

**Micronized Zeolite
Clinoptilolite:
impact-full innovation in
Pet Nutrition**



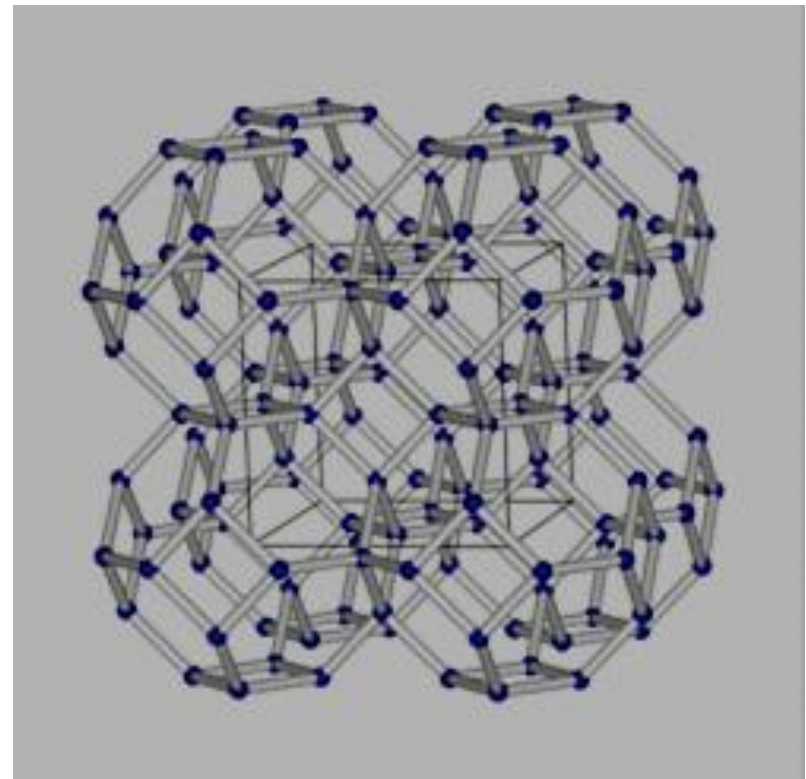
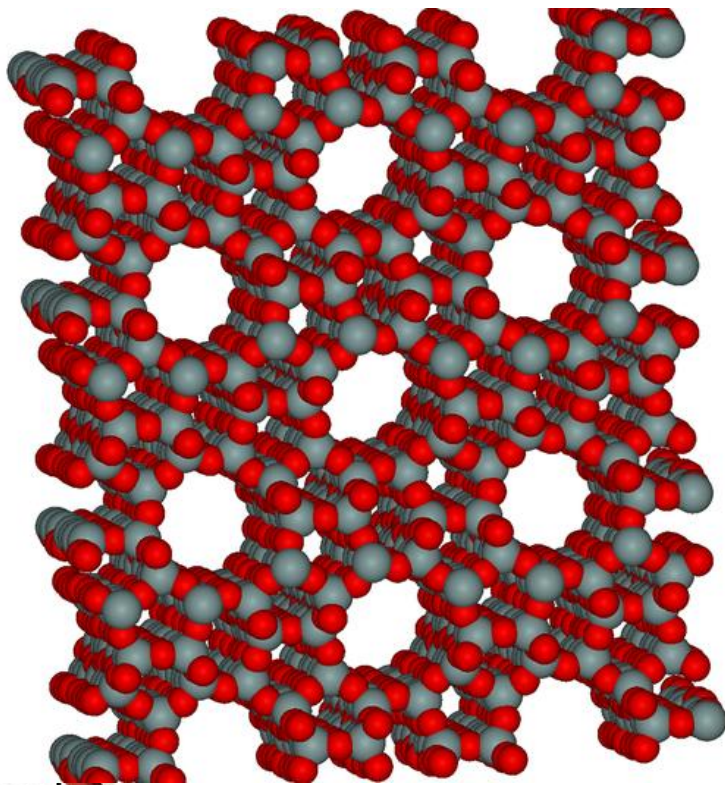
Zeolites

Zeolites are micro porous aluminosilicate minerals. The term zeolite was originally coined in 1756 by Swedish mineralogist Axel Fredrik Cronstedt, who observed that upon rapidly heating the material stilbite, it produced large amounts of steam from water that had been absorbed by the material. Based on this, he called material zeolite, from the Greek **zeo**, meaning “boil” and **lithos**, meaning “stone”

