chlorinated pollutants such as DDT from the environment (Reineke et al., 2011). Biodegradation of DDT by bacteria has been well documented, and DDT-degraders have been isolated (Mwangi et al., 2010; Pan et al., 2016). Strain MY 83295F was found to biodegrade and utilize *p,p'*-DDT as sole carbon and energy source. Though some bacterial species were reported to tolerate < 20 mgL^{-1} of *p,p'*-DDT as sole carbon and energy (Pant et al., 2013; Pan et al., 2016), strain MY 83295F, however, demonstrated higher tolerance and utilization capacity of up to 60 mg L^{-1} of *p,p'*-DDT as the sole carbon source under aerobic condition (**Fig. 3a**).

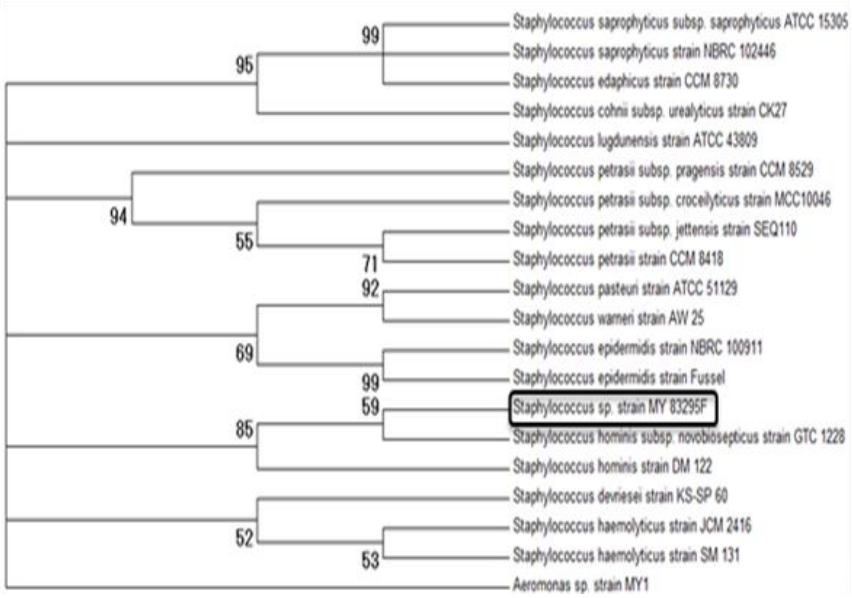

Fig. 2. Phylogenetic and Evolutionary relationships of taxa of strain MY 83295F. The strain's evolutionary position was indicated in a rectangular box. The evolutionary history was inferred using the Neighbor-Joining method.

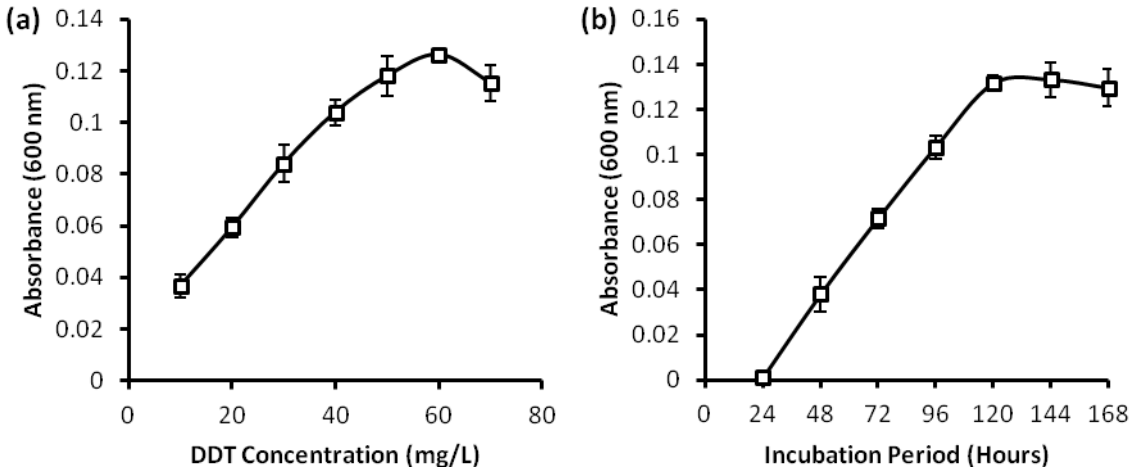

Fig. 3. Effects of *p,p'*-DDT concentration (a) and incubation time (b) on the growth of strain MY 83295F in *p,p'*-DDT enrichment medium. For determination of the effect of the incubation time on the growth of the strain, 60 mgL^{-1} of *p,p'*-DDT was used. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

Some isolates were also documented to tolerate up to 50 mgL^{-1} of DDT when other carbon sources were supplemented (Kantachote et al., 2003; Barragan-Huerta et al., 2007; Fang et al., 2010).

The ability of a microorganism to depend on DDT as a carbon source depends on the organism's capacity to mineralize the DDT and obtain energy from the process (Fang et al., 2010; Pan et al., 2016). Strains MY 83295F demonstrated longer lag phases of nearly 48 h for the initial DDT degradation (Figure 3b). The delay observed in the initial rate of DDT degradation in the strain could be attributed to the delay in the production of enzyme machinery for the degradation. However, the strain was able to moderately biodegraded and utilized *p,p'*-DDT, precipitating total biomass of 0.130 (OD_{600nm}) in 120 h (**Fig. 3b**).

As a complex process, bacterial DDT mineralization is largely influenced by some environmental determinants such as pH, temperature and DDT concentration (Aislabie et al., 1997; Bidlam and Manonmani, 2002). Optimization of these parameters is therefore critical for the application of a microbial entity for the DDT biodegradation. Strain MY 83295F demonstrated a mesophilic behaviour by exhibiting *p,p'*-DDT degradation within a wide range of temperatures between 20-45°C. However, strain MY 83295F showed an optimum temperature of 30°C (**Fig. 4a**). This is not surprising by considering the environmental conditions of the tropical region where this strain was isolated. A fluctuation in temperature, either below or above the optimum value, the strains' DDT degradation capacity is lowered.

Strain MY 83295F showed growth capacity in *p,p'*-DDT enrichment media in both slightly acidic and alkaline conditions, with initial pH ranging from 5.5 to 7.5. However, the strain demonstrated an optimum pH of 6.5 (**Fig. 4b**). A substantial literature has reported an optimal bacterial DDT degradation within a range of pH close to neutral and temperature range around 30°C that correspond to those shown by this strain (Mwangi et al., 2010; Fang et al., 2010; Pan et al., 2016; Raju and Bidlan, 2018).

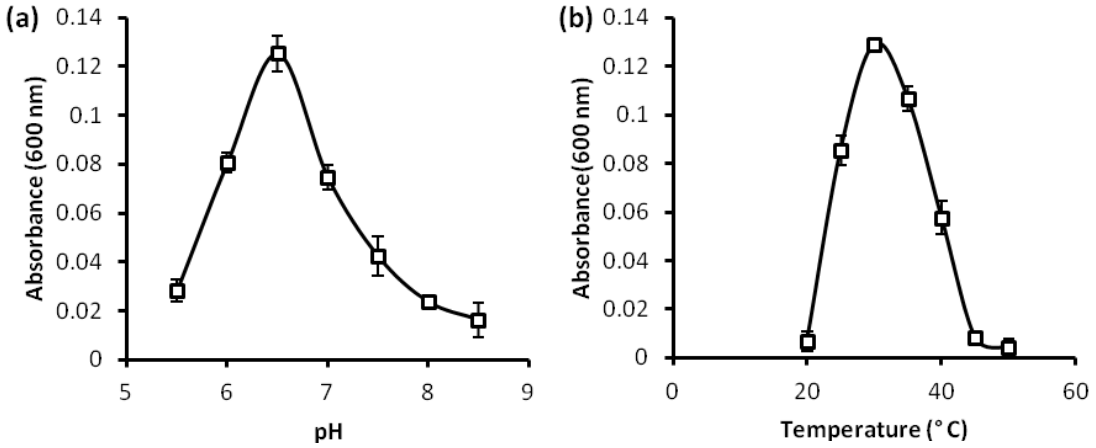

Fig. 4. Effect of pH (**a**) and temperature (**b**) on the growth of strain MY 83295F in *p,p'*-DDT(60 mgL^{-1}) enrichment medium. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

In biological systems, including microorganisms, pH plays a significant role in changing the ionic character of the constituent amino acids in enzymes and other intracellular and membrane proteins. This perhaps, influences DDT degradation capacity of this bacterial strain, either by affecting the DDT membrane transport system or degradation enzymes.

In most cases, organic pollutants such as DDT and heavy metals co-contaminate the environment, and more or less, the later might influence the degradation rate of the former as reported by Lovecke et al. (2015). The effects of heavy metals on DDT degradation capacity of strain MY 83295F showed both stimulatory and inhibitory effects. Only Zn^{2+} at 0.2 mgL^{-1} has demonstrated enhancement effect on *p,p'*-DDT degradation in *Staphylococcus* sp. strain MY 83295F, precipitating an increase in the biomass in *p,p'*-DDT enrichment medium after 168 h. An increase in the Zn^{2+} concentration above 0.2 mgL^{-1} showed a dramatic decline in *p,p'*-DDT degradation, and reduction in the total biomass of the strain MY 83295F (**Fig. 5**).

Zn^{2+} is physiologically essential for bacterial growth. It serves as a cofactor for many microbial metalloenzymes and other structural and regulatory functions. Bacteria have systems called cation diffusion facilitator (CDF) proteins that modulate their survival in the Zn^{2+} contaminated environment (Guffanti et al., 2012). These bacterial proteins are responsible for Zn^{2+} homeostasis via Zn^{2+}-uptake/import and Zn^{2+}-efflux/export mechanisms (Suryawati, 2018). The genus *Staphylococcus* was reported to have these CDF proteins (Nies, 2003).

Thus, *Staphylococcus* sp. strain MY 83295F might have very active Zn^{2+} homeostasis systems that warrant growth enhancement in the Zn^{2+}-*p,p'*-DDT enrichment medium. Indeed, the DDT degradation enzyme machinery might interact with the divalent ion such as zinc as presented by Mansouri et al (2017), leading to the enhancement of catalytic ability by the enzyme machinery.

A pattern of inhibition demonstrated by Fe^{2+}, Cu^{2+} (**Fig. 6a** and **b**) was more favourable to the growth of the strain 83295F relative to that of Pb^{2+}(**Fig. 7a**), Hg^{2+} (**Fig. 7b**), Ag^{2+} (**Fig. 8a**) and Cr^{2+} (**Fig. 8b**). This could be linked to the essentiality of Cu^{2+} for some metabolic processes in bacteria. However, it has been observed that presence of both organic and metal pollutants resulted in metal toxicity in

bacteria, mostly by interacting and inhibiting the bacterial enzymes and thus, inhibiting organic pollutant biodegradation (Angle and Chaney, 1989; Sandrin and Maier**,** 2003; Murata et al., 2005). Also, metal oxyanions, such as chromate, mimic the structure of essential non-metal oxyanions, such as sulfate, and interfere with their biological functions (Sandrin and Maier**,** 2003). Furthermore, mercuric and silver cations form strong toxic complexes which make them dangerous for any physiological functions, in addition to their inhibitory binding to the SH group of the variety of bacterial proteins (Nies, 1999). Metal ions generally affect organic biodegradation by altering both the physiology and ecology of the organic bio-degraders (Sandrin and Maier**,** 2003). The net effect of exposure to Cu^{2+}, Pb^{2+}, Hg^{2+}, Ag^{2+} and Cr^{2+} on the *p,p'*-DDT degradation by strain MY 83295F was thus, reduced biodegradation rates and failure to effectively degrade the *p,p'*-DDT.

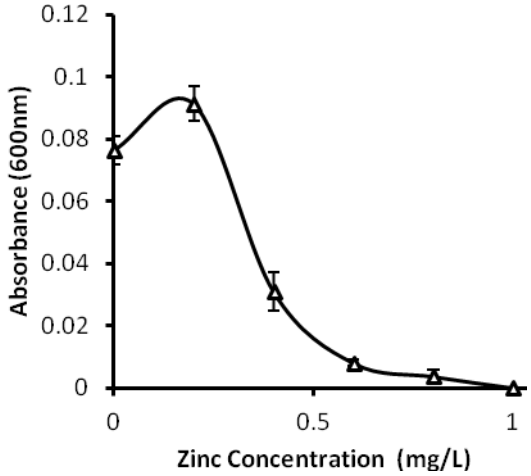

Fig. 5. Effect of Zn^{2+} concentration on the growth of strain MY 83295F in *p,p'*-DDT (60 mgL^{-1}) enrichment medium. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

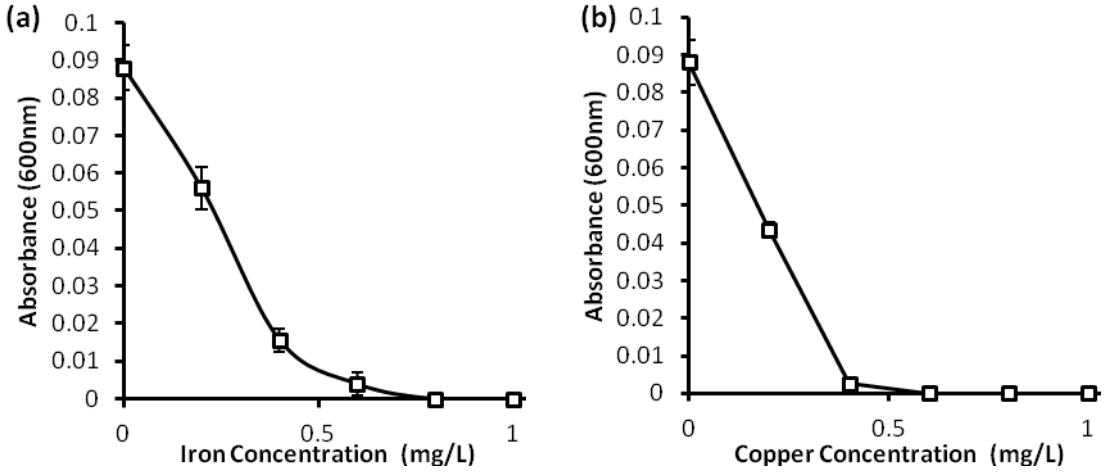

Fig. 6. Effect of Fe^{2+} (a) and Cu^{2+} (b) concentrations on the growth of strain MY 83295F in *p,p'*-DDT(60 mgL^{-1}) enrichment medium. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

