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BRACKEN AS A RISK FACTOR IN DIGESTIVE TRACT TUMORS: STATE OF THE ART

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Summary. - *The present knowledge of the risks of exposure to bracken fern* (Pteridium aquilinum) *is presented. The history of research on bracken toxicity is reviewed, covering the animal observations and chemical analysis, and focusing on the carcinogenic effects in various animal species and on the nature and biological properties of the known carcinogens. The implications of human exposure to bracken are inferred, the possible ways of contamination are discussed, and the existing epidemiological studies are commented. Bracken contribution to digestive tract tumors is deduced, and the need of further research on the plant toxicity and detailed epidemiological investigations with the exposed population is stressed.*

KEY WORDS: bracken, environmental carcinogens, digestive tract tumors.

Riassunto (Felce aquilina come fattore di rischio nei tumori del tratto digerente: conoscenze attuali). - *Nel presente lavoro si riportano le conoscenze attuali sui rischi dovuti all'esposizione alla felce aquilina. Si passa in rassegna la tossicità della felce aquilina sugli animali e le sostanze chimiche in esso presenti, soffermandosi su gli effetti carcinogenici nelle varie specie animali e sulla natura chimica e le proprietà biologiche di noti carcinogeni. Da qui si deducono le implicazioni per la salute dell'uomo, si discutono le possibili vie di contaminazione e si valutano gli studi epidemiologici al riguardo. In particolare si deducono i possibili contributi dell'insorgenza dei tumori dell'apparato digerente come conseguenza dell'uso alimentare umano della felce aquilina e si sottolinea pertanto la necessità di approfondire ulteriormente la tossicità della pianta con studi epidemiologici condotti sulle popolazioni che ne fanno uso alimentare.*

PAROLE CHIAVE: felce aquilina, carcinogeni ambientali, tumori del tratto digerente.

Introduction

Bracken (*Pteridium aquilinum*, L. Kuhn) is a large coarse fern widely distributed in all continents except Antarctica. Usually found in large thickets in open woods, sandy fields and hill sides, it is sometimes used as an ornamental plant in parks or gardens, as winter bedding for farm animals, for thatching and as a source of tannin and potash [1-3]. In many parts of the world, it is also used as human food.

The British navigator and explorer Captain James Cook has recorded the use of bracken rhizomes on Maori diet [2]. The German naturalist Alexander von Humboldt has reported its consume, grinded to powder and mixed with barley, by the inhabitants of Palma and Gomera (Canary Islands) [1]. Others have noticed in Siberia and Norway the employment of unfurled fronds in the brewing of a kind of beer. In the United Kingdom, Culpepper (quoted by Grieve [1]) has given several uses for it: "the roots being bruised and boiled in mead and honeyed water, and drunk kills both the broad and long worms in the body (...). The leaves eaten, purge the belly (...). The roots bruised and boiled in oil or hog's grease make a very profitable ointment (...). The powder of them used in foul ulcers cause their speedier healing".

It was introduced to the biomedical literature in the last decade of the 19th century, when it was recognized as poisonous to cattle [4-6]. Bracken had its carcinogenic content firstly suggested by Rosenberger and Heeschen [7], and evidenced later by the original experiments of Evans and Mason [8], with the development of ileal adenocarcinomas in rats fed dried fronds for about 2 months. So far, it has been incriminated in the causation of chronic bovine enzootic haematuria, often associated with acute enteritis, and eventually followed by bladder cancer

[9, 10], has been connected with the acute bone marrow syndrome and intestinal adenocarcinoma of North Yorkshire Moor sheep [11], and has been shown to induce experimentally mutations in fruit flies and fish, tumors and/or chronic haematuria in toads, quails, mice, rats, hamsters, guinea pigs, rabbits, sheep, pigs, cattle, water buffaloes and monkeys [1, 12, 13].