From Wikipedia, the free encyclopedia



Zeolite Structure

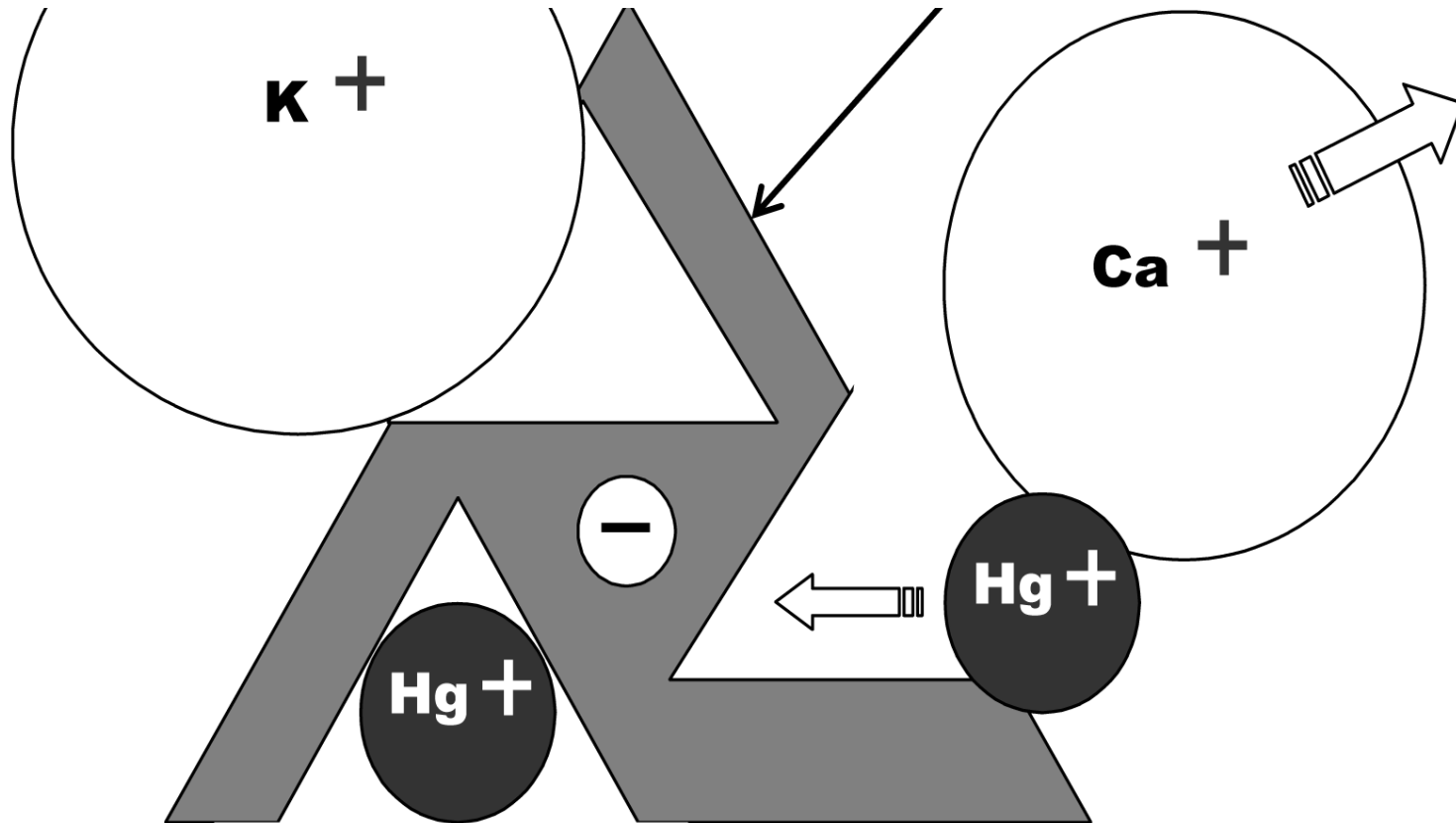


Zeolites

- Zeolites are among the most important inorganic cation exchangers
- Aluminosilicate structure is negatively charged and attracts cations that come to reside inside the pores and channels
- Have large empty spaces, or cages, within their structures that can accommodate large cations such as Na^+ , K^+ , Br^+ , and Ca^{2+}



Ion Exchanging



Zeolites

- The ion exchange process is reversible, allowing for adsorption of ions and molecules, making zeolites useful as filters for dust, toxin removal, and as a chemical sieve
- Subsequently, other solutions can be put through the structure, and thus the zeolite acts as a delivery system for the new fluid
- This process has been exploited and applied in medicine, farm animal feed, and other types of research



Zeolites

It is obvious that the ion accommodation and other remarkable properties of zeolites will be utilized in the near future for the environmental and health care :

- a. their known biological properties accompanied with their long-term chemical and biological stability
- b. zeolites reversibly bind small molecules such as oxygen and nitric oxide
- c. the possibility of metalloenzyme mimicry
- d. immunomodulatory activity



Zeolites in food industry

- Zeolites are already in use in the food industry
- Beer is stabilized with NaA and LiX zeolites, which adsorb the proteins responsible for further degradation
- The de-alcoholization of beer is done with the help of dealuminated zeolite Y
- The fatty acids of comestible oil are eliminated on zeolite X.
- Zeolites are also included in the formulation of toothpaste.