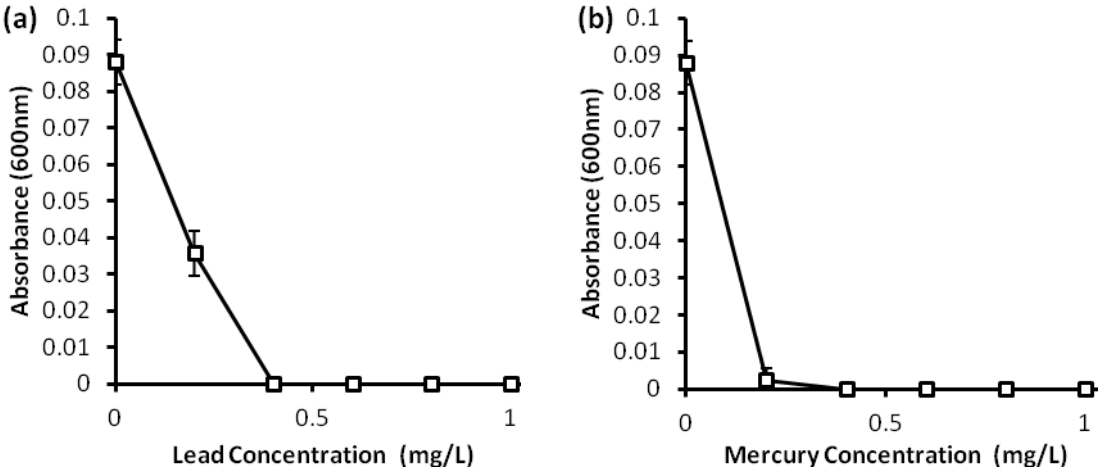

Fig. 7. Effect of Pb^{2+} (a) and Hg^{2+} (b) concentrations on the growth of strain MY 83295F in *p,p'*-DDT(60 mgL^{-1}) enrichment medium. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

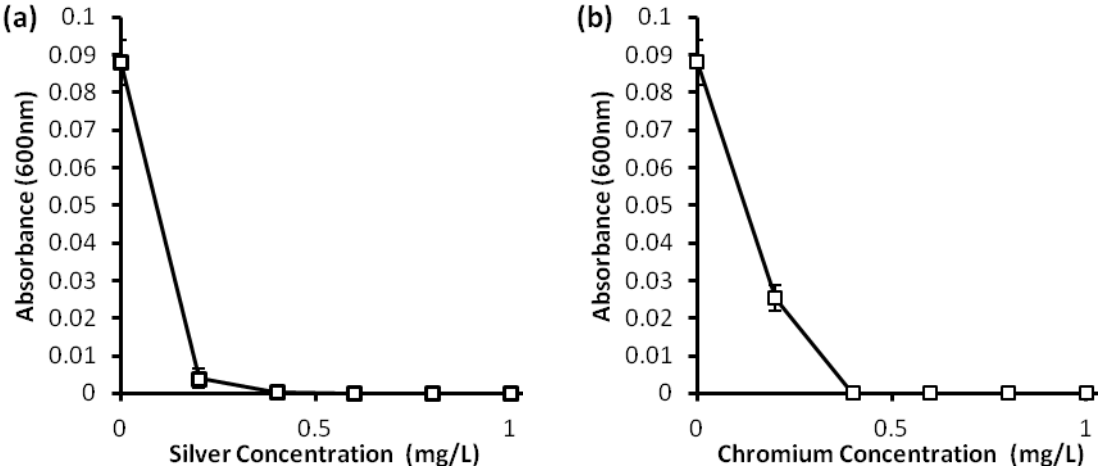

Fig. 8. Effect of Ag^{2+} (a) and Cr^{2+} (b) concentrations on the growth of strain MY 83295F in *p,p'*-DDT(60 mgL^{-1}) enrichment medium. The turbidity of the medium is an index of growth of the isolate in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}. The experinments were conducted in triplicate.

Conclusions

A strain MY 83295F of the genus *Staphylococcus* was isolated from pesticide-contaminated soil. The strain was capable of growing in up to 60 mg L^{-1} of *p,p'*-DDT as the sole carbon and energy source at an optimum pH of 6.5 and optimum temperature of 30 °C within 120 h. Zn^{2+} demonstrated a stimulatory effect on the growth of the strain in the *p,p'*-DDT enrichment medium. However, Fe, Cu, Pb, Hg, Ag and Cr ions showed various patterns of an inhibitory effect on the growth of the strain. Thus, a simultaneous incidence of *p,p'*-DDT and the inhibitory heavy metals in same environment may alter the *p,p'*-DDT biodegradation potentiality of the strain. This strain could be a handy tool for the bioremediation of residual *p,p'*-DDT contaminant.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abdul Kader M (2019) Domination of pollutant residues among food products of South-East Asian countries. Acta Sci Pharm Sci 3(9):75–79.
2. Aislabie JM, Richards NK, Boul HL (1997) Microbial degradation of DDT and its residues-a Review. New Zealand J Agric Res 48:269–282.
3. Angle JS, Chaney RL (1989) Cadmium resistance screening in nitrilotriacetate-buffered minimal media, Appl Environ Microbiol 55:2101–2104.
4. Asogwa EU, Dongo LN (2009) Problems associated with pesticide usage and application in nigerian cocoa production: a review. African J Agric Res 4:675–683.
5. Baczynski TP, Pleissner D, Grotenuis T (2010) Anaerobic biodegradation of organochlorine pesticides in soil - significance of temperature and availability. Chemosphere 78:22–28.
6. Bao P, Hu ZY, Wnag XJ, Chen J, Ba YX, Hua J, Zhu CY, Zhong M, Wu CY (2012) Dechlorination of p,p'-DDTs coupled with sulfate reduction by novel sulfate- reducing bacterium Clostridium sp. BXM. Environ Pollut 162:303–310.
7. Barragan-Huerta BE, Costa-Pe´rezc C, Peralta-Cruza J, Barrera-Cortes J, Esparza-Garcı´a F, Rodrı´guez-Va´zquez R (2007) Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green bean coffee. Inter Biodeter Biodeg 59:239–244.
8. Bascomb S, Manafi M (1998) Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic Gram-positive cocci. Clin Microbiol Rev 11:318–340.
9. Bidlam R, Manonmani HK (2002) Aerobic Degradation of Dichlorodiphenyl-trichloroethane (DDT) by Serratia marcescens DT-1P. Pro Biochem 38:49–56.
10. Bussolaro D, Filipak Neto F, Glinksi A, Roche H,Guiloski IC, Mela M, Silva de Assis HC, Oliveira Ribeiro CA (2012) Bioaccumulation and related effects of pcbs and organochlorinated pesticides in freshwater fish Hypostomus commersoni. J Environ Monit 14(8):2154–2163.
11. Cohn BA, Cirillo PM, Terry MB (2019) DDT and breast cancer: prospective study of induction time and susceptibility windows. J Nat Cancer Inst 111(8):803–810.
12. Cutright TJ, Erdem Z (2012). Overview of the bioremediation and the degradation pathways of DDT: Review. J Adnan Menderes Univ Agric Faculty, 9(2):39–45.
13. Devi NL, (2020) Persistent Organic Pollutants (POPs): environmental risks, toxicological effects, and bioremediation for environmental safety and challenges for future research. In: Saxena G., Bharagava R. (eds) bioremediation of industrial waste for environmental safety. pp 53–76 Springer, Singapore
14. Fang H, Dong B, Yan H, Tang F, Yu Y (2010) Characterization of a bacterial strain capable of degrading DDT congeners and its use in bioremediation of contaminated soil. J Haz Mat 184:281–289.
15. Gao B, Leu W, Jia WB, Jia LJ., Xu L, Xie J (2011) Isolation and characterization of an *Alcaligenes sp*. Strain DG-5 capable of degrading DDTs under aerobic conditions. J Environ Sci Health Part B 46:57–263.
16. Ghebremedhin B, layer F, Konig W, Konig B (2008) Genetic classification and distinguishing of Staphylococcus species

based on the different partial gap, 16S rRNA, *hsp60*, *rpoB*, *sodA* and *tuf* gene sequences. J Clin Microbiol 46:1019–1025.
17. Guffanti AA, Wei Y, Rood SV, Krulwich TA (2002) An antiport mechanism for a member of the cation di!usion facilitator family: divalent cations efflux in exchange for K^+ and H^+. Mol Microbiol 45:145–153.
18. Hadara T, Takeda M, Kojima S, Tomiyama N (2016) Toxicity and carcinogenicity of dichlorodiphenyltrichloroethane (DDT). Toxicol Res 32(1):21–33.
19. Hong S, Farrence CE (2015) Is it essential to sequence the entire 16S RRNA gene for bacterial identification? American Pharm Rev 18(7):1–7.
20. Hu X, Li S, Cirillo P, Krigbaum N, Tran V, Ishikawa T, La Merill M.A, Jones DP Cohn B (2019) Metabolome wide association study of serum DDT and DDE in pregnancy and early postpartum. Rep Toxicol pil: S0890-6238(18):30588-4.
21. Hug LA, Maphosa F, Leys D, Loffler FE, Smidt H, Edwards EA, Adrian L (2013) Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. Phil Transact Royal Soc B 368(1616):20120322.
22. Jill E, Clarridge III (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev 17(4):840–862.
23. Kantachote D, Singleton I, McClure N, Naidu R, Megharaj M, Harch BD (2003) DDT resistance and transformation by different microbial strains isolated from DDT-contaminated soils and compost materials, Compost Sci Util 11:300–310.
24. Karmaker A, Dua P, Ghosh C (2016) Biochemical and molecular analysis of Staphylococcus aureau clinical isolate from hospitalized patients. Canadian J Infect Disease Med Microbiol 2016(3):1–7.
25. Khattak MO, Bilal M, Rizwan M, Ahmad S, Meer A, Ullah I (2015) The sensitivity of different phenotypic tests used for detection of *Staphylococcus* aureus in the coagulase test. J Med Sci (Peshawar) 23(3):125–129.
26. Lovecka P, Pacovska I, Stursa P, Vrchotova B, Kochankova L, Demnerova K (2005) Organochlorinated pesticide degrading microorganisms isolated from contaminated soil. New Biotechnol 32(1):26–31.
27. Mansouri A, Cregut M, Abbes C, Durand MJ, Landoulsi A, Thouand G (2017) The environmental issues of DDT pollution and bioremediation: a multidisciplinary review. Appl Biochem Biotechnol 181(1):309–339.
28. Medehouenou TCM, Ayotte P, Carmichael PH, Kröger E, Verreault R, Lindsay J et al, (2019) Exposure to polychlorinated biphenyls and organochlorine pesticides and risk of dementia, alzheimer's disease and cognitive decline in an older population: a prospective analysis from the Canadian study of health and aging. Environ Health, 18(1):57.
29. Mendes RA, Lima MO, de Deus RJA, Medeiros AC, Faial KCF, Jesus IM, Faial KRF, Santos LS (2019) Assessment of DDT and mercury levels in fish and sediments in the Iriri River, Brazil: distribution and ecological risk, J Environ Sci Health B 9:1–10.
30. Mitra S, Roy P (2010) Molecular identification by 16S rDNA sequence of a novel bacterium capable of degrading trichloroethylene. J Biol Sci 10:637–642.
31. Mnif W, Hassine AH, Bouaziz A, Bargeti A,Thomas O, Roig B (2011) Effect of Endocrine Disruptor Pesticides: A Review. Inter J res Pub Health 8:2236–2303.
32. Murata T, Kanao-Koshikawa M, Takamatsu T (2005) Effects of Pb, Cu, Sb, In and Ag Contamination on the

Proliferation of Soil Bacterial Colonies, Soil Dehydrogenase Activity, and Phospholipid Fatty Acid Profiles of Soil Microbial Communities. Water, Air Soil poll 164:103–118.

33. Mwangi K, Boga HI, Muigai AW, Kiiyukia C, Tsanuo MK (2010) Degradation of Dichlorodiphenyltrichloroethane (DDT) by Bacterial Isolates from Cultivated and Uncultivated Soil. African J Microbiol Res 4 (3):185–196.
34. Nadeau LJ, Sayler GS, Spain, JC (1998). Oxidation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) Ethane (DDT) by *Alcaligenes eutrophus* A5. Arch Microbiol 171:44–49.
35. Naushad S, Barkema HW, Luby C, Condas LAZ, Nobrega DB, Carson DC, Buck, JD (2016) Comprehensive phylogenetic analysis of bovine non-aureus *Staphylococci* species based on whole-genome sequencing. Front Microbiol 7:2016.01990.
36. Nies DH **(**1999) Microbial Heavy-Metal Resistance. Appl Microbiol Biotechnol 51:730–750.
37. Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27: 313–339.
38. Ogbeide O, Tongo I, Ezemonye L (2015) Risk Assessment of Agricultural Pesticides in Water,Sediment, and Fish from Owan River, Edo state, Nigeria, Environ Monit Assess 187:654–666.
39. Pan X, Lin D, Zheng Y, Zhang Q, Yin,Y, Cai L, Fang H, Yu Y (2016). Biodegradation of DDT by *Stenotrophomonas* sp. DDT-1: characterization and genome functional analysis. Sci Reports, 6:21332.
40. Pan X, Xu T, Xu H, Fang H, Yu Y (2017) Characterization and genome functional analysis of the DDT-degrading bacterium *Ochrobactrum* sp. DDT-2, Sci Total Environ 592:593–599.
41. Pant G, Mistry SK, Sibi G (2013). Isolation, identification and characterization of p, p-DDT degrading bacteria from soil. J Environ Sci Technol 6(8):180–187.
42. Piazza MJ, Urbanetz AA (2019) Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. JBRA Assisted Rep 23(2):154–164.
43. Reineke W, Mandt C, Kaschabek SR Pieper DH (2011) Chlorinated Hydrocarbon Metabolism. In: eLS. John Wiley and Sons, Ltd: Chichester. pp. 1-17.
44. Roy RP, Bahadur M, Barat S (2013) Isolation, identification and antibiotic resistance of *Aeromonas* spp. and *Salmonella* spp. from the fresh water loach, *Lepidocephalichthys guntea* and water of Terai River Lotchka, West Bengal, India. Zoologica Poloniae 58:5–17.
45. Sandrin TR, Maier RM (2003) Impact of metals on the biodegradation of organic pollutants**.** Environ Health Pers 111(8): 1093–1101.
46. Sanger F, Nicklen S, Coulson AR (1977) DNA Sequencing with chain-terminating inhibitors. Biochem 74(12):5463–5467.
47. Sangwan P, Kovac S, Kathryn ERD, Sait M, Peter HJ (2005) Detection and cultivation of soil Verrucomicrobia. Appl Environ Microbiol 2005:8402–8410.
48. Saruta K, Matsunaga T, Kono M, Hoshina S, Ikawa S, Sakai O, Machida K (1997) Rapid identification and typing of Staphylococcus aureus by nested PCR amplified ribosomal DNA spacer region. FEMS Microbiol Letters, 146:271–278.
49. Schmidt TM, Delong EF, Pace NR (1991). Analysis of a marine picoplankton community by 165 rRNA gene cloning and sequencing. J Bacteriol 178:4871–4878.