Animal observations

Cattle grazing freely upon bracken have shown co-existance of enzootic haematuria with an increased incidence of papilloma and squamous carcinoma of the tongue, soft palate, oropharynx, oesophagus and rumen, and polyposis or adenoma and adenocarcinoma of the intestine [10, 14, 15]. In one study [10], 30% of the cattle with alimentary squamous carcinomas had also bladder tumors. In the Nasampolai Valley (Narok District) in Kenya Masailand, where bracken fern is found but bovine enzootic haematuria has not been observed, a high incidence of rumenal cancer was reported, together with the finding of similar lesions in giant forest hogs [16]. The consumation of large amounts of bracken is found to produce an haemorrhagic syndrome known as acute bracken poisoning, the animals succumbing after 3 to 10 weeks with mucoidal nasoanal bleeding, severe anorexia and pyrexia, and radiomimetic cytopathological changes consisting of leucopenia and agranulocytosis, thrombocytopenia, aplastic anemia, and widespread haemorrhagic sites, the gut being prticularly ulcerated [10, 17]. The long-term effects of small ingestion may also be radiomimetic, but its mechanism is still obscure [10].

Experimental studies with different strains of rats confirmed the early findings of Evans and Mason. In addition, ileal sarcomas and urinary bladder tumors were reported [18, 19]. Drosophila flies [20] and guppy fish [21] were used to establish the mutagenic potential of bracken. Quails were shown to develop adenocarcinoma predominantly in the caecum, but also in the colon and distal ileum [17], and a sterility effect on the male was observed (Barber, G.D., quoted by Evans [17]). Mice given bracken or concentrated extracts of the plant, orally or intraperitoneally, exhibited haematologic cancers (mainly leukemias of the lymphocytic type), gastric carcinomas, pulmonary adenomas, hepatomas and two rare osteogenic sarcomas with pulmonary metastasis [3]. In hamsters, tumors appear in the caecum and distal ileum. Guinea pigs present predominantly bladder tumors, but also adenocarcinoma of the jejunum. Rabbits are more resistant to tumors, but develop a long-term haematuria [20]. Sheep present intestinal adenocarcinomas and a variety of other tumors [22].

A comparative study between each part of bracken has shown that the carcinogenic activity of the rhizomes is stronger than that of the young curled fronds (croziers), and that the stalks are the less carcinogenic postion [23]. Spores were shown to be powerful inducers of leukemias, gastric, pulmonary and mammary tumors [3].

Chemical analysis

Since the first investigations of the nature of "bracken toxin(s)", many molecules with large variety of biological effects have been isolated and characterized [24-27].

Sugimura *et al.* [28] have shown that kaempferol and quercetin, the product of intestinal hydrolisation of respectively astragalin and isoquercitrin-flavonoids isolated from bracken fronds [29], are mutagenic in the Ames test. In a later paper [30], Sugimura described the induction of sister chromatid exchanges in a human lymphoblastoid cell line by quercetin, and Pamukcu *et al.* [31] found that rats fed with a quercetin diet developed the same tumors as those fed with a bracken diet, with a longer latent period, but other studies [32-34] reported inactivity in other animals.

Japanese researchers have described indanone derivatives from bracken, the pterosins and pterosides, that are toxic against cultured Hella cells [24, 35] but so far were not shown to be carcinogenic. A water soluble precursor of these indanones, ptaquiloside, is thought to correspond with the so-called "guinea pig bleeding factor", a compound that leads to urinary bladder haemorrhage in those animals [36]. It is mutagenic in microbial tests [37, 38] and induces severe haematuria, mammary cancer, and ileal adenocarcinoma in rats [25, 37]. Its conjugated dienone derivative (considered to be the ultimate form) was shown to be a strong alkylating agent [38]. Nevertheless, for its behavior as a weak carcinogen, ptaquiloside does not equate with the powerful one responsible for the major bracken-induced neoplasms [3].