CLINOPTILOLITE

- $\text{CaAl}_2\text{Si}_7\text{O}_{18}$
- Other cations: Na^+ , K^+ , Mg^{2+}
- Applicable for animal and human use
- Ion-exchangers (water softeners); catalysts; adsorbents (filtration, selective separation); animal food additives (anti caking agents - EC12991, No. 2200/2001; October 17, 2001)



Effect of Dietary Inclusions of Clinoptilolite – How it started

- Dietary inclusions of Clinoptilolite could be beneficial for animal production
- Pigs fed Clinoptilolite experience beneficial weight gains and are less subject to disease than pigs fed normal diets
- They also show regular digestion, an appetite increase, and the meat-content increases at the expense of the fat
- New researches shows that zeolites remove toxins and create changes in enzymology and immunological responses

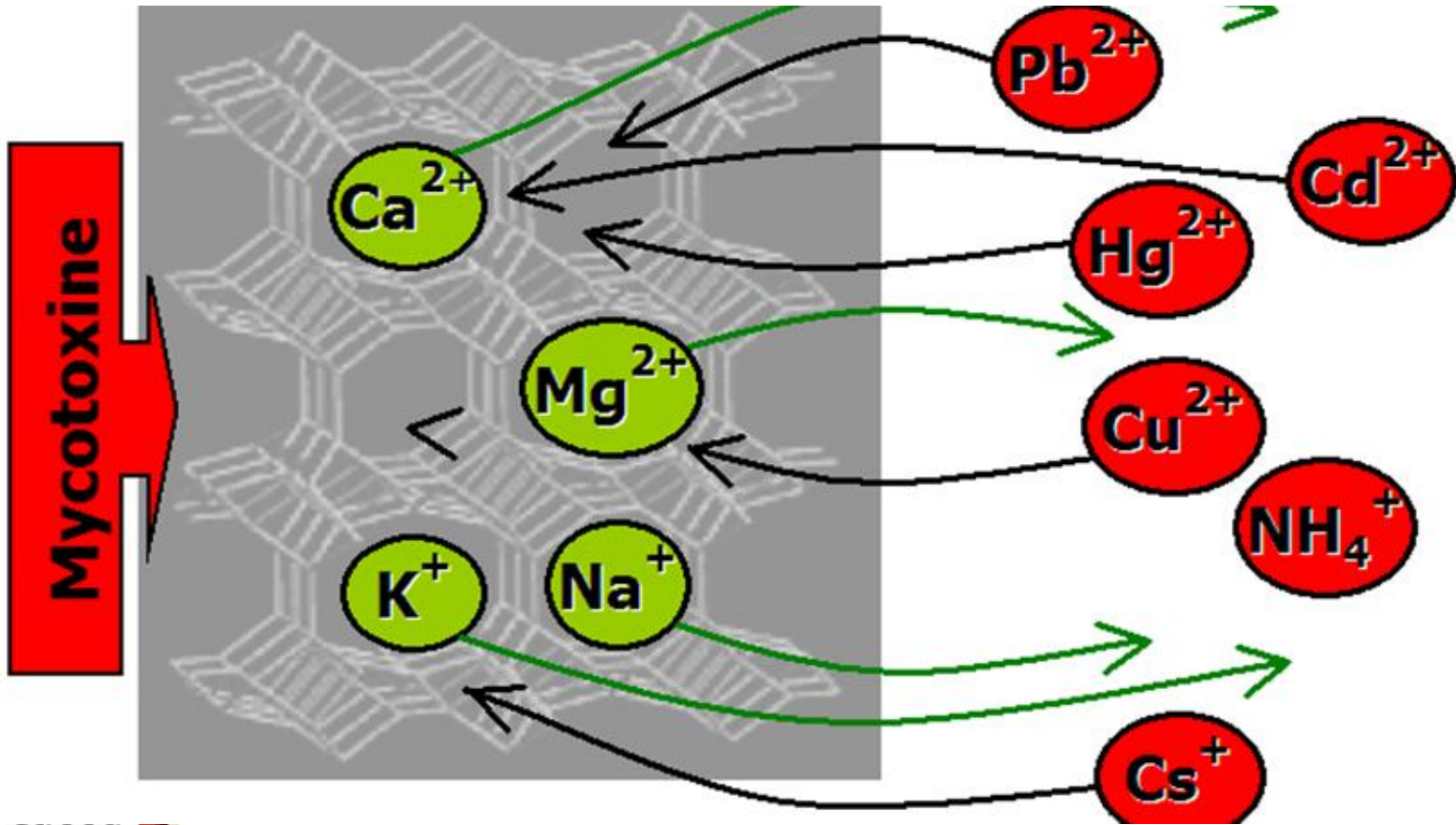


Toxins removal effect

- Clinoptilolite incorporated into the diet can reduce the deleterious effects of aflatoxin because it strongly adsorbs aflatoxins and zearalenone
- Mineral adsorbents based on natural zeolite and bentonite may be used in animal diets in order to prevent poisoning caused by mycotoxins
- Clinoptilolite incorporated into the diet at 1.5 and 2.5% was evaluated for ability to reduce the deleterious effects of 2.5 mg total aflatoxin on broiler chickens



Ion Exchanging



Radioprotection

- After dietary administration with 2.5, 5.0 and 10% zeolite, ^{137}Cs elimination increased, and the radionuclide deposition in liver, kidneys and femoral musculature decreased.
- The Clinoptilolite decontamination effects were observed with preventive administration, as well as with sorbent administration from 24 h after a single contamination of brown rats.



Removal of Heavy Metals and Organopoisoning

- The low resorption rate from the gastrointestinal tract, weaker clinical signs of intoxication, and longer life span for the onset of specific therapy are facts that create conditions for inclusion of natural zeolites in the arsenal of rational prevention and therapy of organophosphate poisoning.



