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**PANORAMIC VISION
OF 20 YEARS OF CSD-RESEARCH
AS INDICATION OF THE POSSIBLE INFLUENCE
OF EXTERNAL ENERGIES ON THE BIO-SUBSTRATA**

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This is a panoramic vision of 20 years of CSD-researches and other 20 years of data-studies on the possible influence of External Energies(EE) on the bio-substrata in comparison also with the studies of several authors on the other substrata, including human health (i. e.: in the space vectors). The author put in evidence Cosmic Rays and Solar Activity as dominant factors of External Energies influencing directly or indirectly the substrata in the biosphere, including CSD.

Key words: sectoring structure of the colonies, microorganisms, external energies, cosmic rays, solar activity.

The research about Colonies with Sector or Sectors Differentiated (CSD) was very simple and was born from some previous observations involuntarily effected on the routine of my microbiological analysis in hygiene laboratory of Milano.

I periodically controlled the bactericide efficacy of laboratory UV lamps. Some year before 1967 I noted the presence of CSD in reduced efficacy of bactericide power of the lamps and after the CSD impressed more particularly my curiosity during the preliminary searchers (1967–1969) about the variability of external air bacteria on the time. The CSD frequency variations were not so casual. And then I took further details about CSD from the microbial literature.

Lamanna and Malette [1] described sectors as: “Discrete portions of colonies from the parent strain growing simultaneous and competing successfully with the parent type. The formation of a sector may be favoured by a gene mutation early in the development of the parent colony. They may be due to gene recombination in a sexually reproducing heterozygous population, to segregation from heterozygous diploids, or to segregation of haploid uninuclear bacteria from multinucleate organisms.” In every way they considered the sectors presence such as a rare event.

I remark that a discernible character of sector may be the colour, the thickness, the transparency, the surface modelling, etc. (figures 1 A, 1 B). A sector-character, in a word, must be well different if compared with remaining part of colony. Therefore it's fundamental that these characters are well marked for the eyes of the researcher. I recommend an observation by stereomicroscope also until at 50x magnifications.

Servin Massieu [2] observed different types of colonies derived from a single strain of *Staphylococcus aureus* that had undergone in successive passages from broth to agar plates. They had sectored colonies on the about 10% of the total colonies. But Shapiro J. A. et al. say also that a sector does not constitute a rigorous proof of a mutation [3]. Witkin E. M. [4] studied cultures subjected to the mutagenic influence of UV light, resulting in formation of lactose positive and lactose negative sectors. The colonies with sector or sectors differentiated or CSD is a complex phenomenon that may involve changes that are not considered at all from several authors under the rubric of mutations such as plasmid loss or chromosome duplication [1–6]. Shapiro J. A. [5, 6] interprets CSD as indicating that certain (internal) regulatory switches during colony development favour the occurrence of the underlying genetic changes.

Seemingly spontaneous and mutagen (i. e., UV light induced sectoring) the CSDs continue to be very interesting.

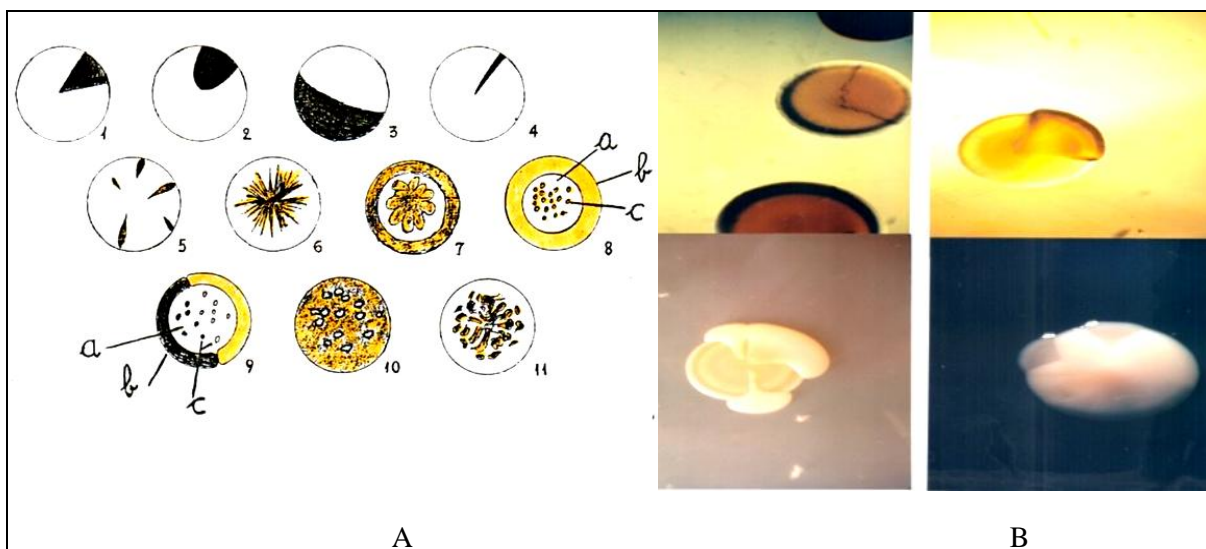


Fig. 1. Atypical colonies from air bacteria of *Staphylococcus aureus* strains (A) and photo of CSD from air bacteria (B) on the surface of the tryptose agar. Atypical colonies considered as CSD: 1, 2, 3 and 4. Other atypical colonies, from 5 to 11, were very exceptional: these were not considered. Microcolonies (c) are noted in lysis area (a), in ring-colonies (b) of 8 and 9; ring not differentiated (8) and ring differentiated (9). The colonies from 6 to 11 were noted in the 1987 when the CSD frequency was higher than in every other years considered. The lysis areas were probably provoked from bacteriophages that may modify the DNA of bacterium through a transduction mechanism. This mechanism may be induced also by mutagenic radiations (i. e.: Cosmic rays, Ultraviolet rays, etc.).