Shikimic acid, present in large amounts in bracken, gives negative results in the Ames microbial mutagen test [39], but gives positive ones with a T4 bacteriophage reversion test [40], is active in the BHK21 cell transformation assay [41], and produces dominant lethal mutations in *Drosophila melanogaster* [3] and leukemias and gastric adenocarcinoma in mice [37]. However, for it does not produce tumor in rats and quails [42, 43], it is not yet the searched "powerful bracken carcinogen" [3], and the compound responsible for the production of gastric cancer and leukemias in mice remains unidentified, since the recognition of 10 previously undescribed water soluble compounds in a volume of water that had been circulating through bracken croziers for two weeks [3]. The biological activity of these molecules has been investigated, with special attention to the ones showing similarities to known carcinogens. The experiments are still going on, and according to Evans [3], a number of these compounds seem promising.

The human risk

It is believed that dietary factors contribute to 30-35% of potentially preventable cancers [44]. Notwithstanding, some portions of the aetiologic cascade depend on the existence of cofactors; the interrelationships between various dietary components are complex and not well understood, and the crudeness of current methods for assessing dietary intake results in large measurement errors [45].

Nowadays, different parts of bracken are eaten by humans in the Society Islands (French Polynesia), Indonesia (Siregar and Sumiartha, personal communication), Northeastern China, Japan, Canada, Western North America, Southeastern Brazil and in certain areas of Europe [1, 3, 10, 46-48]. In Japan, where its use is very diffused, it is eaten as salad and garnish on cooked in various ways [49], the part used is mainly the young unfurled frond [50], the rhizomes being also eaten in some rural areas [25]. In Brazil [51], as in China (personal observation) and Indonesia, curled shoots under 25 cm long are preferred. In its industrially packed presentation, it is sold in Canada, the United States and Japan [47]. Japanese [50] and Brazilian [52] studies have shown that the content of carcinogen after processing is much less compared with unprocessed bracken, but carcinogenic activity still remains. According to Evans [3], the techniques of canning, drying and vacuum packaging that allow the fern to be eaten during all the year and even exported might be less efficacious in the removal of toxic substances than the old ones.

Up to now, we have dealt with the direct exposure to bracken used as a food. An undepreciable route of contamination is that via milk and daily products. In agreement with Turkish and American reports [53, 54], British scientists have detected a low level of carcinogenicity in the milk (fresh or dried) of cows fed bracken [22, 55]. In one of the experiments, a young bull calf fed the milk from cows receiving a bracken supplement developed bone marrow damage (traduced mainly by leucopenia, with a marked decrease in the neutrophil count and the appearance of reticulocytes in the circulation), and signs of early intestinal damage [55]. The incidence of tumors (pulmonary adenoma, leukemias, and gastric adenocarcinoma) in the offspring of mice given bracken during lactation was also found to be increased [3]. The water soluble carcinogenic activity can be retained in the buttermilk, the acidic pH contributing to its stability [3]. The indirect consumption of bracken through milk and daily products would extend the hazard to areas where grazing cattle (specially under free-range conditions) ingest the plant, as in Turkey, Yugoslavia and Bulgaria [47]. Indirect exposure *in vitro* may also occur through the placenta, as demonstrated experimentally in mice [55].

Another possible way of exposure is via the water from wells and springs from bracken rhizomes, and leachates obtained from other parts of the plant were capable of inducing a variety of tumors in mice [56, 57]. This water may constitute a hazard if consumed over extended periods in the early decades of life (it must be remembered that bracken carcinogenicity seem to be age-dependent, the youngs being more vulnerable [3]).

The hypothesis of an aereal contamination by spores (produced in large number during the summer) increased in strength since Evans [3] demonstrated their high carcinogenic potential. Inhaled spores present in the bronchial mucus would eventually reach the stomach after being swallowed [58], thus contributing to broad the risk spectrum.