Toxicology of Clinoptilolite

- In human medicine, zeolites have been used as antidiarrheal remedies, for the external treatment of skin wounds and athletes foot, and in kidney dialyses for the removal of ammonia ions from body fluids
- The beneficial effects of zeolites on hematopoiesis, and various disease states, including tumors, have been observed.
- No toxic effects were observed in toxicology study of Clinoptilolite.
- The physical status of examined animals showed no evidence of any harmful reaction during the studies



Toxicology of Clinoptilolite

- Clinoptilolite is well suited for these applications because of its large pore space, high resistance to extreme temperatures and chemically neutral framework.
- There are a few toxicology studies of Clinoptilolite obtained from different locations.
- The conclusion is that natural Clinoptilolite is not toxic and can be used in human as well as in veterinary medicine



DYNAMICLY MICRONIZED ZEOLITE

RDT



Milling vs. Micronization

- When zeolite rocks are “milled”, they are grinded to powder.
- This is normally takes place at the mine and it the cheaper way to create powder.
- The problem with the milling process is that it becomes unprofitable if smaller, biologically active particles are desired.



Micronization

- “Micronization” is the preferred way to make powdered zeolite while maintaining or even enhancing the benefits of the cage structure
- The micronization process is an expensive quality control
- When faced with the choice of milling the zeolite at the mine or shipping it out to a specialty micronization plant, many zeolite companies choose to mill the zeolite



RDT

RAPID DESINTEGRATION TECHNOLOGY

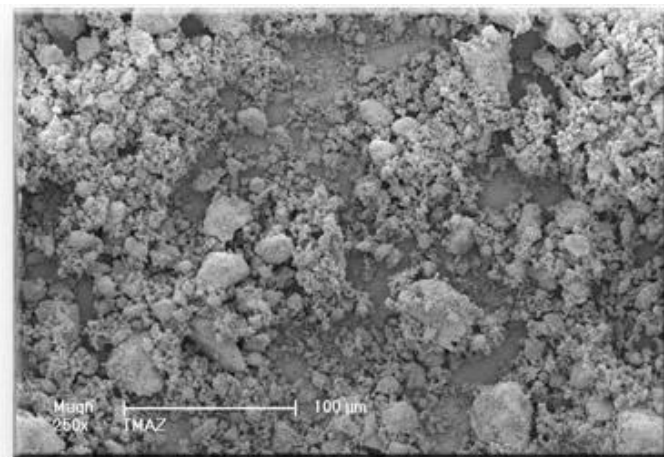
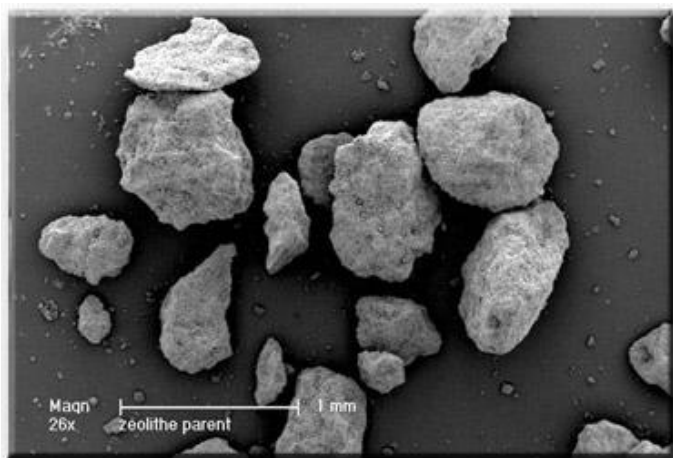
RDT

- Dynamic particle collisions- new approach towards micronisation technology
- Versatile technological platform
- Ability to improve properties of solid materials
- Cost-efficient production process (high level material throughput)



CHARACTERISTICS OF MICRONIZED ZEOLITE

- Particle size: 200 nm – 2 μm, Gauss distribution, 900nm peak
- Increased mesoporosity: portion of total mesoporous surface within BET total surface are exceeds 50%
- Due to mentioned characterists, the adsorption capacity is increased



Biological advantages of zeolite micronized with RDT



Bone formation

- Silicon in trace amounts enhances bone formation
- Micronized zeolite have therapeutic utility in osteoporotic individuals because of its ability to stimulate bone formation
- Micronized zeolite increases proliferation, differentiation, and transforming growth factor b production in normal, adult human osteoblast-like cells *in vitro*.
- In concentrations from 0.1 - 100 $\mu\text{g}/\text{m}^1$, zeolite induces a dose-dependent increase in DNA synthesis of normal human osteoblast-like cells.



Effects on gastrointestinal disorders

- Ten percent of Clinoptilolite as dietary supplements for swine and poultry showed that animals generally grew faster, and the number and severity of intestinal diseases were decreased
- In 1997, a new anti-diarrheic drug for humans were introduced based on the physical and chemical properties of a purified natural Clinoptilolite
- Zeolites can adsorb cholera toxin and *Escherichia coli* enterotoxins.
- This also have a beneficial role for children suffering spontaneous diarrhea by reducing enterotoxin activity.