50. Sheldon M, Pinion JC, Klyza J, Zimeri A (2019) Pesticide contamination in Central Kentucky urban honey: a pilot study. J Environ Health 82(1):8–13.
51. Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Inter J Sys Bacteriol 44:846–849.
52. Suryawati B (2018) Zinc homeostasis mechanism and its role in bacterial virulence capacity. The 8th *Annual Basic Science International Conference. AIP Conference Proceedings*, Universitas Brawijaya, Indonesia. 2021:070021-1-070021-7.
53. Takahashi T, Satoh I, Kikuchi N (1999) Phylogenetic relationships of 38 taxa of the genus *Staphylococcus* based on 16S rRNA gene sequence analysis. Inter J Sys Bacteriol 49:725–728.
54. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30(12):2725–2729.
55. Truong KM, Cherednichenko G, Pessah IN (2019) Interactions of dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) with skeletal muscle ryanodine receptor type 1. Toxicol Sci 170(2):509–524.
56. UNEP, (2019) DDT expert group and its report on the assessment of scientific, technical, environmental and economic information on the production and use of DDT and its alternatives for disease vector control. *Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants Ninth meeting*, Geneva, 29 April-10 May 2019.
57. Wang B, Liu W, Liu X, Franks AE, Teng Y, Luo Y (2017) Comparative analysis of microbial communities during enrichment and isolation of DDT-degrading bacteria by culture-dependent and – independent methods. Sci Total Environ 590–591:297–303.
58. Xie H, Zhu L, Wang, J (2018) Combined treatment of contaminated soil with a bacterial *Stenotrophomonas* strain DXZ9 and ryegrass (*Lolium perenne*) enhances DDT and DDE remediation. Environ Sci Pollut Res 25:31895–31905.
59. You G, Sayles GD, Kupferle MJ, Kim IS, Bishop PL (1996) Anaerobic DDT biotransformation: enhancement by application of surfactants and low oxidation reduction potential. Chemosphere 32:2269–2284.
60. Yu X, Yu RQ, Zheng X, Zhan F, Sun X, Wu Y (2019) DDT exposure induces cell cycle arrest and apoptosis of skin fibroblasts from Indo-Pacific Humpback dolphin via mitochondria dysfunction. Aqua Toxicol 213:105229.

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PROTECTIVE EFFECT OF NATURALLY-DERIVED ANTIOXIDANTS AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY: A REVIEW

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Abstract: Acetaminophen (APAP) is a commonly used over-the-counter (OTC) drug known to induce hepatotoxicity when consumed in excess. Formation of reactive oxygen species (ROS) and oxidation of cellular proteins and enzymes are directly involved in its toxic mechanisms. However, antioxidants can be helpful to inhibit or restrict the oxidative damage. Besides synthetic antioxidants, naturally-derived substances can be used to serve the purpose. In this paper, a thorough literature review revealed that APAP combined with food-derived natural antioxidants exhibit a protective effect against APAP-induced hepatotoxicity.

Keywords: acetaminophen, chemoprevention, hepatotoxicity, free radical, natural antioxidants, redox homeostasis.

Introduction

Acetaminophen (APAP) is a well-known and frequently used antipyretic and analgesic drug by people all over the world (Bessems et al., 2001; Dargan & Jones, 2002; James et al., 2003; Yang et al., 2013). It is normally prescribed to relieve conditions such as mild to moderate pain from headaches, muscle aches, toothaches, backaches, menstrual cramp, common colds, sore throats, reaction to vaccines and fever reduction (Medline Plus drug information). At therapeutic dose, acetaminophen is conjugated to glucuronic acid and eliminated in bile as part of metabolism. Nevertheless, 7.0 mg/day for adults and 150 mg/kg for children are considered to be toxic for the liver (Hazai et al., 2002; Kon et al., 2007).

The liver is one of the most vital organs of the body, carrying out over 500 functions including metabolism of ingested substances and detoxification of toxic substances (Almeer et al., 2018). Extensive use of acetaminophen in thousands of prescriptions and over-the-counter drugs has escalated the risk of hepatotoxicity (Larson, 2007; Hinson et al., 2010).

Though the exact mechanism of APAP-induced liver injury is yet unclear, the idea is that hepatotoxicity starts from the moment its metabolic activation is set in motion. About 95% of the therapeutic dose is converted into inactive metabolites however, CYP1A2, CYP2E1 coverts the rest of the dose into a toxic metabolite named N-acetyl-*p*-benzo-quinone imine (NAPQI) (Corcoran et al., 1985; Esterline et al., 1989; Nelson, 1990; James et al., 2003) (**Fig. 1.**). In some cases involving APAP overdose, alcohol abuse, hepatic impairment, and starvation, a low reduced glutathione (GSH) level is seen which exponentially multiplies the pernicious effect of NAPQI. As the GSH level is always low, the NAPQI targets cytosolic and mitochondrial proteins and lipids and disrupts the function of several pro (Bcl-Xs, Bad, Bax, Bid, BAK, BIM) and anti-cell death (Bcl-2, Bcl-XL) genes. This series of incidences gradually leads to mitochondrial dysfunction and results in hepatotoxicity and hepatocellular death (Ray & Corcoran, 2009; Ghosh et al., 2010; Jaeschke et al., 2012; McGill et al., 2012). NAPQI on the other hand can also increase the formation of reactive oxygen species (ROS) that includes-superoxide ions, hydrogen peroxide, and hydroxyl radical leading to lipid peroxidation and decreased antioxidant enzymes (Michael et al., 1999; Hinson et al., 2002; Hinson 2004).

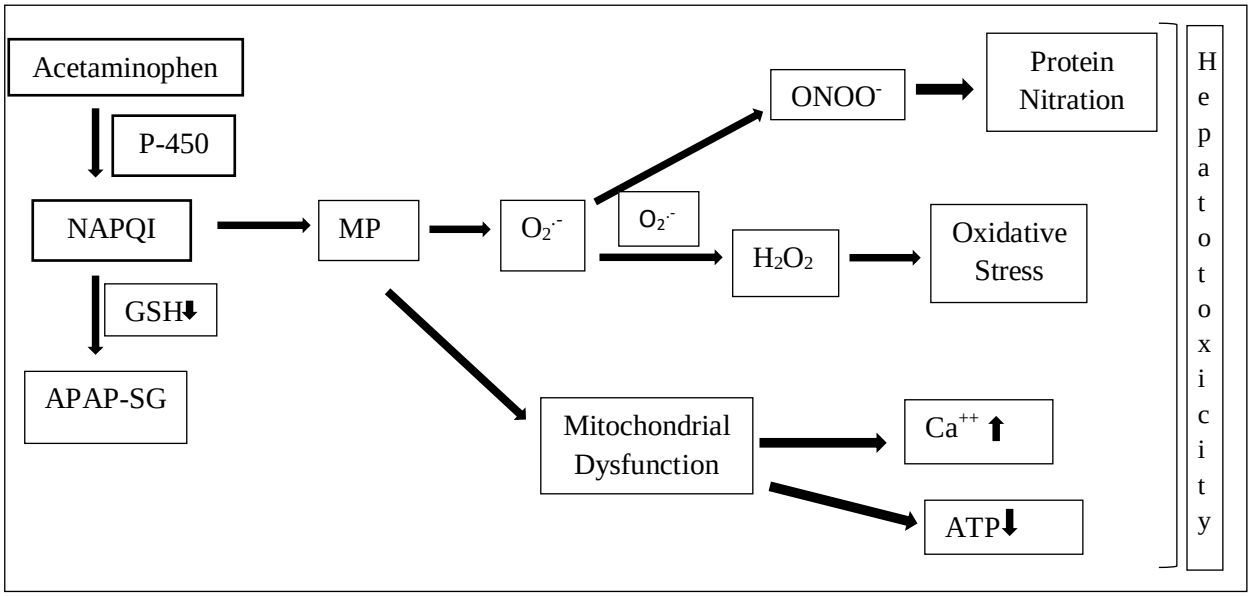

Fig. 1. Acetaminophen (APAP) metabolism and induction of hepatotoxicity

As the redox reaction is directly involved with hepatotoxicity, antioxidants application could be a potent alternative. Antioxidants are generally known as substances or compounds that have free radical scavenging capacity while inhibiting oxidative progression (Antioxidants: In Depth. NCCIH. 2010). Different naturally-derived compounds like organosulfur compounds, triterpenoids, sulphoraphae, resveratrol, saponins, lipids and different acids are more likely to act as potent antioxidants (Adeyemi et al., 2018; Atolani et al., 2019; 2020; Wang et al., 1996; Kumari & Kakkar, 2012; Noh et al., 2015; Du et al., 2015; Xu et al., 2017; Pang et al., 2016; Elshazly et al., 2014). These compounds generally inhibit the acetaminophen-induced oxidative reaction in the liver and confer hepatoprotection. This review aims to enlighten the capacity of

naturally-derived antioxidants against APAP-induced hepatotoxicity.

Hepatoprotective activity of naturally-derived antioxidants:

Several naturally derived substances showed great antioxidant activity when tested on animals with APAP-induced liver toxicity. Kumari & Kakkar (2012) claimed in their study that, lupeol (150 mg/kg), a naturally occurring triterpenoid derived from olive, mango, crataeva, strawberry, and fig reduced oxidative damage by scavenging free radicals and prevented alteration in the antioxidant defense, inhibited depolarization of the mitochondria, prevented down-regulation of Bcl-2, up-regulation of Bax and activation of caspases, prevented DNA damage and cell death in rats (**Fig. 2.**). Honey is another natural substance derived from the floral nectar that prevented an increase of the serum levels of hepatic enzyme markers, reduce both oxidative stress and inflammatory cytokines thus confirming hepatoprotection (Galal et al., 2012). Different acids derived from natural sources also have great antioxidant activity when tested against APAP-induced hepatotoxicity. Arjunolic acid (AA) found in the bark of *Terminalia arjuna*potentially inhibited P450-mediated APAP bio-activation, and c-Jun N-terminal kinase (JNK)-mediated activation of mitochondrial permeabilization (Ghosh et al., 2010). Alpha-Lipoic acid another naturally derived compound is found to have hepatoprotective activity at a dose of 20 or 100 mg/kg (Elshazly et al., 2014). Caffeic acid generally found in coffee decreases Kcap 1 expression, inhibits binding of Kcap 1 to nuclear factor erythroid 2-related factor 2 (Nrf2) which activates Nrf2, increases expression of anti-oxidative signals including heme oxygenase-1 (HO-1), NAD(P)H Quinone Dehydrogenase 1 (NQO1), thus protects the liver from APAP-induced toxicity (Pang et al., 2016).

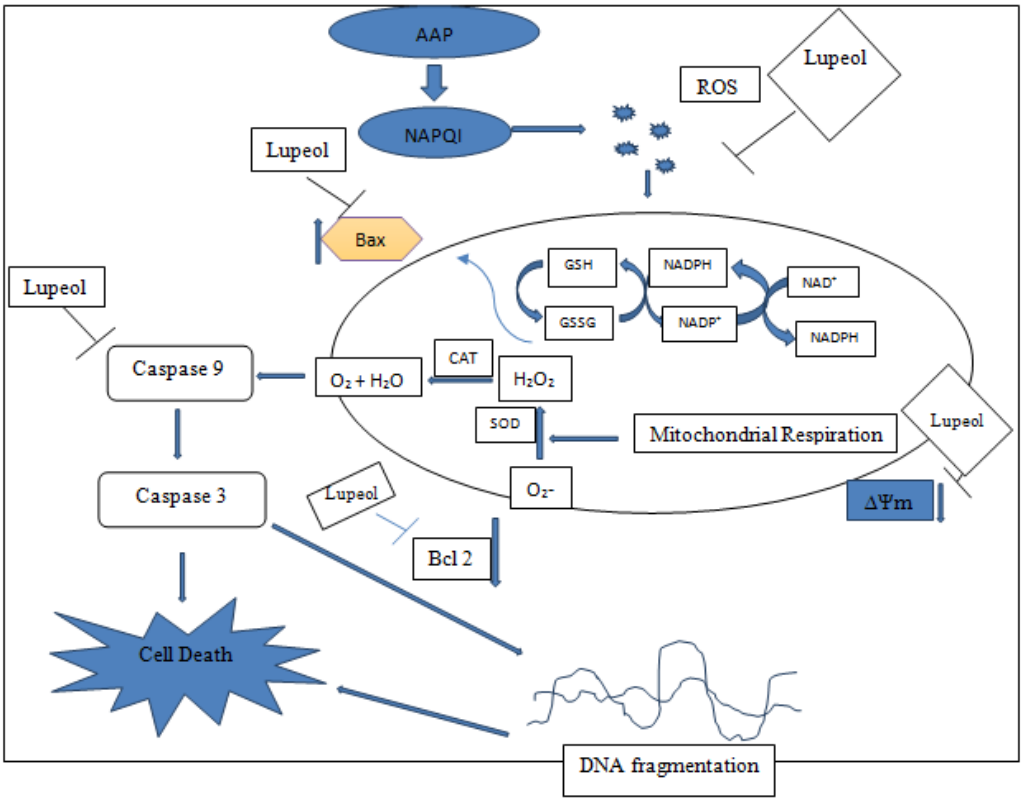

Fig. 2. Protective effect of lupeol in acetaminophen induced hepatotoxicity

Extracts of different fruits and plants are reported to have free-radical scavenging capacity. Yen et al (2008), reported that ethanolic extract of *Cuscuta chinensis* (CE) and nanoparticles are potential antioxidants as they enhanced antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and diminished lipid peroxidation (MDA) that resulted in hepatoprotective and antioxidant activity. Polyphenol extract of *Hibiscus sabdariffa* L. (HPE) at a dose of 100, 200, or 300 mg/kg reduced APAP-induced death of BABL/c in normal liver cells (BNLs), restored lost mitochondrial potency, and improved anti-oxidative status (Lee et al., 2012). Polyphenol enriched fraction from *Folium Microcos* (FM) also acts as a great antioxidant and safeguards the liver (Wu et al., 2017). A detailed summary of the hepatoprotective tendencies of some naturally derived molecules is presented in **Table 1**.