Epidemiological studies

Since 1977, when Pamucku *et al.* [47] registered "a paucity of epidemiologic data concerning tumor evidence in the human population in areas of the world where bracken fern is directly or indirectly consumed", few reports have contributed to change this picture. An early epidemiological study in Japan (where the variety of bracken found is the *latiusculum*), a country with a high rate of upper digestive tract malignancies, has favored the idea that bracken consumption could be one of the factors contributing to the augmented occurence of oesophagal cancer in some prefectures of central Japan [59], a continuation of this study has demonstrated that daily bracken intake elevates the relative risk of this cancer to 2.10 in men and 3.67 in women [60], but another case-control study suggested that eating bracken does not constitute a risk [61]. Nevertheless, a recent investigation in Northern Japan has evidenced the large difference of dietary mutagenicity between the inhabitants of high and low risk areas of stomach cancer, the mutagenicity being caused by an inbalance on the intake of mutagen-positive and mutagen-depressive foods, and corresponding to the mortality [62].

In North Wales, where bracken coverage (of the *aquilinum* variety) is substantially great [63], the incidence of gastric cancer is strikingly higher than the national average, the incidence of eoesophageal cancer being also elevated [64]. A study conducted in Gwynedd (NW Wales) [58] has shown that bracken exposure in childhood (determined through a special questionnaire), and length of residence in the area (covered with bracken in 20% or more of its surface [63]), were associated with an increased risk of gastric cancer. Consumption of buttermilk in childhood and adulthood was also attended by increased risk. No clear evidence for or against any particular vector was inferred, however.

Villalobos-Salazar has demonstrated that in the mountainous regions of Costa Rica (rich in the *caudatum* variety of bracken), the age-adjusted rates for oesophageal and gastric carcinoma are increased when compared with the bracken-free lowlands [65, 66], what has been attributed to the consumption of bracken-contaminated milk (shown to be carcinogenic to mice [67]).

In Southeastern Brazil, where the local plant (var. *arachnoideum*) was shown to be carcinogenic to animals [68, 69], epidemiological studies with the bracken-eating population are in course. Australasia, where bracken (of the *esculentum* variety) was found to contain a very high amount of ptaquiloside (670 μg/g of dry solid residue, on a wet matter basis [70]), and other areas of the world where the plant is commonly used as food supplement (like the Indonesian islands of Sulawesi and Bali, the eastern slopes of the Great Khingan Range in China, and even the Japan of the annual gastric mass screening programs) are lacking detailed investigation within bracken-exposed communities. The question of the undirectly exposed population (including those employed in the bracken processing industry), for the complexity of variables involved, is much more difficult to assess, but not less important to elucidate.

Despite decades of research, much of the bracken toxicity remains to be understood, and further investigations are necessary in order to provide a better comprehension of the bracken risk to human health.

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REFERENCES

1. GRIEVE, M. 1971. *A modern herbal*. Vol. 1 (A-H). Dover Publications Inc., New York. pp. 305-306.

2. RYMER, L. 1976. The history and ethnobotany of bracken. *Bot. J. Linn. Soc.* **73**: 151-176.

3. EVANS, I.A. 1987. Bracken carcinogenicity. In: *Quarterly Scientific Reviews, "Reviews on Environmental Health"*, Vol. VIII, No. 3/4. G. V James (Ed.). Scientific Publications Division, Freund Publishing House Ltd., Tel-Aviv. pp. 161-179.

4. PENBERTHY, J. 1893. Vegetable poisoning (?) simulating anthrax in cattle. *J. Comp. Pathol.* **6**: 266-275.

5. STORRAR, D.M. 1893. Cases of vegetable poisoning in cattle. *J. Comp. Pathol.* **6**: 266-275.

6. ALMOND, N. 1894. Fern poisoning. *J. Comp. Pathol.* **7**: 165-167.

7. ROSENBERGER, VON G. & HEESCHEN, W. 1960. Adlerfarn (*Pteris aquilina*) die Ursache des sog. Stallroter der Rinder (*Haematu vesicalis bovis chronica*). *Dtsch. Tierärztl. Wochenschr.* **67**: 201-208.