Effects on Gastrointestinal Disorders

Factors that create conditions for inclusion of natural micronized zeolite in the arsenal of rational prevention and therapy of organophosphate poisoning:

- Lower toxins resorption rate from the gastrointestinal tract,
- Weaker clinical signs of intoxication
- Longer time span for the onset of specific therapy



TOXIN REMOVAL

Micronized Zeolite efficiently adsorbes following toxins:

- Mycotoxins (ochratoxin and aflatoxin B1) / AFB1 >OCHRA> ZEN
- Polycyclic aromatic hydrocarbons (benz-o-pyrene)
- Nitrosamines
- Acrylamide
- Dioxines
- Heavy metals (Pb, Hg, Cd, As) and ammonia

- Colloids Surf B Biointerfaces. 2005 Nov 25;46(1):20-5. Epub 2005 Sep 28. **Adsorption of mycotoxins by organozeolites**. Daković A, Tomasević-Canović M, Dondur V, Rottinghaus GE, Medaković V, Zarić S. Institute for Technology of Nuclear and Other Mineral Raw Materials, P.O. Box 390, 11000 Belgrade, Serbia and Montenegro.
- Chemosphere 58 (2005) 109–114 **Adsorption of nitrosamines in acidic solution by zeolites** Chun Fang Zhou, Jian Hua Zhu Department of Chemistry, Nanjing University, Nanjing 210093, China
- Journal of Colloid and Interface Science 280 (2004) 309–314. **The removal of heavy metal cations by natural zeolites** E. Erdem a, N. Karapinar b, R. Donat a, a Department of Chemistry, Faculty of Science and Arts, Pamukkale University, 20017 Denizli, Turkey-Department of Chemistry Engineering, Faculty of Engineering, Pamukkale University, 20017 Denizli, Turkey



ADSORPTION OF MICROORGANISMS

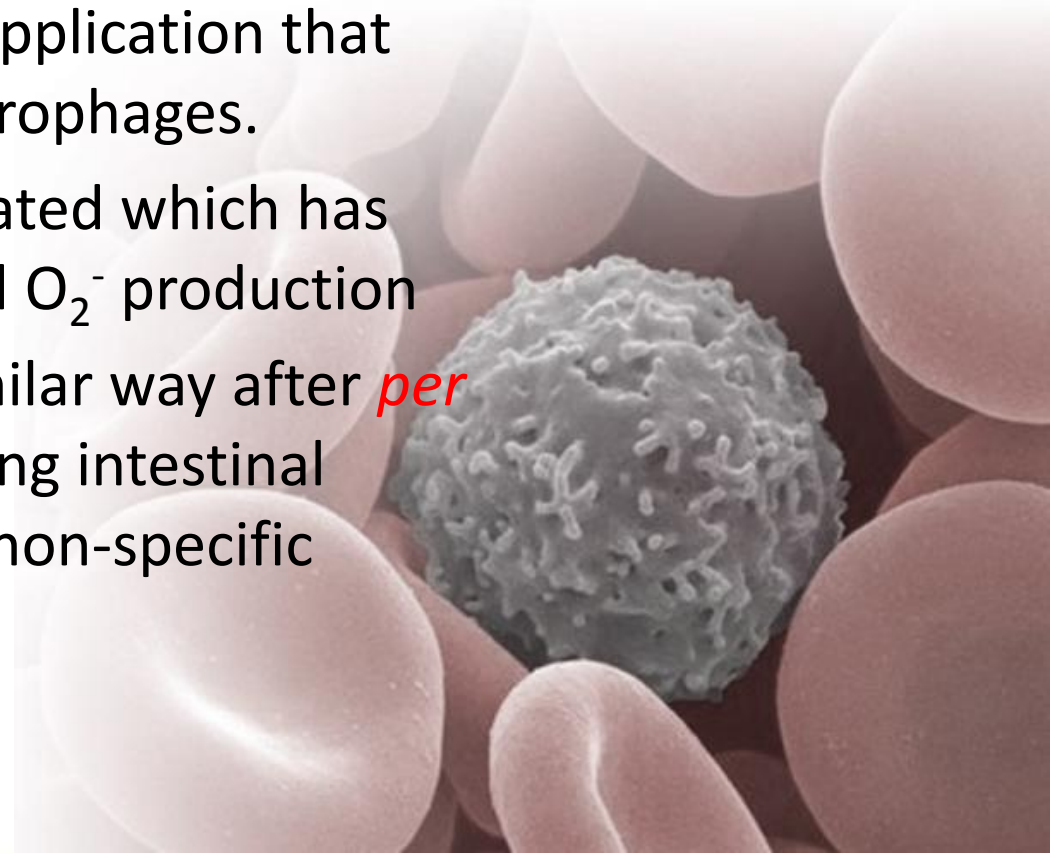
Micronized Zeolite (MZ) acts as efficient adsorber of:

- Bacteria (and bacterial toxins)
- Viral particles
- Colloids Surf B Biointerfaces. 2008 Jun 15;64(1):88-97. Epub 2008 Jan 25. **Selective adsorption of bacterial cells onto zeolites.** Kubota M, Nakabayashi T, Matsumoto Y, Shiomi T, Yamada Y, Ino K, Yamanokuchi H, Matsui M, Tsunoda T, Mizukami F, Sakaguchi K. Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba-ken 278-8510, Japan.
- Science Direct: Microporous Materials: 1387/1811 . **Antiviral properties of clinoptilolite,** Grce, Pavelic, Ruder Bosković Institute, Division of Molecular Medicine, Zagreb, Croatia



Immunomodulatory effect

- Micronized Clinoptilolite caused local reaction at the place of application that attracted peritoneal macrophages.
- Macrophages were activated which has been shown by increased O_2^- production
- Clinoptilolite acts the similar way after *per os* administration, affecting intestinal lymphocytes, producing non-specific immune response.