Table 1. Some reported hepatoprotective potencies of naturally-derived antioxidants

Antioxidants	**Sources**	**Dose/ concentration (R/A)**	**Protective effect (possible mechanism of action)**	**Reference**
Fresh garlic homogenates (FGH) and related organosulfur compounds	Garlic bulbs	2.5 or 5.0 g/kg	Inhibited P450 2E1-mediated APAP bio-activation displays protective activity.	Wang et al., 1996
Cuscuta chinensis ethanolic extract (CE) &*Cuscuta chinensis* nanoparticles (CN)	Seeds of *Cuscuta chinensis* Lam. (Convolvulaceae)	125 & 250 mg/kg for CE, 25 & 50 mg/kg for CN	Enhanced antioxidant enzymes (SOD, CAT, GPx), and diminished lipid peroxidation (MDA) results in hepato-protective and antioxidant activity.	Yen et al., 2008
Sesamol	Sesame oil	10 mg/kg, i.p.	Maintained mitochondrial aconitase activity in the liver, ferrous ions (Fe^{2+}), hydrogen peroxide levels, and inhibited hydroxyl-radical-associated lipid peroxidation and hepatic injury.	Chandrasekan et al., 2009
Spirulina fusiformis	*Spirulina fusiformis*	800 mg/kg/b. wt	Decreased liver marker enzymes activity, tumor necrosis factor-alpha (TNF-α), and lipid peroxidation level with increased antioxidant status.	Sabina et al., 2009
Anthocyanin fraction (AF)	Purple fleshed sweet potato	800 mg/kg	Blocked bio-activation of APAP by inhibiting CYP2E1 activity, up-regulated GSH level, and glutathione (GST)	Choi et al., 2009

			activity which increases free radical scavenging capacity and inhibits lipid peroxidation.	
Arjunolic acid (AA)	Bark of *Terminalia arjuna*	80 mg/kg	Inhibited P450-mediated APAP bio-activation and JNK-mediated activation inhibition of mitochondrial permeabilization.	Ghosh et al., 2010
Curcumin (CUR; diferuloylmethane)	*Curcuma longa*	17 mg/kg	Blockage of APAP induced oxidative stress by decreasing the several pro-injury parameters (alanine aminotransferase (ALT), nitrate/ nitrite levels, lipid peroxidation, DNA fragmentation), and several protective parameters (GSH content, SOD activity).	Bulku et al., 2012
Honey	Honey	5, 10 and 20 g/kg	Prevention of increase in the serum levels of hepatic enzyme markers, reduction in both oxidative stress and inflammatory cytokines.	Galal et al., 2012
Polyphenol extract of *Hibiscus sabdariffa* L. (HPE)	*Hibiscus sabdariffa L.*	100, 200 or 300 mg/kg	Reduced APAP-induced death of BABL/c in normal liver cells (BNLs), restored lost mitochondrial potency, and improved anti-oxidative status.	Lee et al., 2012
Lupeol, a naturally occurring triterpenoid	Olive, mango, crataeva, strawberry, and fig.	150 mg/kg	Reduced oxidative damage by scavenging free radicals and preventing alteration in the antioxidant defense, inhibited depolarization of the mitochondria, prevented down-regulation of Bcl-2, up-regulation of Bax and activation of caspases, prevented DNA damage and cell death.	Kumari & Kakkar, 2012

Red ginseng extract	Roots of *Panax ginseng* C. A Meyer	10, 30, 100, 300, 500 mg/kg	Suppressed hepatotoxicity, suppressed hepatic CYP2E1 leading to high retention of intact APAP in plasma and GSTA2 gene induction with transcriptional activation of Nrf2 and/ or C/ EBPb downstream of multiple signaling pathways that facilitate conjugation of GSH with NAPQI.	Gum & Cho, 2013
Silymarin	*Silybum marianum* (milk thistle)	100 mg/kg	Inhibition of migrating neutrophils, protection against GSH depletion that elevates nitric oxide (NO) levels in tissue along with antioxidant and free radical scavenging properties.	Bektur et al., 2013
Ginger	*Zingiber officinale* Roscoe	100 mg/kg	Reduced hepatic marker enzymes (aspartate aminotransferase, serum alanine aminotransferase, and arginase) and total bilirubin in plasma, improved paracetamol (PARA)-induced oxidative stress by inhibiting lipid peroxidation malondialdehyde (MDA), restored triacylglycerols (TAGs), and total protein levels.	Abdel-Azeem et al., 2013
Apigenin	Parsley, onions, oranges, tea, and chamomile	100 and 200 mg/kg	Increased hepatic glutathione reductase (GR) activity, reduced GSH content, and decreased hepatic malondialdehyde content.	Yang et al., 2013
Glossogyne tenuifolia (GT) Cassini	*Glossogyne tenuifolia*	100 or 300 mg/kg	Decreased ALT, aspartate aminotransferase (AST) in serum, alleviated GSH depletion, and	Tien et al., 2014

			inhibited lipid peroxidation leads to free radical scavenging and antioxidant activity.	
Alpha-Lipoic acid	Naturally found in plants and animals.	20 or 100 mg/kg	Reduction in APAP-induced liver injury is seen by restoring the changes in ALT, total protein, GSH, MDA, GSH synthase, crystathionine β-synthase, NADPH oxidae, and nuclear factor kappa B (NF-κB) towards control value. Also improves the hepatic histopathology with an increase in the expression of HO-1, Nrf2.	Elshazly et al., 2014
Aloe vera	*Aloe vera*	150 mg/kg	Improved level of serum alanine aminotransferase, hepatic MDA, number of interleukin-12 (IL-12), and interleukin-18 (IL-18) positive-stained cells, and hepatic GSH along with improved liver histopathology.	Werawatgann et al., 2014
Sulphoraphae (SFN)	Cruciferous vegetables of the genus Brassica such as cauliflower, kale, broccoli, cole crops, cabbage, collards, brussels sprout, mustard, cress, and even radish.	5 mg/kg	Antioxidant activity against APAP-induced liver injuries by blocking ROS generation, GSH depletion, and lipid peroxidation followed by up-regulation of Nrf2-targeted cytoprotective genes such as HO-1.	Noh et al., 2015
Resveratrol	Skin of grape	50 mg/kg	Reduced hepatotoxicity by scavenging peroxynitrite and preventing apoptosis-inducing factor (AIF), EndoG release from mitochondria, and subsequent nuclear DNA fragmentation.	Du et al., 2015

Caffeic acid	Coffee, some fruits, and traditional Chinese medicines	10, 30 mg/kg	Decreased Kcap 1 expression, inhibited binding of Kcap 1 to Nrf2 which activates Nrf2, increased expression of anti-oxidative signals including HO-1, NQO1.	Pang et al., 2016
Black ginseng (BG)	Roots of *Panax ginseng* C. A Meyer	600 mg/kg	Decreased level of ALT, AST, decreased lipid peroxidation, increased hepatic antioxidants GSH, apoptotic pathway suppression by increasing Bcl-2, and decreasing Bax protein expression which resulted in inhibited APAP-induced necrosis and inflammatory infiltration in the liver tissue.	Hu et al., 2017
Saponins (ginsenosides)	Leaves of *Panax quinquefolius* (PQS)	150 and 300 mg/kg	Ameliorated oxidative stress via lipid peroxidation suppression, down-regulation of pro-inflammatory factors disrupted apoptotic signal pathway by Bcl-2 overexpression, and Bax low-expression, prevented caspase-3 release.	Xu et al., 2017
Polyphenol enriched fraction from *Folium Microcos* (FM)	Leaves of *Microcos paniculata* L.	100, 200 & 400 mg/kg body weight	Modified ROS/ mitogen-activated protein kinase (MAPK)/ apoptosis axis, and Nrf2-mediated antioxidant response by four phenolic compounds: narcissin, isorhamnetin-3-O-β-D-glucoside, isovitexin, and vitexin.	Wu et al., 2017
Nutmeg	Kernel extract of *Myristica fragrans*	300 mg/kg	Suppressed oxidative stress, inflammation, and apoptosis, promoted Nrf2/antioxidant responsive element	Dkhil et al., 2019

			(ARE) pathway which leads to hepatoprotection.	
Sonneratia apetala	*Sonneratia apetala*	100, 200 & 400 mg/kg	Decreased ALT, AST level in serum, reduced MDA in the liver, increased glutathione (GSH), glutathione peroxidase (GPx) activity, enhanced catalase and antioxidant capacity, and inhibited TNF-α, IL-6, myeloperoxidase (MPO) formation in liver inhibits APAP induced liver injury.	Liu et al., 2019

Conclusions

The liver is a major organ of our body that performs several functions including metabolism and detoxification of substances. Acetaminophen is a globally used drug for different health issues. Exceeding the therapeutic level, a higher dose may often induce severe toxicity in the liver. Researchers from time to time have discovered the direct connection of redox reaction with hepatotoxicity. However, this situation can be controlled by using antioxidants that scavenge free radicals produced during the oxidative reaction. As enumerated in this review, antioxidants derived from natural sources like saponins, acids, triterpenoids, polyphenols seem to have a significant effect against APAP-induced hepatotoxicity.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Abdel-Azeem AS, Hegazy AM, Ibrahim KS, Farrag ARH, El-Sayed EM (2013) Hepatoprotective, antioxidant, and ameliorative effects of ginger (*Zingiber officinale* Roscoe) and vitamin E in acetaminophen treated rats. Journal of dietary supplements 10(3):195–209.
2. Almeer RS, Alarifi S, Alkahtani S, Ibrahim SR, Ali D, Moneim A (2018) The potential hepatoprotective effect of royal jelly against cadmium chloride-induced hepatotoxicity in mice is mediated by suppression of oxidative stress and upregulation of Nrf2 expression. Biomedicine & Pharmacotherapy 106:1490–1498.
3. Adeyemi OS, Atolani O, Banerjee P, Arolasafe G, Preissner R, Etukudoh P, Ibraheem O (2018) Computational and experimental validation of antioxidant properties of synthesized bioactive ferulic acid derivatives. International Journal of Food Properties 21(1):86–88.
4. Antioxidants: In Depth. NCCIH (2010) Archived from the original on 25 August 2018. Retrieved 20 June 2018.

5. Atolani O, Areh ET, Oguntoye OS, Zubair MF, Fabiyi OA, Oyegoke RA, Tarigha DE, Adamu N, Adeyemi OS, Kambizi L, Olatunji GA (2020) Chemical characterization, antioxidant, cytotoxicity, anti-toxoplasma gondii and antimicrobial potentials of the *Citrus sinensis* seed oil for sustainable cosmeceutical production. Heliyon, 6(2):e03399.
6. Atolani O, Oguntoye H, Areh ET, Adeyemi OS, Kambizi L (2019) Chemical composition, anti-toxoplasma, cytotoxicity, antioxidant, and anti-inflammatory potential of *Cola gigantea* seed oil. Pharmaceutical Biology 57(1):154–160.
7. Bektur NE, Sahin E, Baycu C, Unver G (2016) Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. Toxicology and Industrial Health 32(4):589–600.
8. Bessems JG, Vermeulen NP (2001) Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical reviews in toxicology 31(1):55–138.
9. Bulku EJ, Stohs S, Cicero L, Brooks T, Halley H, Ray DS (2012) Curcumin exposure modulates multiple pro-apoptotic and anti-apoptotic signaling pathways to antagonize acetaminophen-induced toxicity. Current neurovascular research 9(1):58–71.
10. Chandrasekaran VRM, Hsu DZ, Liu MY (2009) The protective effect of sesamol against mitochondrial oxidative stress and hepatic injury in acetaminophen-overdosed rats. Shock 32(1):89–93.
11. Choi JH, Choi CY, Lee KJ, Hwang YP, Chung YC, Jeong HG (2009) Hepatoprotective effects of an anthocyanin fraction from purple-fleshed sweet potato against acetaminophen-induced liver damage in mice. Journal of medicinal food 12(2):320–326.
12. Corcoran GB, Racz WJ, Smith CV, Mitchell JR (1985) Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. Journal of Pharmacology and Experimental Therapeutics 232(3):864–872.
13. Dargan P, Jones A (2002) Paracetamol: balancing risk against benefit. Qjm 95(12):831–832.
14. Dkhil MA, Abdel Moneim AE, Hafez TA, Mubaraki MA, Mohamed WF, Thagfan FA, Al-Quraishy S (2019) Myristica fragrans kernels prevent paracetamol-induced hepatotoxicity by inducing anti-apoptotic genes and Nrf2/HO-1 pathway. International journal of molecular sciences 20(4):993.
15. Du K, McGill MR, Xie Y, Bajt ML, Jaeschke H (2015) Resveratrol prevents protein nitration and release of endonucleases from mitochondria during acetaminophen hepatotoxicity. Food and Chemical Toxicology 81:62–70.
16. Elshazly SM, El-Moselhy MA, Barakat W (2014) Insights in the mechanism underlying the protective effect of α-lipoic acid against acetaminophen-hepatotoxicity. European journal of pharmacology, 726:116–123.
17. Esterline RL, Ray SD, Ji S (1989) Reversible and irreversible inhibition of hepatic mitochondrial respiration by acetaminophen and its toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI). Biochemical pharmacology 38(14):2387–2390.
18. Galal RM, Zaki HF, Seif ENMM, Agha AM (2012) Potential protective effect of honey against paracetamol-induced hepatotoxicity. Archives of Iranian medicine 15(11):674–80.

19. Ghosh J, Das J, Manna P, Sil PC (2010) Arjunolic acid, a triterpenoid saponin, prevents acetaminophen (APAP)-induced liver and hepatocyte injury via the inhibition of APAP bioactivation and JNK-mediated mitochondrial protection. Free Radical Biology and Medicine 48(4):535–553.
20. Gum SI, Cho MK (2013) Korean red ginseng extract prevents APAP-induced hepatotoxicity through metabolic enzyme regulation: The role of ginsenoside Rg3, a protopanaxadiol. Liver International 33(7):1071–1084.
21. Hazai E, Vereczkey L, Monostory K (2002) Reduction of toxic metabolite formation of acetaminophen. Biochemical and Biophysical Research Communications 291(4):1089–1094.
22. Hinson JA, Bucci TJ, Irwin LK, Michael SL, Mayeux PR (2002) Effect of inhibitors of nitric oxide synthase on acetaminophen-induced hepatotoxicity in mice. Nitric Oxide 6(2):160–167.
23. Hinson JA, Reid AB, McCullough SS, James LP (2004) Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. Drug metabolism reviews 36(3-4):805–822.
24. Hinson JA, Roberts DW, James LP (2010) Mechanisms of acetaminophen-induced liver necrosis. In: Adverse drug reactions, Springer, Berlin, Heidelberg. pp 369–405.
25. Hu JN, Liu Z, Wang Z, Li XD, Zhang LX, Li W, Wang YP (2017) Ameliorative effects and possible molecular mechanism of action of black ginseng (*Panax ginseng*) on acetaminophen-mediated liver injury. Molecules 22(4):664.
26. Jaeschke H, Williams CD, Ramachandran A, Bajt ML (2012) Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. Liver International 32(1):8–20.
27. James LP, Mayeux PR, Hinson JA (2003) Acetaminophen-induced hepatotoxicity. Drug metabolism and disposition 31(12):1499–1506.
28. Kon K, Ikejima K, Okumura K, Aoyama T, Arai K, Takei Y, Sato N (2007) Role of apoptosis in acetaminophen hepatotoxicity. Journal of gastroenterology and hepatology 22:S49–S52. doi: 10.1111/j.1440-1746.2007.04962.x
29. Kumari A, Kakkar P (2012) Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. Life sciences 90(15-16):561–570.
30. Larson AM (2007) Acetaminophen hepatotoxicity. Clinics in liver disease 11(3):525–548.
31. Lee CH, Kuo CY, Wang CJ, Wang CP, Lee YR, Hung CN, Lee HJ (2012) A polyphenol extract of *Hibiscus sabdariffa* L. ameliorates acetaminophen-induced hepatic steatosis by attenuating the mitochondrial dysfunction in vivo and in vitro. Bioscience, biotechnology, and biochemistry 76(4):646–651.
32. Liu J, Luo D, Wu Y, Gao C, Lin G, Chen J, Su Z (2019) The protective effect of *Sonneratia apetala* fruit extract on acetaminophen-induced liver injury in mice. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2019/6919834
33. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H (2012) The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. The Journal of clinical investigation 122(4):1574–1583.

34. Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA (1999) Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology 30(1):186–195.
35. Nelson SD (1990) Molecular mechanisms of the hepatotoxicity caused by acetaminophen. In: Seminars in liver disease, Thieme Medical Publishers, Inc., Vol. 10, No. 04, pp 267–278.
36. Noh JR, Kim YH, Hwang JH, Choi DH, Kim KS, Oh WK, Lee CH (2015) Sulforaphane protects against acetaminophen-induced hepatotoxicity. Food and Chemical Toxicology 80:193–200.
37. Pang C, Zheng Z, Shi L, Sheng Y, Wei H, Wang Z, Ji L (2016) Caffeic acid prevents acetaminophen-induced liver injury by activating the Keap1-Nrf2 antioxidative defense system. Free Radical Biology and Medicine 91:236–246.
38. Ray SD, Corcoran GB (2009) Apoptosis and cell death. General and Applied Toxicology 247–312.
39. Sabina E, Samuel J, RajappaRamya S, Patel S, Mandal N, Pranatharthiiharan P, Mishra PP, Rasool M (2009) Hepatoprotective and antioxidant potential of *Spirulina fusiformis* on acetaminophen-induced hepatotoxicity in mice. IJIB 6(1):1–5.
40. Tien YH, Chen BH, Wang Hsu GS, Lin WT, Huang JH, Lu YF (2014) Hepatoprotective and anti-oxidant activities of *Glossogyne tenuifolia* against acetaminophen-induced hepatotoxicity in mice. The American journal of Chinese medicine 42(06):1385–1398.
41. Wang EJ, Li Y, Lin M, Chen L, Stein AP, Reuhl KR, Yang CS (1996) Protective effects of garlic and related organosulfur compounds on acetaminophen-induced hepatotoxicity in mice. Toxicology and applied pharmacology 136(1):146–154.
42. Werawatganon D, Linlawan S, Thanapirom K, Somanawat K, Klaikeaw N, Rerknimitr R, Siriviriyakul P (2014) Aloe vera attenuated liver injury in mice with acetaminophen-induced hepatitis. BMC complementary and alternative medicine 14(1):229.
43. Wu H, Zhang G, Huang L, Pang H, Zhang N, Chen Y, Wang G (2017) Hepatoprotective effect of polyphenol-enriched fraction from Folium Microcos on oxidative stress and apoptosis in acetaminophen-induced liver injury in mice. Oxid Med Cell Longev 2017:3631565.
doi: 10.1155/2017/3631565.
44. Xu XY, Hu JN, Liu Z, Zhang R, He YF, Hou W, Wang ZQ, Yang G, Li W (2017) Saponins (Ginsenosides) from the leaves of *Panax quinquefolius* ameliorated acetaminophen-induced hepatotoxicity in mice. Journal of agricultural and food chemistry 65(18):3684–3692.
45. Yang J, Wang XY, Xue J, Gu ZL, Xie ML (2013) Protective effect of apigenin on mouse acute liver injury induced by acetaminophen is associated with increment of hepatic glutathione reductase activity. Food & function, 4(6):939–943.
46. Yen FL, Wu TH, Lin LT, Cham TM, Lin CC (2008) Nanoparticles formulation of Cuscuta chinensis prevents acetaminophen-induced hepatotoxicity in rats. Food and chemical toxicology 46(5):1771–1777.
47. ***https://medlineplus.gov/druginfo/meds/a681004.html#:~:text=Acetaminophen%20is%20used%20to%20relieve,)%2C%20and%20to%20reduce%20fever.

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EFFECTS OF PRIMING WITH ASCORBIC ACID, L-CYSTEIN AND TRIACONTANOL ON GERMINATION OF RAPESEED (*BRASSICA NAPUS* L.)

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Abstract: The germination of seed batches of two rapeseed (*Brassica napus* L.) hybrids ('Hybrirock' and 'Factor') were investigated in response to priming in aqueous solutions of ascorbic acid (10 mM), L-cysteine (10 mM) and triacontanol (1 μM), respectively tap water (as control treatment). Investigations were focused on seed quality parameters, such as germination percentage, mean germination time, germination index, uniformity of germination, and seedling shoot and root growth. Germinated seeds were counted every 24 hours for a period of seven days, recording the final germination percentage (FGP), mean germination time (MGT), the coefficient of velocity of germination (CVG), germination rate index (GRI), germination index (GI), uncertanty (U), and synchrony (Z). In a separate trial fresh shoot- and root length, respectively the dry weight of the shoots and roots were also determined after a 14 day period. Germination tests were performed to examine the quality of seeds in response to different priming treatments. The results demonstrated that priming improved the germination parameters of seeds of both hybrids. Furthermore, the use of ascorbic acid, L-cysteine and triacontanol also enhanced seedling growth. The results may be used to rank seed lots by vigor, and decisions can be made regarding planting potential of each seed lot.

Keywords: seed priming, germination, rapeseed, ascorbic acid, triacontanol, L-cystein.

1. Introduction

Rapeseed or canola (*Brassica napus* L.) belongs to the Brassicaceae family, and it is the second most important oilseed crop of the world, with an oil content of 40% (Bhuiyan et al., 2019). Rapeseed is used as an oil plant for nutritional and industrial purposes, and also as protein crop for animal feed (Nesi et al., 2008).

Plants are regularly exposed to various adverse environmental conditions. The abiotic stresses (drought, salinity, frost, and high temperatures) adversely affect the plant growth and productivity. High-quality seeds have the capability to germinate under adverse growing conditions (Ozbay, 2018).

Rapid germination, high seed germination percentage, low mean germination time, respectively rapid and uniform seedling emergence are important aspects of canola production (Devaiah et al., 2007). Slow, delayed germination results in slow growth, and decreased number of normal seedlings (Walters, 1998).

Seed priming is an effective technique that can be used to enhance germination percentage and growth, and to achieve uniform plant stand and better yield in stress conditions (Kandil et al., 2018).

Poor and/or uneven germination followed by an unsynchronized seedling emergence are a major cause of losses in crop productions and yields (Dutta, 2018). Priming with nutrients is an innovative practice that holds positive influences in terms of enhanced nutrient supply. Plants with better nutrient supply possess greater potential to tolerate abiotic stress (Ashraf et al., 2018).

Priming with ascorbic acid, an important antioxidant and a promising priming agent (Jisha et al., 2013), showed significant effects on germination percentage, shoot- and root length, vigor index in case of rapeseed plants in drought conditions (Razaji et al., 2014).

Triacontanol, a long-chain aliphatic alcohol was found in 1933 (Ries et al.) as a component of the epicuticular waxes of alfalfa (*Medicago sativa* L.). Since then, its growth regulating effects have been shown in several greenhouse and field experiments and a number of attempts were made to clarify its mode of action as well. Growth stimulating effects of triacontanol in the micropropagation process of *Melissa officinalis* L. and fruit rootstocks are already demonstrated (Tantos et al., 1999).

Cellular redox state regulates important processes that mediate growth and development under stressful environment (Miller et al., 2009). Sulfur (S) is a key ameliorating agent for reducing the effect of heavy metals in plants. It is one of the essential micronutrients that regulate photosynthesis under normal and stress condition (Saha et al., 2019). Sulfur forms the basic structural component for certain amino acids e.g cysteine and methionine (Khan et al., 2014). Seed priming with cysteine alleviated injury due to gamma radiation, and caused more conspicuous effects in the elongation of primary roots (Reddy and Smith, 1978).

Many experiments investigated the effects of seed treatments on germination parameters. Ansari et al. (2013) and Seiadat et al. (2012) reported that priming improves germination parameters in many crops.

The result is measured in terms of the extent to which seeds have germinated. But not just the final germination percentage attained is of great interest, but also the mean germination time, germination index, germination rate index, coefficient of velocity of germination, synchrony, and uncertainty are often used to judge the agronomic relevance of treatments (Al-Mudaris, 1998).

Various parameters for measuring or estimating germination speed have been developed. Mean germination time can be used as an indicator of seed vigor in canola (Amirmoradi and Feizi, 2017). The germination index (GI) emphasizes on both the percentage of germination and its speed. A higher GI value denotes a better percentage and rate of germination (Kader, 2005). The velocity of germination (CVG) coefficient indicates the rapidity of germination (Al-Mudaris, 1998). Higher germination rate index (GRI) values indicate higher and faster germination (Kader, 2005).

Based on germination characters and seedling growth parameters it can be concluded which varieties are more tolerant, under salinity stress. Seedling growth parameters as shoot and root length, fresh weights, and dry weights, were affected significantly by the interaction

between cultivars and salinity concentrations (Kandil et al., 2016).

The aim of the study was to test priming with ascorbic acid, L-cysteine, and triacontanol solution, at very low (millimolar/ppm) concentration, on germination and seedling growth processes of two rapeseed hybrids in order to optimize the rapeseed plants growth.

2. Materials and Methods

The experiment was performed in the laboratory of Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Târgu-Mureș, in 2019.

2.1 Seed materials

Seeds of two rapeseed (*Brassica napus* L.) hybrids, (Hybrirock and Factor) provided by KWS Romania were used in this study.

2.2 Priming

Priming was made with the following substances:

1. Ascorbic acid ≥99.0% (ASA) was purchased from VWR,
2. L-Cysteine 97.0% (L-cyst) was purchased from Merck,
3. Triacontanol 2.5% (Tria) was purchased from Nutri-Tech-Solution.

During the experiment batches of 25 seeds each, in four replications were used. The seeds were immersed prior to the start of the germination trial for 24 hours at 24 ºC in the priming solutions of ascorbic acid (10 mM), L-cysteine (10 mM) or triacontanol (1 µM). Seeds of the control treatment were submersed in tap water.

2.3 Germination test

After priming, the seed cohorts were placed in Linhardt vessels in laboratory conditions (20°C, 75% relative humidity, 16 h photoperiod), according to national germination standards (SR 1634/ 1999). To determine the germination indices, observations were performed every 24 hours for a seven day period. The germination indices and seed growth including final germination percentage (FGP), mean germination time (MGT), the coefficient of velocity of germination (CVG), germination rate index (GRI), germination index (GI), uncertanty (U), and synchrony (Z) were calculated.

2.4 Seedling growth rate

Twenty-five seeds per each replication were placed in Linhardt vessels under the same conditions (20°C, 75% relative humidity, 16 h photoperiod) for a 14 days period. At the end of the growth period the seedlings shoot and root lengths were measured using a caliper and expressed in mm.

Fresh and dry shoot and root mass were weighed using an analytical balance, and expressed in g. The shoots and roots of the seedlings were cut from the axis and were dried in an oven at 75ºC for 48 h.

2.5 Methods of calculation of germination parameters

Seven different germination parameters were assessed. The methodology of calculations of parameters (2), (3), (4), (5) followed Al-Mudaris (1998), (6) and (7) Ranal et al. (2009), and (1) followed Aravid et al. (2020).

(1) Final germination percentage (FGP)

$$FGP = \frac{Ng}{Nt} x100$$

Ng = Number of germinated seeds

Nt = Total number of seeds

(2) Mean germination time (MGT)

$$MGT=\Sigma f \cdot x/\Sigma f$$

f=Seed germinated on day x

(3) Coefficient of velocity of germination (CVG)

$$CVG= 100 \times \Sigma Ni / \Sigma NiTi$$

Ni = Number of germinated seeds per day

Ti = Number of days from the start of the experiment

(4) Germination rate index (GRI)

GRI = G1/1+G2/2+....+Gx/x

G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on

(5) Germination index (GI)

GI = (7 × N1) + (6 × N2) + ... + (1 × N7)

N1, N2 ...N7 is the number of germinated seeds on the first, second and subsequent days until 7th day: 7, 6… and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively.

(6) Uncertanty (U)

$$U = -\sum_{i=1}^{k} fi \log 2\, fi$$

Where, *f*i is the relative frequency of germination (estimated as $f_i = \frac{Ni}{\sum_{i=1}^{k} Ni}$),

Ni is the number of seeds germinated on the ith time interval, and *k* is the total number of time intervals

7. Synchrony (Z)

$$Z = \frac{\sum_{i=1}^{k} Ni,2}{C\sum Ni,2}$$

Where, CNi,2 is the partial combination of the two germinated seeds from among Ni, the number of seeds germinated on the *i*th time interval (estimated as $CNi,2 = \frac{Ni(Ni-1)}{2}$), and CΣNi,2 is the partial combination of the two germinated seeds from among the total number of seeds germinated at the final count, assuming that all seeds that germinated did so simultaneously.

2.6 Statistical Analyses

The SPSS software was used to perform a descriptive analysis based of means of each germination index. The data obtained were subjected to one-way Analysis of Variance (ANOVA). After assessing the distribution of the datasets, post-hoc tests were run, namely Tukey pairwise test in the case of normally distributed data and Mann-Whitney pairwise test, in the case of non-normally distributed data ($p \leq 0.05$).

3. Results and discussions

In the majority of cases the final germination percentage (FGP) of both hybrids was improved due to priming (**Table 1**).

Positive effects were observed on ‘Factor’ hybrid seeds in case of treatments with ASA and L-cyst, while treatment with Tria had negative effect on the seeds, although the differences were not significant. The seeds of ‘Hybrirock’ variety also responded positively to priming, the FGP having higher values compared to the control. The differences were significant in case of ASA and L-cyst ($p \leq 0.05$).

The mean germination time (MGT) decreased in both hybrids in response to priming, excepting ’Factor’ hybrid treated with L-cyst, where the MGT value increased compared to the control (**Table 1**). ’Factor’ hybrid had higher MGT values than ’Hybrirock’ and also, the differences among treatments had higher variation. The lowest (ASA treatment) and highest (L-cyst) values of MGT parameter varied significantly ($p \leq 0.05$) for ’Factor’ seeds.

Table 1. The results of one-way ANOVA analysis and the Tukey post-hoc test, (in the case of the * Mann-Whitney test) on the germination parameters; different letters denote significant differences at $p \leq 0.05$.

Hybrid	Treatment	FGP %	MGT * (day)	GI	CVG*	GRI (%/day)	U (bit)	Z
'Factor'	ASA	99 [a]	1.202 [a]	118.75 [a]	83.83 [a]	23.41 [a]	0.4375 [b]	0.8435 [a]
	L-cyst	96 [a]	1.532 [b]	106.25 [b]	66.21 [b]	19.70 [b]	1.1300 [a]	0.5285 [b]
	Tria	93 [a]	1.315 [ab]	109.75 [ab]	79.28 [ab]	21.80 [ab]	0.4350 [b]	0.8432 [a]
	Control	94 [a]	1.365 [ab]	108.75 [ab]	74.13 [ab]	20.92 [ab]	0.8187 [ab]	0.6810 [ab]
	Treatment	**FGP %**	**MGT * (day)**	**GI**	**CVG***	**GRI (%/day)**	**U (bit)**	**Z***
'Hybrirock'	ASA	99.5 [ab]	1.04 [ab]	124.50 [a]	96.36 [ab]	24.68 [a]	0.121 [ab]	0.9600 [ab]
	L-cyst	98 [ab]	1.08 [ab]	123.00 [ab]	93.01 [b]	24.38 [a]	0.148 [ab]	0.9207 [ab]
	Tria	99 [b]	1.00 [a]	123.70 [ab]	100 [a]	24.75 [a]	0.0142 [b]	1.0000 [a]
	Control	90 [b]	1.19 [b]	108.25 [b]	86.48 [b]	21.27 [b]	0.471 [a]	0.8420 [b]

Note: FGP-final germination percentage; MGT-mean germination time; GI-germination index; CVG-coefficient of velocity of germination; GRI-germination rate index; U-uncertanty; Z-synchrony

Tria and Control treatments had similar values and no significant differences were observed. All of the used substances have reduced the MGT of 'Hybrirock' seeds but only seeds treated with Tria differed significantly from untreated seeds. In case of ASA and L-cyst treated seeds no significant differences were found regarding the MGT compared to control.