8. EVANS, T.A. & MASON, J. 1965. Carcinogenic activity of bracken. *Nature* **208**: 913-914.

9. PAMUKCU, A.M., GOKSOY, S.K. & PRICE, J.M. 1967. Urinary bladder neoplasms induced by feeding bracken fern *(Pteris aquilina)* to cov *Cancer Res.* **27**: 917-924.

10. JARRET, W.F.H., McNEIL, P.E., GRIMSHAW, W.T.R., SELMAN, T.E. & McINTYRE, W.I.M. 1978. High incidence of cattle cancer w a possible interaction between an environmental carcinogen and a papilloma virus. *Nature* **274**: 215-217.

11. PARKER, W.H. & McCREA, C.T. 1965. Bracken *(Pteris aquilina)* poisoning of sheep in the North York moors. *Vet. Rec.* **77**: 861-86

12. PAMUKCU, A.M., PRICE, J.M. & BRYAN, G.T. 1976. Naturally occurring and bracken-fern induced bovine urinary bladder tumors, clin and morphological characteristics. *Vet. Pathol.* **13**: 110-122.

13. DONIGIEWICZ, K. 1987. Correlation between bracken and the development of cancer. *Med. Weter.* **43**: 608-611.

14. DÖBEREINER, J., TOKARNIA, C.A. & CANELLA, C.F.C. 1967. Ocorrencia de hematùria enzoòtica e de carcinomas epidermòides no digestivo superior em bovinos no Brasil. *Pesqui. Agropecu. Bras.* **2**: 489-504.

15. TOKARNIA, C.H., DÖBEREINER, J. & CANELLA, C.F.C. 1969. Ocorréncia de hematùria enzoòtica e de carcinomas epidermòides no digestivo superior em bovinos no Brasil. II. Estudos Complementares. *Pesqui. Agropecu. Bras.* **4**: 209-234.

16. PLOWRIGHT, W., LINSELL, C.A. & PEERS, F.G. 1971. A focus on rumenal cancer in Kenyan cattle. *Br. J. Cancer* **25**: 72-80.

17. EVANS, I.A. 1968. The radiomimetic nature of bracken toxin. *Cancer Res.* **28**: 2252-2261.

18. PAMUKCU, A.M. & PRICE, J.M. 1969. Induction of urinary bladder cancers in rats by feeding bracken fern *(Pteris aquilina)*. *J. Natl. Can Inst.* **43**: 275-281.

19. HIRONO, I., SHIBUYA, C., FUSHIMI, K. & HAGA, M. 1970. Studies on carcinogenic properties of bracken, *Pteridium aquilinum*. *J. N Cancer Inst.* **45**: 179-188.

20. EVANS, I.A., WIDDOP, B. & BARBER, G.D. 1967. Carcinogenic activity of bracken. In: *British Empire Cancer Campaign for Rese Annual Report*. Part II. pp. 411-412.

21. MATSUSHIMA, T., SATO, S., HARA, K., SUGIMURA, T. & TAKASHIMA, F. 1975. Bioassay on environmental carcinogens with guppy. *Lebistes reticulatus*. *Mutat. Res.* **31**: 265.

22. EVANS, I.A. 1984. Bracken carcinogenity. In: *Chemical carcinogens. ACS Monograph 182*. C.E. Searle (Ed.). Vol. II, 2nd. Ed., Ame Chemical Society, Washington D.C. pp. 1171-1204.

23. HIRONO, I., FUSHIMI, K., MORI, H., MIWA, T. & HAGA, M. 1973. Comparative study of carcinogenic activity in each part of bracken. *J. Natl. Cancer Inst.* **50**: 1367-1371.