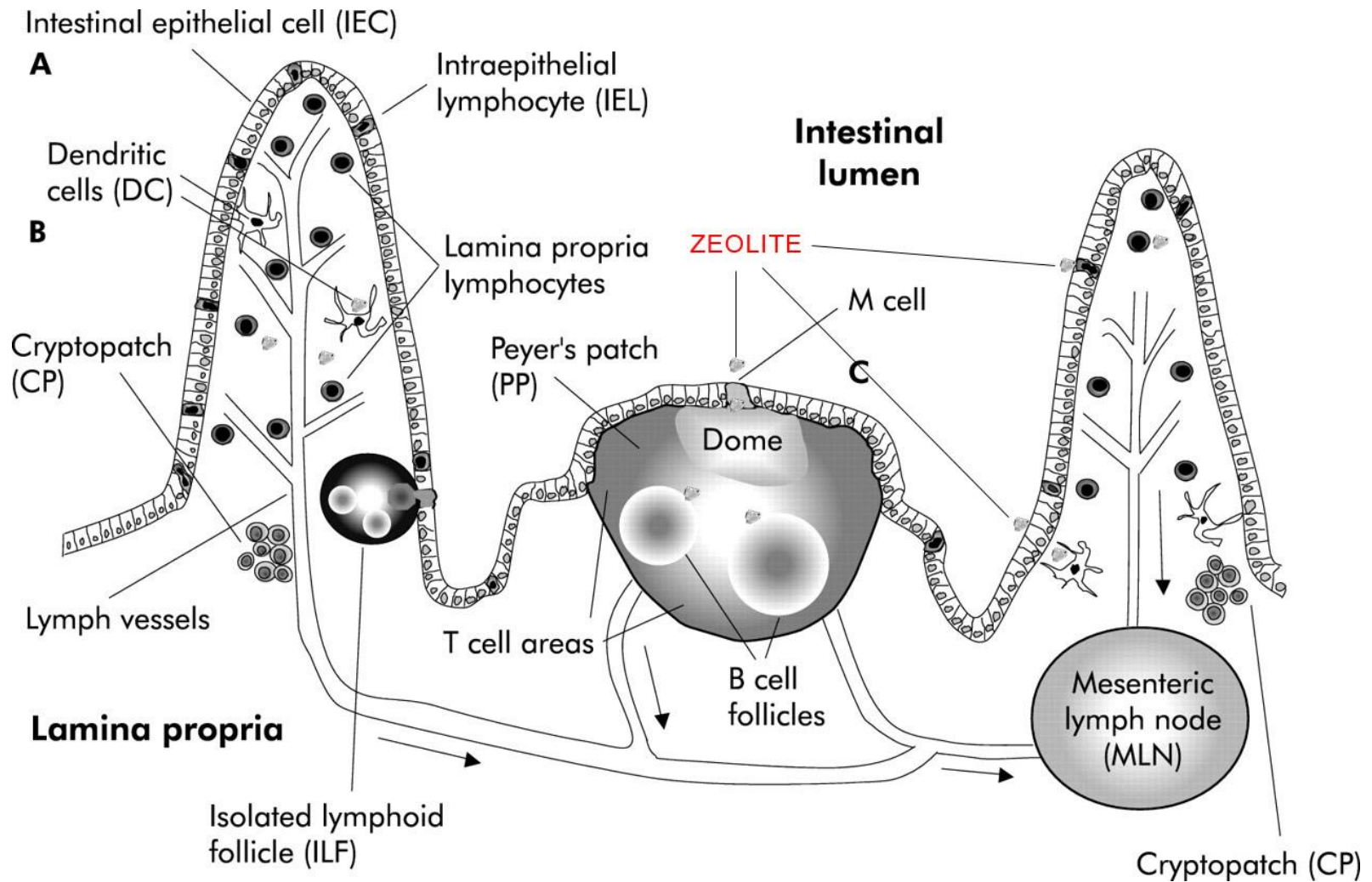


IMMUNE STIMULATION

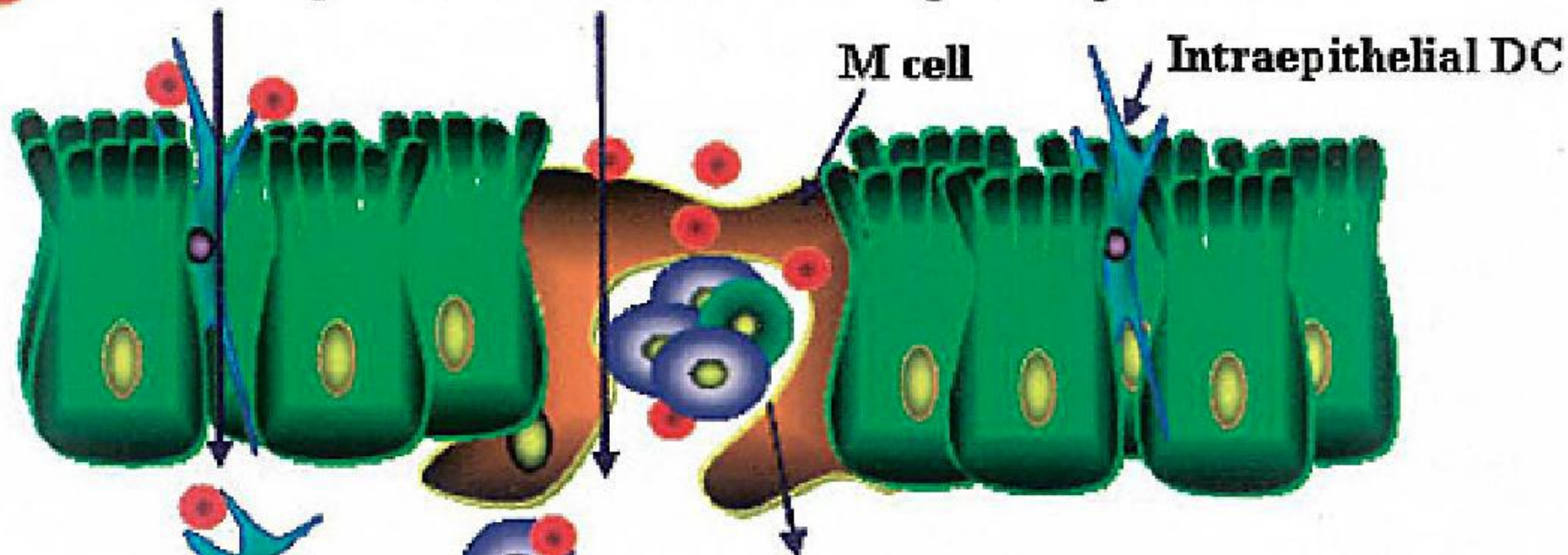
Mincronized Zeolite interacts with GALT (gutt associated lymphoid tissue) which induces:

- Increased macrophage and Dendritic Cells response (superoxide production)
- CD4 CD8 (cytotoxic T cells) proliferation
- J Cancer Res Clin Oncol. 2002 Jan;128(1):37-44. Epub 2001 Nov 10. **Immunostimulatory effect of natural clinoptilolite as a possible mechanism of its antimetastatic ability.** Pavelic K, Katic M, Sverko V, Marotti T, Bosnjak B, Balog T, Stojkovic R, Radacic M, Colic M, Poljak-Blazi M. Rudjer Boskovic Institute, Division of Molecular Medicine, Bijenicka 54, HR-10000 Zagreb, Croatia.
- Adv Ther. 2004 Mar-Apr;21(2):135-47. **Dietary supplementation with the tribomechanically activated zeolite clinoptilolite in immunodeficiency: effects on the immune system.** Ivkovic S, Deutsch U, Silberbach A, Walraph E, Mannel M. Megamin GmbH, Berlin, Germany





→ Nanoparticle interaction and capture by APCs



M cell

Intraepithelial DC

M cell pocket containing lymphocytes

Uptake of nanoparticles by APCs

Migration of APCs to the mesenteric lymph nodes



Effect on Tumor Growth

- Finely ground Clinoptilolite could serve as a new adjuvant in anticancer therapy.
- Such treatment of mice and dogs suffering from a variety of tumor types led to improvement in the overall health status, a prolonged life-span, and a decrease in tumor size.
- Local application of Clinoptilolite to skin cancers of dogs effectively reduced tumor formation and growth



Tumor Growth *in Vivo*

- The range of effects on tumor growth *in vivo* are diverse, ranging from partial reduction of tumor size, normalization of biochemical parameters and prolongation of life span.
- The best results in animal models were observed in the treatment of skin cancer in dogs, suggesting that adsorption of some active components is responsible for micronized clinoptilolite activity (direct contact action)