The seeds of rapeseed varieties responded differently to priming regarding the germination index (GI). The used substances increased the GI parameter of 'Hybrirock' seedbatches in a more pronounced manner than in the case of 'Factor' variety. 'Factor' seeds had the highest GI values when treated with ASA, followed by Tria and the Control treatment, while seeds of L-cyst presented the lowest GI values. Among ASA and L-cyst treatments there were statistically significant differences ($p \leq 0.05$). All treatments resulted in higher GI values for 'Hybrirock' variety compared to the untreated batch. Furthermore the ASA and Tria treatments values differed significantly from the control batch (**Table 1**).

'Hybrirock' variety had higher values than 'Factor' also regarding the CVG parameter. Seeds treated with L-cystein had the lowest CVG in the case of 'Factor' hybrid, followed by the untreated seed batch, seeds treated with Tria and ASA, respectively. There were statistically significant differences among the lowest (L-cyst) and highest (ASA) values. 'Hybrirock' seeds reacted differently to the different treatments. In this case the untreated seeds were characterized with the lowest CVG values, followed by L-cyst, ASA and finally Tria treatments. The results with Tria were significantly different from the untreated seeds and those of L-cyst treatment (**Table 1**).

'Hybrirock' variety also had superior germination rate index (GRI) compared to 'Factor' variety. 'Factor' seeds responded negatively to L-cyst treatment, the GRI values of this treatment being lower than those of the untreated seeds. On the contrary, with Tria and ASA superior results were obtained compared to control treatment. ASA treatment resulted in significantly higher GRI values compared to those of L-cyst treatment. In the case of 'Hybrirock' variety, all of the used substances had positive effect on the GRI parameter. The results of the three treatments presented higher variation, but all of them differed significantly from the control seeds (**Table 1**).

Table 2. The results of the one-way ANOVA analysis and the Tukey post-hoc test (in the case of the * Mann-Whitney test) on seedling growth parameters of 'Hybrirock' variety, different letters denote significant differences at $p \leq 0.05$.

Hybrid	Treatment	SL (mm)	RL (mm)	SFW* (g)	RFW* (g)	SDW * (g)	RDW* (g)
'Hybrirock'	ASA	22.99 ^{b}	48.95 ^{a}	0.499 ^{ab}	0.245 ^{a}	0.085 ^{a}	0.0165 ^{a}
	L-cyst	22.61 ^{bc}	38.61 ^{b}	0.368 ^{b}	0.167 ^{ab}	0.062 ^{b}	0.0082 ^{b}
	Tria	31.48 ^{a}	41.32 ^{b}	0.531 ^{a}	0.226 ^{ab}	0.080 ^{ab}	0.0117 ^{ab}
	Control	18.97 ^{c}	24.90 ^{c}	0.401 ^{ab}	0.128 ^{b}	0.069 ^{ab}	0.0092 ^{b}

Note: SL- shoot length, RL- root length; SFW- shoot fresh weight; RFW- root fresh weight ; SDW- shoot dry weight; RDW- root dry weight

The uncertainty (U) values of 'Factor' variety are at least two times higher than the values of 'Hybrirock' variety. L-cystein had negative effects on 'Factor' seeds, raising their U values, while ASA and Tria treatments decreased the parameter, compared to the control seeds. The differences between L-cystein, respectively ASA and Tria treatments were statistically significant ($p \leq 0.05$). 'Hybrirock' seeds reacted in a positive manner to all treatments, thus the U value decreased at least three folds (**Table 1**).

The synchrony (Z) of germination was also higher in 'Hybrirock', and lower in 'Factor' variety. As a result of priming in L-cyst solution, the Z value of 'Factor' hybrid decreased, on the other hand, priming in Tria, or ASA solutions increased the Z value of the seed cohorts. Statistically significant differences can be spotted among the results of L-cyst, respectively Tria and ASA treatments. 'Hybrirock' seeds reacted positively to all substances, with Tria treatment having the most positive effect, resulting in significant differences compared to the untreated seeds (**Table 1**).

Due to an unexpected fungal contamination of the dishes containing the seeds of 'Factor' variety, the results of shoot and root biometrics are not presented.

Priming had a positive effect on shoot length of 'Hybrirock' seedlings. Tria treatments produced the highest shoot elongation and shoot fresh weight (SFW), the results being significantly different of those of control treatment. Also all of the priming substances had positive effect on root fresh (RFW) and dry weight (RDW) excepting L-cyst. In this case the most remarkable results were obtained in seed cohorts treated with ASA, followed by Tria. There were statistically significant differences among ASA, respectively Tria and L-cyst group, and the control treatment (**Table 2**). The results regarding the dry weights of shoots (SDW) and roots (RDW) of the seedlings were somewhat similar. Seed batches treated with L-cystein have developed organs with smaller dry weight than the untreated seeds. On the contrary seeds treated with Tria and ASA are characterized with superior dry shoot and root weights. There are significant differences in both cases, namely between the results of L-cyst and ASA treatments in the case of SDW, respectively between L-cyst-treated and untreated and ASA treated seeds (**Table2**).

Altogether it can be concluded that to maximize seedling establishment and germination parameters in 'Factor' and 'Hybrirock' hybrids, priming with ASA or Tria are recommended.

Conclusions

The results of the study show that canola seeds respond differently to the priming substances. In most of the cases the

germination and seedling growth parameters were increased compared to the control due to priming.

Overall, it can be said that seed priming with ascorbic acid performed better in 'Factor' rapeseed hybrid, by indicators MGT, GI, CVG, GRI, and Z. Triacontanol priming produced also good results, while L-cysteine treatments results were weaker compared to the control.

'Hybrirock' seeds responded better to triacontanol treatments, followed by priming with ascorbic acid and L-cystein.

Results concerning seedling biomass production indicate that the average shoot and root length were lowest in the untreated seeds, while shoot and root dry weight gain was the lowest in the L-cyst treated group of seeds of 'Hybrirock' variety.

The seed priming can improve the seeds germination parameters and induce early, synchronized and healthier crop stand. Priming with ascorbic acid, L-cysteine and triacontanol could also invigorate the seeds at early seedling stage.

Further experiments are required to clarify the effects of the above mentioned substances.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

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References

1. Al-Mudaris M (1998) Notes on various parameters recording the speed of seed germination. In: Der Tropenlandwirt - Journal of Agriculture in the Tropics and Subtropics, Vol 99, No2, pp 147–154.
2. Amirmoradi S, Feizi H (2017) Can mean germination time predict seed vigor of canola (*Brassica napus* L.) seed lots? Acta Agrobot. 70(4):1729. https://doi.org/10.5586/aa.1729
3. Ansari O, Sharif-Zadeh F, Moradi A, Azadi MS, Younesi E (2013) Heat shock treatment can improve some seed germination indexes and enzyme activity in primed seeds with gibberellin of mountain rye (*Secale montanum*) under accelerated aging conditions. Cercet. Agron. Moldova 156 (4):21–30.
4. Aravind J, Vimala Devi S, Radhamani J, Jacob S R, and Kalyani Srinivasan (2020) germinationmetrics: Seed Germination Indices and Curve Fitting. R package version 0.1.4.9000 https://github.com/aravind-j/germinationmetricshttps://cran.rproject.org/package=germinationmetrics
5. Ashraf MA, Akbar A, Askari SH, Iqbal M, Rasheed R, Hussain I (2018) Recent Advances in Abiotic Stress Tolerance of Plants Through Chemical Priming: An Overview. In: Rakshit A., Singh H. (eds) Avances in Seed Priming. Springer, Singapore. https://doi.org/10.1007/978-981-13-0032-5_4
6. Bhuiyan TF, Ahamed KU, Nahar K, Al Mahmud J, Bhuyan MB, Anee TI, Fujita M, Hasanuzzaman M (2019). Mitigation of PEG-induced drought stress in rapeseed (*Brassica rapa* L.) by exogenous application of osmolytes. Biocatal. Agric. Biotechnol. 20:1–10. https://doi.org/10.1016/j.bcab.2019.101197
7. Saha B, Chowardhara B, Kar S, Devi SS, Awasthi JP, Moulick D, Tanti B, Panda SK (2019) Advances in Heavy Metal-Induced Stress Alleviation with Respect to Exogenous Amendments in Crop Plants. In: Hasanuzzaman M., Fotopoulos V. (eds) Priming and Pretreatment of Seeds and

Seedlings. Springer, Singapore. https://doi.org/10.1007/978-981-13-8625-1_15
8. Deviah SP, Pan X, Hong Y, Roth M, Welti R, Wang X (2007) Enhancing seed quality and viability by suppressing phospholipase D in Arabidopsis. The Plant J. 50: 950–957.
9. Dutta P (2018) Seed Priming: New Vistas and Contemporary Perspectives. In: Rakshit A., Singh H. (eds) Advances in Seed Priming. Springer, Singapore. https://doi.org/10.1007/978-981-13-0032-5_1
10. Jisha KC, Vijayakumari K, Puthur JT (2013) Seed priming for abiotic stress tolerance: an overview. Acta Physiol Plant 35(5):1381–1396. https://doi.org/10.1007/s11738-012-1186-5
11. Kader M. A (2005) A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal & Proceedings of the Royal Society of New South Wales 138:65–75.
12. Kandil AAEN, Sharief AES, Botabaah AKD (2018) Effect of antioxidants and salinity stress on seedling parameters of some wheat cultivars. Res. J. Seed Sci. 11:12–21.
13. Kandil AA, Sharief AE, Kasim MF (2016) Germination characters as affected by seed priming of some safflower cultivars under salinity stress. Int. J. Agron. Agric. Res. 9:65–80.
14. Khan NA, Khan MI, Asgher M, Fatma M, Masood A, Syeed S (2014) Salinity tolerance in plants: revisiting the role of sulfur metabolites. J Plant Biochem Physiol 2(120):2.
15. Nesi N, Delourme R, Bregeon M, Falentin C, Renard M (2008) Genetic and molecular approaches to improve nutritional value of *Brassica napus* L. seed. C R Biol 331:763–771.
16. Ozbay N (2018) Studies on Seed Priming in Pepper (*Capsicum annuum* L.). In: Rakshit A., Singh H. (eds) Advances in Seed Priming. Springer, Singapore. https://doi.org/10.1007/978-981-13-0032-5_12
17. Ranal MA, Garcia de Santana D, Ferreira WR, Mendes-Rodrigues C (2009) Calculating germination measurements and organizing spreadsheets. In: Revista Brasiliera Botanica 32, pp 849–855.
18. Razaji A, Farzanian M, Sayfzadeh S (2014) The effects of seed priming by ascorbic acid on some morphological and biochemical aspects of rapeseed (*Brassica napus* L.) under drought stress condition. Int J Biosci 4(1):432–442.
19. Reddy CS, Smith JD (1978) Effects of delayed post treatment of gamma-irradiated seed with cysteine on the growth of Sorghum bicolor seedlings. Environ Exp Bot 18(4):241–243. https://doi.org/10.1016/0098-8472(78)90050-3
20. Seiadat SA, Moosavi A, Sharafizadeh M (2012) Effect of seed priming on antioxidant activity and germination characteristics of Maize seeds under different aging treatments. Research Journals of Seed Scienc 5(2):51–62.
21. Tantos Á, Mészáros A, Kissimon J, Horváth G, Farkas T (1999) The effect of triacontanol on micropropagation of balm, *Melissa officinalis* L. Plant Cell Rep. 19:88–91.
22. Walters, C (1998) Understanding the mechanisms and kinetics of seed aging. Seed. Sci. Res. 8:223–244.
23. *** SR (Standard Român) 1634, (1999) Seeds for sowing. Germination test

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PRELIMINARY STUDY OF THE EFFECT OF CHEMICAL AND ORGANIC FERTILIZERS ON A SEMI-NATURAL GRASSLAND IN VLĂHIȚA, HARGHITA MOUNTAINS, ROMANIA

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Abstract: The aim of the research was to examine the effect of different fertilizers on the floristic composition and biomass yield of the semi-natural grassland used as hayfield near Vlăhița locality (Harghita County, Romania) in order to improve its management. In the same time the qualitative structure of the studied grassland was evaluated after the first application of the fertilizers. The vegetation survey was made before mowing, and a total of 84 plant taxa were identified. The studied plant community belongs to the association *Festuco-Agrostetum capillaris* Horv. 1951. From the 31 fodder species, 13 had very good or good nutritional value. The qualitative structure analysis indicates that the vegetation has developed under moderate anthropogenic pressure. Beside the Euro-Asian elements the Circumpolar and the Cosmopolite elements were well represented. Many species with high tolerance for temperature, soil pH, and soil mineral nitrogen content were registered. After the first application of fertilizers in all treated plots the biodiversity increased compared to the control plot. Fertilized plots also had a significantly higher biomass yield than the control plot. All fertilizers reduced the proportion of the species with good nutritional value from the Poaceae family. Organic fertilizer affected positively the proportion of other plant families than Poaceae and Fabaceae. Long-term experiments are necessary to evaluate the response of the vegetation on treatment with organic fertilizer in order to optimize productivity of the hayfield and sustain species richness.

Keywords: fertilizers, grasslands, floristic composition, biomass yield, *Festuca rubra*, *Agrostis capillaris*.

1. Introduction

Vlăhița locality is situated in Harghita Mountains, in the upper part of Homorodul Mic and Pârâul Vârghiș Rivers. It is the city with the highest altitude (860 meters above sea level) from Harghita County, Romania (Vofkori, 1998). According to the geological map (http://atlas.anpm.ro/atlas#) the territory is characterized by volcanogenic-sedimentary formations, pyroclastic breccias, agglomerates, pyroclastic microbresches, microconglomerates and tuffs alternating with conglomerates, microconglomerates, sandstones, and sands of andesitic nature. In the area haplic luvisols and albic luvisols (World Reference Base for Soil Resources, WRB-SR-1998) are dominating, that correspond to luvosol according to the Romanian Soil Taxonomy System (Florea and Munteanu, 2012). The average annual temperature is 4-6 °C, while the average annual rainfall is 1000-1200 mm (Burus, 2007). The

herbaceous vegetation is represented by various associations dominated mostly by *Agrostis capillaris*, *Festuca rubra*, and *Nardus stricta* (Csűrös, 1963).

The grasslands from Vlăhița locality are commonly used for mowing, thus fertilizers are applied every second or third year.

The aim of the research was to examine the effect of different fertilizers on the floristic composition and biomass yield of the semi-natural grassland used as hayfield near Vlăhița locality (Harghita County, Romania) in order to improve its management. In the same time the qualitative structure of the studied grassland was evaluated after the first application of the fertilizers.

2. Materials and Methods

The experiment was conducted in 2020 near Vlăhița locality (46°20'44.85"N; 25°32'39.76"E) on a plane territory used as hayfield, at 857 m above sea level. In the hayfield, five experimental plots were delimited, with 10 m distance between them. The size of one plot was of 225 m^2 (22.5 m long and 10 m wide). In each plot different type of fertilizer was applied. One plot was kept as control. The utilized fertilizers and the doses were: NPK 12:12:17, 150 kg/ha + NH_4NO_3 150 kg/ha (chemical fertilizer), diluted cattle manure 50 m^3/ha (diluted fertilizer type I), diluted cattle manure 100 m^3/ha (diluted fertilizer type II), and cattle manure 20 t/ha (organic fertilizer). All fertilizers were applied on one occasion in spring (of the year 2020). In the previous years, on the studied territory, diluted manure from cattle was applied for one time every second year.