24. YOSHIHIRA, K., FUKUOKA, M., KUROYANAGI, M. & NATORI, S. 1978. Chemical and toxicological studies on bracken fern (*Pteridium aquilinum* var. *latiusculum*). I. Introduction, extraction and fractionation of constituents and toxicological studies including carcinogenic tests. *Chem. Pharm. Bull. (Tokyo)* **26**: 2346-2364.

25. HIRONO, I., YAMADA, K., NIWA, H., SHIZURI, Y., OJIMA, M., HOSAKA, S., YAMAJI, T., WAKAMATSU, K., KOGOSHI, H., NIIAMA, K. & YOSAKI, Y. 1984. Separation of carcinogenic fraction of bracken fern. *Cancer Lett.* **21**: 239-246.

26. GOTO, I. 1984. Carcinogenic compound of bracken (in feed). *Kagaku* **39**: 429-430.

27. OJIMA, M., KIGOSHI, H., KUYAMA, H., NIWA, H. & YAMADA, K. 1985. Studies on *Pteridium aquilinum* var. *latiusculum*. Isolation of 3-para-hidroxystyrene and an efficient method for isolation of Ptaquiloside, an unstable bracken carcinogen. *J. Natl. Prod.* **48**: 634-638.

28. SUGIMURA, T., NAGAO, M., MATSUSHIMA, T. *et al.* 1977. Mutagenicity of flavone derivatives. *Proc. Jpn. Acad.* **53**: 194-197.

29. NAKABAYASHI, T. 1955. Isolation of astragalin and isoquercitrin from bracken, *Pteridium aquilinum*. *Bull. Agric. Chem. Soc. Jpn.* **19**: 104-109.

30. SUGIMURA, T. 1979. Naturally occurring genotoxic carcinogens. In: *Naturally occurring carcinogens-mutagens and modulators of carcinogenesis*. E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura and S. Takayama (Eds). Baltimore University Park Press. pp. 241-261.

31. PAMUKCU, A M., YALCINER, S., HATCHER, J.F. & BRYAN, G.T. 1980. Quercitin, a rat intestinal and bladder carcinogen present in bracken fern *(Pteridium aquilinum)*. *Cancer Res.* **40**: 3468-3472.

32. AMBROSE, A.M., ROBBINS, D.J. & DeEDS, F. 1952. Comparative toxicities of quercitin and quercitrin. *J. Am. Pharm. Assoc.* **41**: 119-122.

33. HIRONO, I., UENO, I., HOSAKA, S., TAKANASHI, M., MATSUSHIMA, T., SUGIMURA, T. & NATOMI, S. 1981. Carcinogenic examination of quercitin and rutin in ACI rats. *Cancer Lett.* **13**: 15-21.

34. MORINO, K., MATSUKURA, N., KAWACHI, T., OHGAKI, H., SUGIMURA, T. & HIRONO, I. 1982. Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration. *Carcinogenesis* **3**: 93-97.

35. SAITO, M., UMEDA, M., ENOMOTO, M. HATANAKA, Y. NATORI, S. YOSHIHIRA, K. FUKOKA, M. & KUROYANAGI, M. 1975. Citotoxicity and carcinogenicity of pterosins and pterosides, 1-indanone derivatives from bracken. *(Pteridium aquilinum)*. *Experientia* **31**: 829-831.

36. EVANS, I.A., PROROK, J.H., COLE, R.C. AL-SALMANI, M.H., AL-SAMARRAI, A.M.H., PATEL, M.C. & SMITH, R.M.M. 1982. The carcinogenic, mutagenic and teratogenic toxicity of bracken. *Proc. Br. Royal Soc. Edinburgh* **81**: 65-77.