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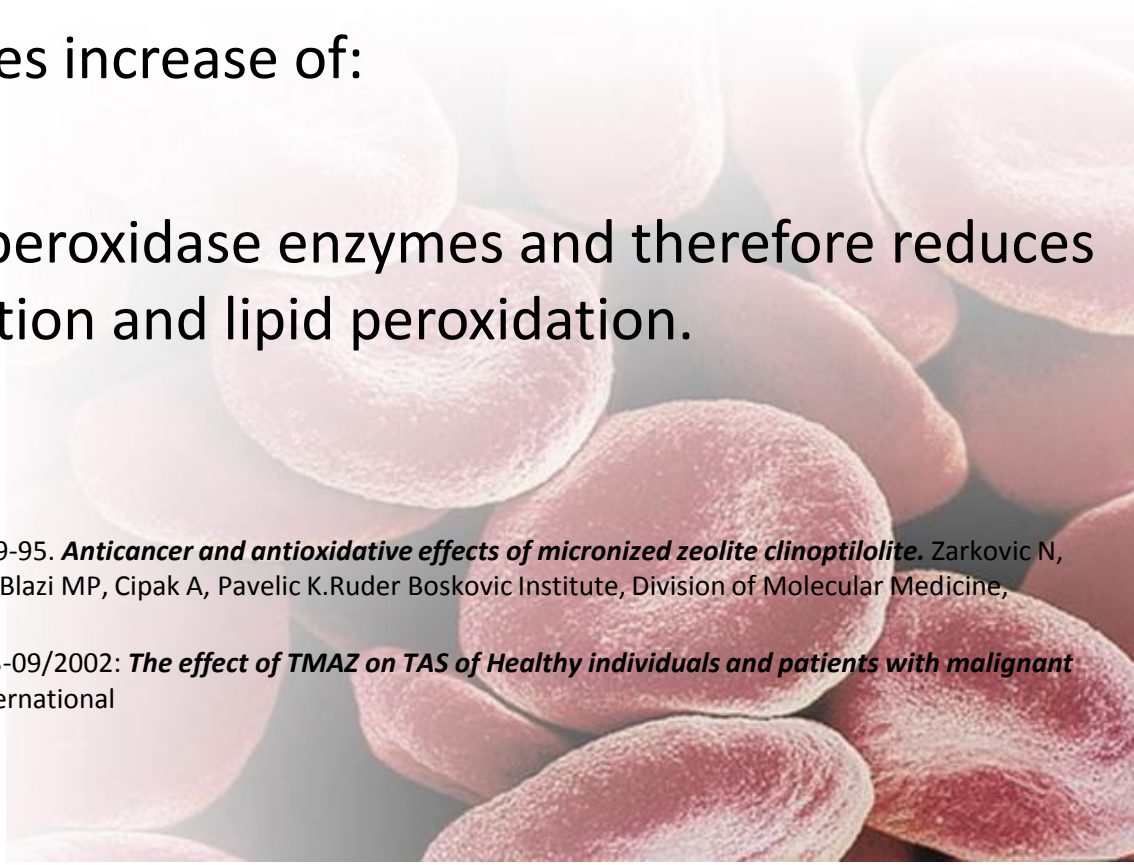
Date: February 2001 Pages: 708 – 720 **Natural zeolite clinoptilolite: new adjuvant in anticancer therapy**



PROMOTING ANTIOXIDATIVE ENZYMES

MZ indirectly stimulates increase of:

- SOD, Catalase and peroxidase enzymes and therefore reduces oxidation, peroxidation and lipid peroxidation.
- Anticancer Res. 2003 Mar-Apr;23(2B):1589-95. **Anticancer and antioxidative effects of micronized zeolite clinoptilolite.** Zarkovic N, Zarkovic K, Kralj M, Borovic S, Sabolovic S, Blazi MP, Cipak A, Pavelic K. Ruder Boskovic Institute, Division of Molecular Medicine, Bijenicka 54, HR-10000 Zagreb, Croatia.
- FREE RADICAL BIOLOGY AND MEDICINE 33-09/2002: **The effect of TMAZ on TAS of Healthy individuals and patients with malignant diseases.** Ivkovic S, Zabcic D. Megamin International



IMPROVED SKIN HEALING

MZ promotes faster skin injury repair through:

- Adsorption of exudate
 - Adsorption of inflammatory cytokines
 - Distribution of bioavailable silicon (collagen synthesis)
 - Activation of skin dendritic cells
 - Haemostatic activity
- J Trauma. 2007 Aug;63(2):276-83; discussion 283-4. **Comparison of a new hemostatic agent to current combat hemostatic agents in a Swine model of lethal extremity arterial hemorrhage.** Ward KR, Tiba MH, Holbert WH, Blocher CR, Draucker GT, Proffitt EK, Bowlin GL, Ivatury RR, Diegelmann RF. Department of Emergency Medicine, Virginia Commonwealth University, Virginia Commonwealth University Reanimation Engineering Shock Center, Richmond, Virginia 23298, USA.



IMPROVED BONE RECOVERY

MZ promotes faster bone growth and reduces bone resorption:

- Osteoblast differentiation
 - Osteoclast inhibition
 - Distribution of bioavailable silicon (collagen synthesis)
 - Better nutrient availability (food conversion)
-
- J Cell Biochem. 1995 May;58(1):39-46. **Zeolite A inhibits osteoclast-mediated bone resorption in vitro**. Schütze N, Oursler MJ, Nolan J, Riggs BL, Spelsberg TC. Department of Biochemistry, Mayo Clinic, Rochester, Minnesota 55905, USA.
 - **Autocrine signals promote osteoblast survival in culture** A Tumber, M C Meikle and P A Hill Bone Biology Unit, Department of Orthodontics and Paediatric Dentistry, Guy's, King's and St Thomas' Hospital's Medical and Dental Schools, Guy's Hospital, St Thomas' Street, London SE1 9RT, UK
 - Adv Drug Deliv Rev. 2005 May 25;57(7):959-71. Epub 2005 Apr 15. **Mechanism of osteoclast mediated bone resorption--rationale for the design of new therapeutics**. Väänänen K. Department of Anatomy, Institute of Biomedicine, University of Turku, FI-20700 Turku, Finland. kalervo.vaananen@utu.fi



MZ IN PET FOOD - OVERAL BENEFITS

- **Absorbent of (pathogenic) bacteria and bacterial toxins**
- **Anti viral properties**
- **Mycotoxins absorbent and removal**
- **Stimulation of immune system**
- **Promotion of antioxidant enzymes in the body**
- **Improved skin healing**
- **Improved bone recovery**



THANK YOU!



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