The identification of the studied association was made based on the Braun-Blanquet method and the work *Les associations vègètales de Roumanie Tome 2 Les associations anthropogènes* (Coldea, 2012). The qualitative structure of the grassland was assessed before mowing (when grasses were in flowering stage), according to Cristea et. al (2004), but also the work of Păcurar and Rotar (2014) was used. The relevés size was 25 m^2, and 2 relevés were effectuated in case of all experimental plots.

The produced biomass was estimated by sampling with mowing from 1 square meter surface. In case of all plots 4 biomass samples were taken. The obtained biomass was immediately weighed and expressed as biomass yield in t/ha. To compare biomass yields One-way-ANOVA and Tukey's pairwise test of the software Past (version 4.03) were used.

3. Results and discussions

The studied association was included in the following phytocoeno-system (Coldea, 2012):

Cls. Molinio-Arrhenatheretea R. Tx. 1937
Ord. Arrhenatheretalia R. Tx. 1931
All. Cynosurion R. Tx. 1947
Ass. *Festuco-Agrostetum capillaris* Horv. 1951

All vegetation surveys were made at one time, before mowing, and a total of 84 plant taxa were identified: 38 taxa in the control plot, 50 taxa in the plot with chemical fertilizer, 48 taxa in the plot with diluted fertilizer type I, 44 taxa in the plot with diluted fertilizer type II, and 55 taxa in the plot with organic fertilizer. The diversity index (Shannon) was higher in the fertilized plots (2.575-chemical fertilizer, 2.642-diluted fertilizer type I, 2.752-diluted fertilizer type II, and 2.637-organic fertilizer) than in the control plot (1.825). This result is in accordance with other studies that report the increase in species richness by the utilization of organic fertilizer (Tong et al., 2019) or different type of fertilizers (mineral nitrogen or manure) (Samuil et al., 2013). However mineral fertilizers are responsible for long-term

nitrogen deposition and can decrease biodiversity (Stevens et al. 2004; Pallett et al., 2016).

The characteristic and dominant species of the association were *Agrostis capillaris* and *Festuca rubra*. In the composition of the association many species from the Cynosurion alliance (*Trifolium repens*, *Cynosurus cristatus*, and *Phleum pratense*) and from the Arrhenatheretalia order were present (*Achillea millefolium*, *Leucanthemum vulgare*, *Lotus corniculatus*, *Trifolium hybridum*, *Senecio jacobaea*, *Leontodon hispidus*, *Plantago media*, *Veronica chamaedrys*, *Knautia arvensis*, *Daucus carota*, and *Dactylis glomerata*). Other species with high abundance-dominance values were: *Deschampsia cespitosa*, *Holcus lanatus*, *Centaurea nigrescens*, *Anthoxanthum odoratum*, and *Carex hirta*.

The spectrum of bioforms shows that hemicryptophytes (H) are in high number (**Fig. 1**). The altitudinal index (K_a = 12.69) indicates that the vegetation has developed in a mountain area, with moderate climate, and moderate anthropogenic pressure (Cristea et. al 2004).

The spectrum of floristic elements shows that the Euro-Asian (Euras.) elements are in high number. The species number is high also for the European (Eur.), Circumpolar (Circ., cold climate species), and Cosmopolite (Cosm.) elements (**Fig. 2**).

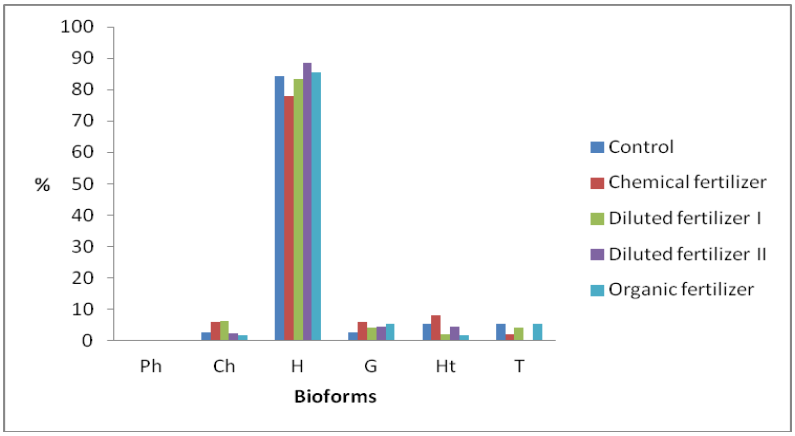

Fig. 1. The spectrum of bioforms in function of the applied fertilizer

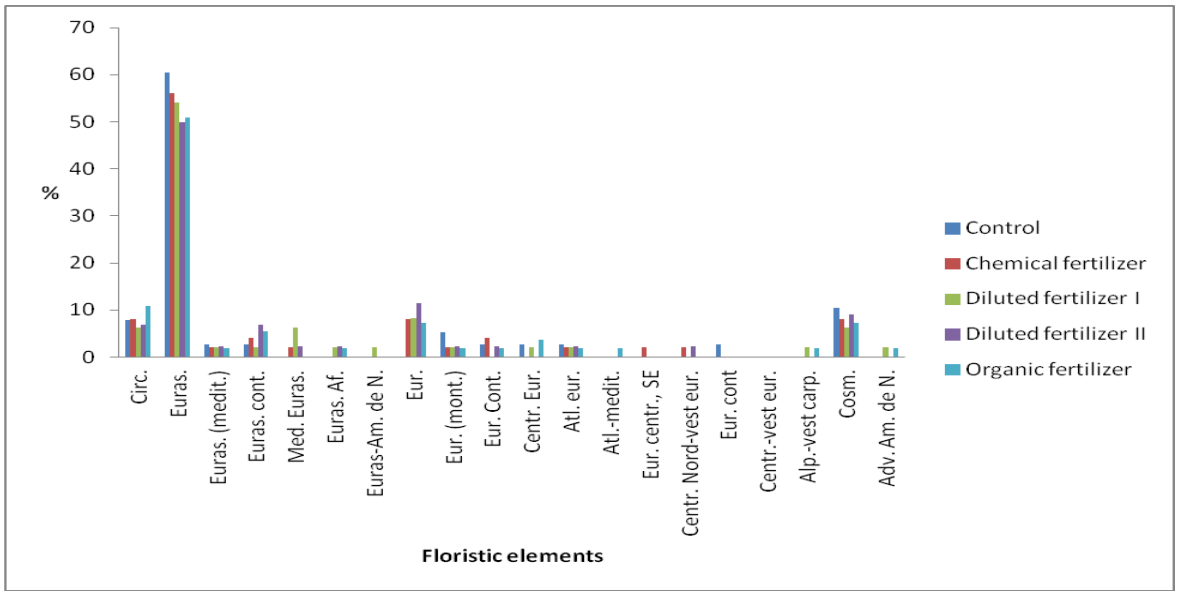

Fig. 2. The spectrum of floristic elements in function of the applied fertilizer

In the association two adventitious species (*Cuscuta campestris* and *Erigeron annus*) were identified. One taxa listed in the Romanian Red List was found (*Achillea ptarmica*) (Oltean et al., 1994).

The spectrum of edaphic humidity, highlights the slightly (U_4) and moderately moist soil species (U_5), together with the well-drained soil species (U_6). The euryhydric species (U_x) are also in high number. The plot with organic fertilizer had the highest number of wet soil species, while the plot with chemical fertilizer had the highest number of slightly and moderately moist soil species (**Fig. 3**).

In the spectrum of air temperature the euriterm species (T_x) are in high number, followed by species of temperate zones, characteristic to the hilly (T_6) and sub-mountainous floor (T_5) (**Fig. 4**).

In all plots the euryionic species (R_x) were in high number, followed by the species of moderately and slightly acid soils (R_5, R_6) (**Fig. 5**).

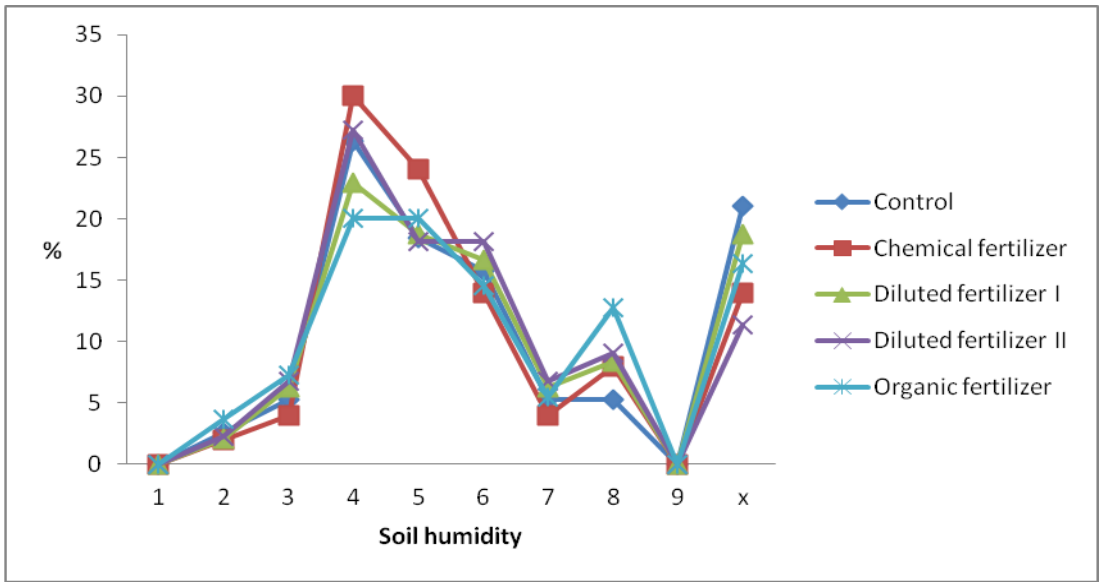

Fig. 3. The ecological spectrum for the edaphic humidity in function of the applied fertilizer

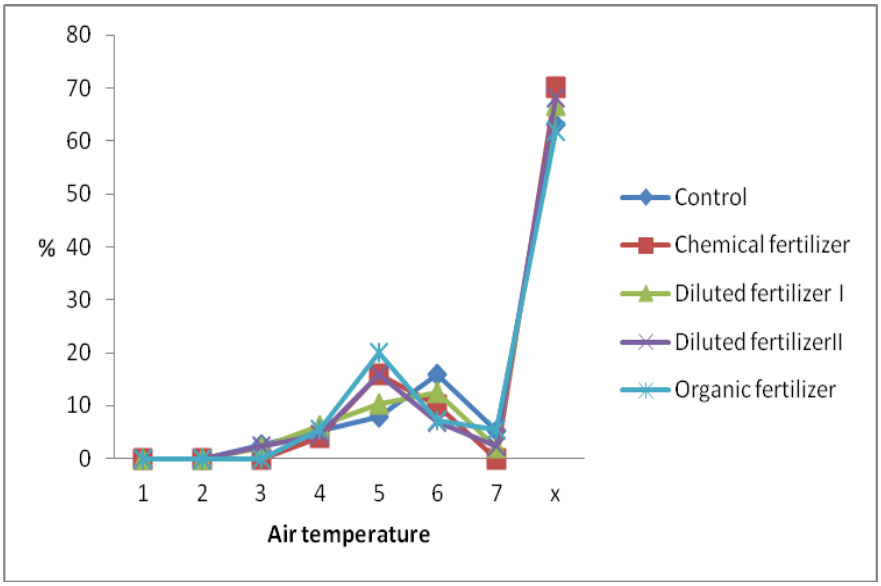

Fig. 4. The ecological spectrum for the air temperature in function of the applied fertilizer

Regarding the spectrum for soil mineral nitrogen content, in all plots the eurinitrofil species (N_x) were better represented, followed by the species spread on soil with moderate mineral nitrogen content (N_5). However in case of the organic fertilizer and diluted fertilizer type II, the species spread on soil with poor mineral nitrogen (N_2 and N_3) were in higher number compared to the other treatments. According to the field observations in the studied plots the species spread on excessive nitrogen-rich soil indicating deposition and pollution were not present (**Fig. 6**).

The values of the diploid index (the ratio between diploid and polyploid species) varied between 0.44 and 0.58. In all plots the polyploid species were in higher number (**Fig. 7**).

The fodder plants from the studied *Festuco-Agrostetum capillaris* Horv. 1951 association were listed in **Table 1**, together with their nutritional values (Pop, 1982; Cristea 2004).

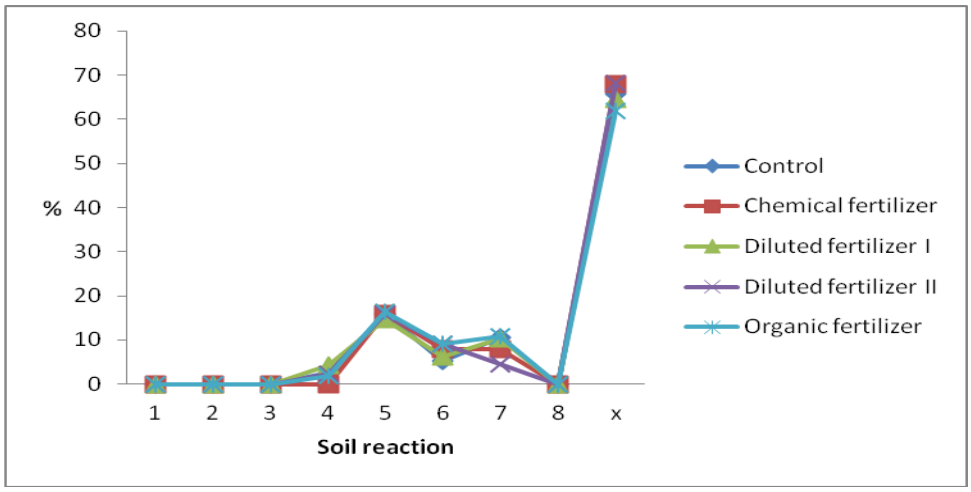

Fig. 5. The ecological spectrum for soil reaction in function of the applied fertilizer

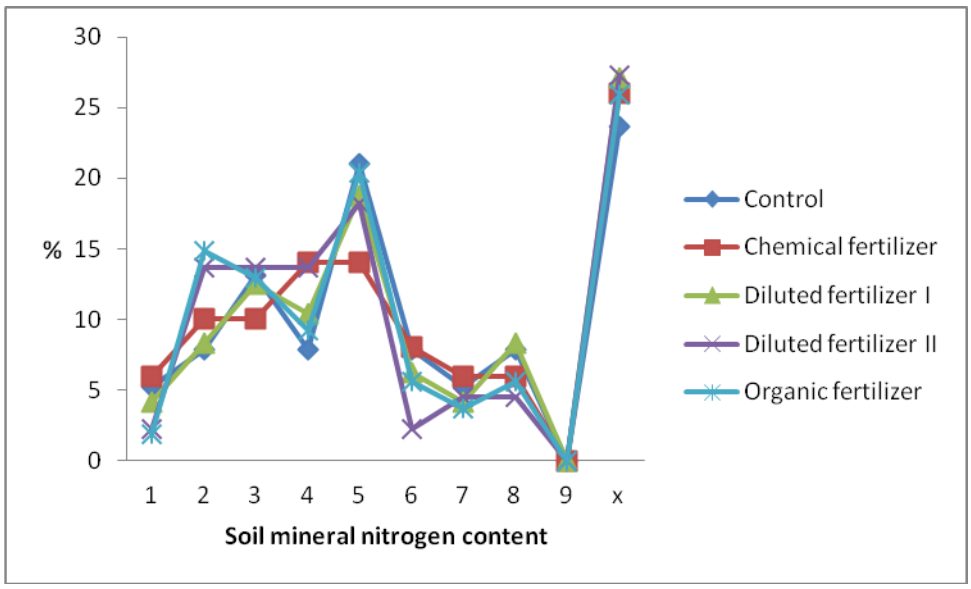

Fig. 6. The ecological spectrum for soil mineral nitrogen content in function of the applied fertilizer