37. EVANS, I.A. & OSMAN, M.A. 1974. Carcinogenicity of bracken and shikinic acid. *Nature* **25**: 348-349.

38. MATOBA, M., SAITO, E., SAITO, K. KOYAMA, S., NATORI, S. MATSUSHIMA, T. & TAKIMOTO, M. 1987. Assay of ptaquiloside, the carcinogenic principle of bracken, *Pteridium aquilinum*, by mutagenicity testing in *Salmonella typhimurium*. *Mutagenesis* **2**: 419 424.

39. JACOBSON, L.B., RICHARDSON, C.L. & FLOSS, H.G. 1978. Shikimic acid and quinic acid are not mutagenic in the Ames test. *Lloydia* **41**: 450-452.

40. ROBERTS, I.M., SHAW, D.S. & EVANS, W.C. 1971. A T4 bacteriophague reversion test for the naturally occurring mutagen present in bracken fern *(Pteridium aquilinum)*. *Biochem. J.* **124**: 13.

41. JONES, R.S., ALI, M., IOANNIDES, C. STYLES, J.A., ASHBY, J., SULEJ, J. & PARKE, D.V. 1983. The mutagenic and cell transforming properties of shikimic acid and some of its bacterial and mammalian metabolites. *Toxicol. Lett.* **19**: 43-50.

42. HIRONO, I., FUSHIMI, K. & MATSUBARA, N. 1977. Carcinogenicity test of shikimic acid in rats. *Toxicol. Lett.* **1**: 9-10.

43. EVANS, I.A. 1979. Bracken carcinogenicity. *Res. Vet. Sci.* **26**: 339-348.

44. MILLER, A.B. 1990. Diet and cancer, a review. Reviews in Oncology, 3:1. In: *Acta Oncologica* **29**: 87-95.

45. HENDERSON, B.H. 1990. Summary report of the sixth symposium on cancer registries and epidemiology in the Pacific Basin. *J. Natl. Cancer Inst.* **82**: 1187-1190.

46. MEDSGER, O.P. 1939. *Edible Wild Plants*. McMillan Co., New York. p. 135.

47. PAMUKCU, A.M., YALCINER, S. & BRIAN, G.T. 1977. Inhibition of carcinogenic effects of bracken fern *(Pteridium aquilinum)* by various chemicals. *Cancer* **40**: 2450-2454.

48. SANTOS, R.C., HOJO, E.S. & BRASILEIRO-FILHO, G. 1986. Studies on the possible carcinogenicity of bracken fern *(Pteridium aquilinum)* from Ouro Preto, Minas Gerais, Brazil. *Cienc. Tecnol. Aliment.* **6**: 93-98.

49. AOKI, K. & OHNO, Y. 1982. Bracken fern, phenacetin and cancer of the urinary tract. *Natl. Cancer Inst. Monograph.* **62**: 191-195.

50. HIRONO, I., SHIBUYA, C., SHIMIZU, M. & FUSHIMI, K. 1972. Carcinogenic activity of processed bracken used a human food. *J. N Cancer Inst.* **48**: 1245-1250.

51. HiRONO, I., FUSHIMI, K., MORI, H., MIWA, T. & HAGA, M. 1973. Comparative study of carcinogenic activity in each part of bracke *Natl. Cancer Inst.* **50**: 1367-1371.

52. SANTOS, R.C. 1990. Consideracoes sobre o uso do broto de samambaia na alimentacäo humana. *Ciencia e Cultura* **42**: 216219.

53. PAMUKCU, A.M., ERTÜRK, E., YALCINER, S. & BRYAN, G.T. 1978. Carcinogenic and mutagenic activities of milk from cows fed brac fern *(Pteridium aquilinum)*. *Cancer Res.* **38**: 1556-1560.

54. PAMUKCU, A.M., WANG, C.Y., HATCHER, J. *et al.* 1980. Carcinogenicity of tannin and tannin-free extracts of bracken fern *(Pterid aquilinum)* in rats. *J. Natl. Cancer Inst.* **65**: 131-136.

55. EVANS, I.A., JONES, R.S. & MAINWARING-BURTON, R. 1972. Passage of bracken fern toxicity into milk. *Nature* **237**: 107-108.