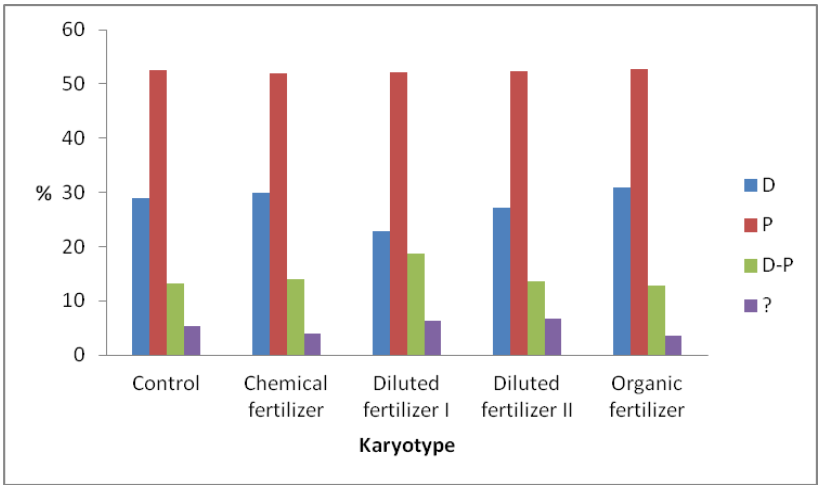

Fig. 7. The caryologic spectrum in function of the applied fertilizer

Table 1. Fodder plants identified in the *Festuco-Agrostetum capillaris* Horv. 1951 association near Vlăhița (Harghita County, Romania) and their nutritional values: 1-poor; 2- mediocre; 3-good; 4-very good (Pop, 1982; Cristea 2004)

Species	**Nutritional value**	**Species**	**Nutritional value**
Dactylis glomerata	4	*Plantago lenceolata*	2
Festuca pratensis	4	*Sanguisorba officinalis*	2
Phleum pratense	4	*Trifolium montana*	2
Trifolium hybridum	4	*Anthoxanthum odoratum*	1
Trifolium pratense	4	*Carex hirta*	1
Trifolium repens	4	*Chenopodium album*	1
Trisetum flavescens	4	*Deschampsia cespitosa*	1
Agrostis capillaris	3	*Holcus lanatus*	1
Cynosurus cristatus	3	*Leontodon hispidus*	1
Festuca rubra	3	*Plantago major*	1
Lotus corniculatus	3	*Plantago media*	1
Taraxacum officinale	3	*Potentilla erecta*	1
Alchemilla xanthochlora	2	*Symphytum officinale*	1
Daucus carota	2	*Tragopogon pratensis*	1
Elymus repens	2	*Vicia cracca*	1
Phleum phleoides	2		

The proportion of the main fodder plants (species from the Poaceae and Fabaceae family) was determined considering the abundance-dominance values of the species. In plots with diluted fertilizer type II and organic fertilizer a lower proportion of the family Poaceae and a higher proportion of other plant families (**Fig. 8**) could be observed. The differences in floristic composition could be partially explained by the differences found at the level of the abiotic factors. Most of the identified species from both plots indicated the poor mineral nitrogen content of the soil. In case of the plot with organic fertilizer a higher number of plants indicated the wet soil. In the plot with diluted fertilizer type II the species *Carex hirta* presented high abundance-dominance values compared to the other studied plots.

Although plant diversity increased in all treated plots compared to the control, the coverage (abundance-dominance) of the

species with good nutritional value decreased (**Table 2**).

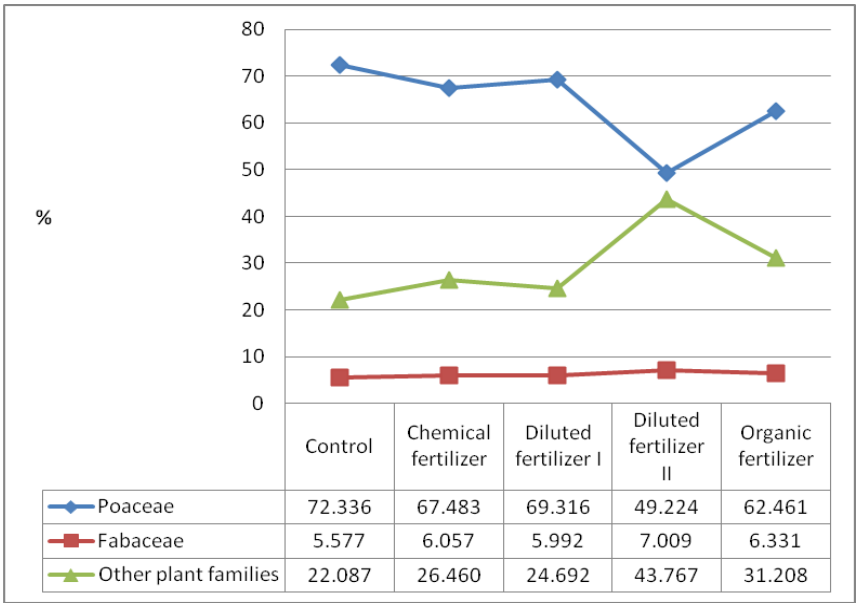

Fig. 8. The proportion (considering the abundance-dominance indices) of the main fodder species (Poaceae and Fabaceae) in function of the applied fertilizer

Table 2. The composition in economical categories: N-number of species; %-proportion of the categories considering the abundance-dominance indices

		Control		Chemical fertilizer		Diluted fertilizer I		Diluted fertilizer II		Organic fertilizer	
		N	%	N	%	N	%	N	%	N	%
Fodder	**1**	8	2.56	9	20.61	6	26.45	8	31.06	8	30.98
	2	5	2.42	5	3.90	6	7.52	5	6.06	7	9.56
	3	4	57.37	4	29.11	4	23.16	5	18.08	5	7.75
	4	5	7.11	5	6.07	6	7.82	2	4.73	6	4.46
Melliferous	**1**	2	0.09	3	0.35	2	0.36	1	0.04	2	0.06
	2	7	5.26	9	5.85	8	2.02	7	2.62	6	1.78
	3	1	0.47	1	0.32	1	3.26	1	4.30	2	3.00
Toxic		11	16.20	12	15.76	11	15.31	10	12.03	14	8.43
Spiny		2	0.95	3	3.55	2	0.65	2	4.73	2	3.00
Medicinal		8	2.56	6	4.89	8	7.30	10	6.66	8	6.65
Industrial	**Tc**	2	0.09	3	0.38	3	0.39	3	0.90	3	0.36
Alimentary	**Cu**	2	0.52	3	6.74	2	3.29	3	0.90	2	0.33
	Ol	0	0	0	0	0	0	0	0	1	0.03
Decorative		1	0.47	2	0.35	2	0.65	3	0.52	3	0.62
Other plants		7	3.93	12	2.11	11	1.82	10	7.39	13	22.99

Note-Fodder and melliferous plant categories: 1-poor, 2-mediocre, 3-good, 4-very good; Industrial plants: Tc-tinctorial; Alimentary plants: Cu-culinary, Ol-oleifer.

In a similar study with grassland edified by *Agrostis capillaris* and *Festuca rubra* it was found that manure improved mainly the growth of plants from other botanical families and no or less influence on the functional groups was observed. Manure had the highest positive

effect on biodiversity by changing the soil nutrient composition and also by introducing new species that were present in the applied fertilizer (Samuil, 2013).

The biomass yield varied between 18.9 and 29.1 t/ha. All fertilized plots produced a significantly higher biomass yield compared to the control plot ($F = 14.4$, $p < 0.0005$) (**Fig. 9**).

The positive effect of fertilizers on green biomass and floristic composition was observed after two years treatments in the *Festuca rubra-Agrostis capillaris* grassland from Băișoara (Cluj County, Romania). Application of manure (from cattle and horses) and/or mineral fertilizers increased the coverage of the Poaceae and Fabaceae family in the second and the third fertilization year (Rotar et al., 2016; Cirebea et al., 2020). Substantial increases in biomass yield in the following years after manure application were reported in many other grassland studies (Djukic et al., 2008; Štýbnarová, 2014; Botiș et al., 2015). Long-term studies (87-150 year) on the utilization of fertilizers demonstrated that chemical fertilizers reduce the soil pH, increase soil organic carbon stock mainly in the soil fractions with coarse particle- and fine particle size, and have a negative effect on diversity and abundance of plants from the Fabaceae family and other forbs. Farmyard manure, instead increases soil organic carbon stock also in the very fine particle size soil fractions, prevents soil acidification, and has a positive effect on hey yield (Silvertown et al., 2006; Hejcman et al., 2014; Kidd et al., 2017).

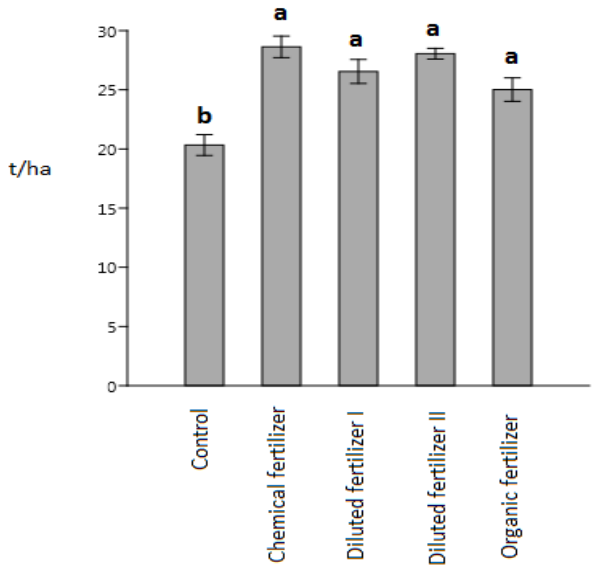

Fig. 9. Biomass yield (t/ha) in function of the applied fertilizer (different letter indicate significant differences)

Conclusions

The studied semi-natural grassland presented a relatively high number of plant species, considering other similar associations. The qualitative structure analysis indicates that the vegetation has developed under moderate anthropogenic pressure. Beside the Euro-Asian elements the cold climate species (Circumpolar) and the Cosmopolite elements were well represented. Many species with high tolerance for temperature, soil pH, and soil

mineral nitrogen content were registered. No mineral nitrogen content deposition or pollution indicating species were found. After the first application of fertilizers in all treated plots the biodiversity increased compared to the control plot. Fertilized plots also had a significantly higher biomass yield than the control plot. Organic fertilizer reduced the proportion of the species with good nutritional value from the Poaceae family, but affected positively the proportion of other plant families than Poaceae and Fabaceae. Long-term experiments are necessary to evaluate the response of the vegetation on treatment with organic fertilizer in order to optimize productivity of the hayfield and sustain species richness.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Vofkori L (1998) Székelyföld útikönyve I-II., Cartographia Kft., Budapest
2. Florea N, Munteanu I (2012) Sistemul român de taxonomie a solurilor (SRTS), Institutul Național de Cercetare-Dezvoltare pentru pedologie, agrochimie și protecția mediului - ICPA București
3. Burus T (2007) Szentegyháza és környéke idegenforgalmi lehetőségei, Magánkiadás
4. Csűrös Ș (1963) Scurtă caracterizare generală a vegetației din Transilvania. Acta Horti Bot București 2:825–853.
5. Coldea GH (ed.) (2012) Les associations végétales de Roumanie, Tome 2 Les associations anthropogènes Presa Universitară Clujeană, Cluj-Napoca
6. Cristea V, Gafta D, Pedrotti F (2004) Fitosociologie, Ed. Presa Universitară Clujeană, Cluj-Napoca, 2004
7. Păcurar F, Rotar I (2014) Metode de studiu și interpretare a vegetației pajiștilor, Ed. Risoprint, Cluj-Napoca
8. Tong Z, Quan G, Wan L, He F, Li X (2019) The effect of fertilizers on biomass and biodiversity on a semi-arid grassland of Northern China. Sustainability 11:2854. doi:10.3390/su11102854
9. Samuil C, Vintu V, Sirbu C, Stavarache M (2013) Influence of fertilizers on the biodiversity of semi-natural grassland in the Eastern Carpathians. Not Bot Horti Agrobo 41(1):195–200.
10. Stevens CJ, Dise NB, Owen MJ, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303. doi: 10.1126/science.1094678
11. Pallett DW, Pescott OL, Schäfer SM (2016) Changes in plant species richness and productivity in response to decreased nitrogen inputs in grassland in southern England. Ecological Indicators 68:73–81.
12. Oltean M, Negrean G, Popescu A, Roma N, Dihoru G, Sanda V, Mihăilescu S (1994) Lista roșie a plantelor superioare din România – Studii, sinteze, documentații de ecologie, Ed. Acad. Română, București 14–52.
13. Pop I (1982) Plante spontane și subspontane cu valoare economică din flora R. S. România. Contrib. Bot, 22:131–142.
14. Rotar I, Cirebea M, Păcurar F, Vidican R, Malinas A, Ranta O (2016) Mineral and organic fertilization influence on *Festuca rubra-Agrostis capillaris* natural meadow. Romanian Journal of Grassland and Forage Crops 13:39–46.

15. Cirebea M, Rotar I, Vidican R, Pleșa A, Morea A, Ranta O (2020) Impact of organo-mineral fertilization upon phytocoenosis and feed quality of the grasslands in the region of Transylvania. Romanian Agricultural Research 37
16. Djukic D, Stevovic V, Djurovic D, Ilic O (2008) The effect of organic fertilizer on biomass yield and quality of natural meadows. Options Méditerranéennes: Série A. Séminaires Méditerranéens 79:431–434.
17. Štýbnarová M, Mičová P, Fiala K, Karabcová H, Látal O, Pozdíšek J. (2014) Effect of organic fertilizers on botanical composition of grassland, herbage yield and quality. Agriculture (Poľnohospodárstvo) 60(3):87–97.
18. Botiș AL, Mihai GH, Sima N, Criste D, Mihalca I, Medrea I, Făgădar B (2015) Studies regarding the effect of organic fertilizers on a permanent grassland in Petrova, Maramureș. Bulletin UASVM Animal Science and Biotechnologies 72(2). doi:10.15835/buasvmcn-asb:11420
19. Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The Park Grass Experiment 1856–2006: its contribution to ecology. Journal of Ecology 94:801–814.
20. Hejcmana M, Sochorováa L, Pavlu V, Štrobach J, Diepolderc M, Schellberg J (2014) The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application Agriculture. Ecosystems and Environment 184:76–87.
21. Kidd J, Manning P, Simkin J, Peacock S, Stockdale E (2017) Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. PLoS ONE 12(3): e0174632. https://doi.org/10.1371/journal.pone.0174632

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