56. GLIESSMANN, S.R. 1976. Allelopathy in a broad spectrum of environments as illustrated by bracken. *Bot. J. Linn. Soc.* **73**: 95-104.

57. EVANS, I.A., AL-SAMARRAI, A.M.H. & SMITH, R.M.M. 1984. Bracken toxicology. Identification of some water soluble compounds f crozier and rhizome. *Res. Vet. Sci.* **37**: 261-266.

58. GALPIN, O.P., WHITAKER, C.J., WHITAKER, R. & KASSAB, J.Y. 1990. Gastric cancer in Gwynedd. Possible links with bracken. *B Cancer* **61**: 737-740.

59. KANON, S. & HIRAYAMA, T. 1980. Epidemiologic survey on oesophageal cancer (originally presented in 1975, at the 34th Annual Mee of the Japanese Cancer Association in Osaka, as "Epidemiology of cancer of the oesophagus in Mie, Nara and Wakayama prefecture with sp reference to the role of bracken fern"). In: *Cancer, Japan and the World.* T. Nagayo and S. Tominaga (Eds). Shinohara, Tokyo. pp. 279 (in Japanese).

60. HIRAYAMA, T. 1979. Diet and cancer. *Nutr. Cancer* **1**: 67-81.

61. OKADA, H., AOKI, K., OHNO, Y. & KUROISHI, K. 1980. Esophageal cancer and environmental factors. No. II. In: *Proceedings of the Annual Meeting of the Japanese Cancer Association*. Tokyo, 1980. University of Tokyo Press, Tokyo. p. 367.

62. MICHIOKA, S. 1990. Mechanism and structure of the mutagen-depressing compound in vegetables. *J. Cancer Res. Clin. Oncol.* **116**(Sup 76.

63. TAYLOR, J.A. 1985. Bracken encroachment rates in Britain. *Soil Use and Management* **1**: 53.

64. GARDNER, M.J., WINTER, P.D., TAYLOR, C.P. & ACHESON, E.D. 1983. *Atlas of cancer mortality in England and Wales.* W Chichester.

65. VILLALOBOS-SALAZAR, J. 1987. *Pteridium aquilinum* and gastric cancer. In: *Abstracts of the XVIII World Veterinary Congress.* Mon 16-21 August 1987. p. 90.

66. VILLALOBOS-SALAZAR, J. 1988. Bracken derived carcinogens as affecting animal health and human health in Costa Rica. In: *Br toxicity and carcinogenicity as related to animal and human health.* J. Taylor (Ed.). Institute of Earth Studies, Bangor. pp. 40-51.

67. VILLALOBOS-SALAZAR, J. 1985. Carcinogenicidad del *Pteridium aquilinum* y alta incidencia del cancer gastrico en Costa Rica. *Rev Cienc. Med.* **6**: 131-135.

68. SANTOS, R.C., BRASILEIRO-FILHO, G. & HOJO, E.S. 1987. Induction of tumors in rats fed bracken fern *(Pteridium aquilinum)* from Preto (Minas Gerais, Brazil). *Braz. J. Med. Biol. Res.* **20**: 73-77.

69. SANTOS, R.C., BRASILEIRO-FILHO, G. & SILVA, M.E. 1990. Oncogenicity of bracken fern *(Pteridium aquilinum)* from Ouro Preto, for rats fed low protein diets. In: *Bracken control and management.* J.A. Thomson and R.T. Smith (Eds). Australian Institute of Agri Science, Sydney. pp. 253 257.

70. SMITH, B.L., LAUREN, D.R., HOLLAND, P.T., EMBLING, P.P. & AGNEW, M.P. 1990. Ptaquiloside in Australasian ferns as a ca neoplasia. In: *Bracken control and management.* J.A. Thomson and R.T. Smith (Eds). Australian Institute of Agricultural Science, S