I experimentally individualized CSD as a detector of biological type in negative correlation with Solar Cycle [7–18]. A detector that would represent also, in according with the literature, the presence of a possible mutation and a possible involvement of the DNA to favour a selection in the bacterial population. (Relationship of some external signal with living beings in the Bacterial-Cell-Population (BCP). External Energies (EE) as factor influencing from space on Biosubstrata).

In the BCP, the mechanism that preserves his kind does not utilize Self-Biological Compensation (SBC) that may preserve the life of the single cell, but utilizes only a selection of the whole population pro-someone that may correspond again to a new environmental situation. This in damage of the BCP, where many bacteria are dying soon.

The quick rule that seems to be absolutely valid for bacteria cells species is: to a fast extinction must correspond a fast new reproduction. In More Complex Beings (MCB) on the contrary, the SBC of the species requires less hecatomb of the single one and more tendency on individual Auto Compensation Mechanism (ACM). In fact the ACM is very more valid as anymore if a living being is complex and his species is evolved. This ACM may more support the life and the evolution of living organisms.

Concluding, the results are here a document that remarks as natural physical influence from the space (EE) may be involved in modulating environmental changing. The bacteria suffering changing (i. e.: giving sectors in CSD) may survive or not through a selection on his whole population more congenial to the Species-outliving.

Chizevskiy [19] already hypothesized since 1930, a possible correlation between solar activity and biological phenomena (big epidemics in the USSR). Moreover he compared the solar activity also with social and medical phenomena. He made a long and careful investigation ascertaining on the basis of epidemiological data, that some of the epidemics followed a cyclical pattern similar to the another one expressed from SA (Solar Activity / Wolf's Number). In 1965, a year after his death, his researches were recognized as studies of considerable scientific value (Commission set up by the USSR–Academy of Sciences).

I knew Prof. Piccardi G. and his studies after the beginning of CSD experiments with agar tryptose plate-expositions to external air. The preliminaries of this research were before, in November

of 1969 [7] during a research on the seasonal variability of air bacteria. But a standard CSD-research was only from February of 1970. I met Piccardi past a year about. It was in the University of Milan, through common colleagues and with big surprising of me, Piccardi was very gentleman and was very interested of experimental trials on CSD of this young researcher. He said to me vigorously: “you are on the right road, you must insist, you are bound to insist...”. We at last, together shook warmly our hands. The moving meeting, face to face, was concluded wishing ourselves in this way: “At the best!”. And then I continued daily my research till 1991, conquered from the remarkable life of Piccardi and his extraordinary researches. Piccardi [20–25], supported the hypothesis of Chizevskiy with his experimental data. He experimentally devised a detector colloidal system that showed a correlation between colloidal precipitations and Solar Activity. Moreover as Piccardi said, we can remember also the Cosmic Radiations (CR) as influencing directly the Electromagnetic Waves of Low Frequency (3 Hz and 10 Hz) and the VLF waves (3–30 KHz) in the ionosphere.

My research was daily conducted from 1970 to 1991 [7–18] on more than 4 million of microbial colonies, showing cyclical variations of frequency of colonies that I called CSD. The CSD numeration was considered as percentage of total counted colonies (excluding micromycetes) and independently from the number of sectors in this sense not considered. After the preliminary searches 1967–1969, I continued systematically my experimental study about CSD on three phases: with the external air bacteria from 1970 to 1983 (the first phase) with the *S. aureus* strains from 1984 to 1991 (the second phase), In fact these bacteria showed a greater frequency of the CSD during the 1st phase in confronting the other bacteria. The *S. aureus* strains had curves of CSD frequency at all similar to those ones found for the external air bacteria (fig. 2) in spite of standard conditions of these strains culture of *S. aureus* always maintained in laboratory room. In this way I concluded that the factors, influencing the CSD, proceeded also through the several screens of a confined-site (i. e.: room, thermostat, refrigerator, etc.). And then I carried out another research in Gran Sasso National Laboratories, (1–11 November 1990) [11], on CSD given from *S. aureus* strains simultaneously under several experimental conditions (the third phase). The various monitory points were:

- a) 1000m above sea level in Gr. Sasso laboratories on superficial ground;
- b) 1000m above sea level, in Gr. Sasso laboratories under rock 1400m thick;
- c) Sea level in laboratories of my Institute of Rome (Presidium Multizonal of Health Prevention).

The results of CSD frequency were medially: (a) 11%, (b) 4% and (c) 9%.

The data before said were calculated as percentage of total *S. aureus* colonies counted daily of the *S. aureus* tested point by point; the colonies counted in this trial were in total 50 685. It was very interesting to note that the lowest percentage of *S. aureus* tested was always to referring to the point (b). It was to considering especially that the 4% of (b)-point, showed the presence of a very penetrating EE with possible “mutagenic” activity even also under rock 1400m thick.

I also noted a positive correlation of CSD frequency with Cosmic Rays (neutron flux) and a negative correlation with Solar Activity (Wolf’s N) (fig. 2). Subsequently I thought opportunely to have a deep statistical study of data collected, through the collaboration of other researchers to confirming his results-interpretation.

And then my collaborators confirmed the interpretation of me and my principal intuitions advanced previously in 1971 and in 1974 [7, 8], as the ELF, the CR, the SA and the GM (geo magnetic activity). In this collaboration it appeared confirmed clearly that the CSD frequency is essentially a fluctuating phenomenon with the typical periodicity occurring every eleven years; in significant correlation negative with the SA (solar activity expressed from the variations of the sunspots); the SF (solar flow in the range of 2800MHz), the ELF (electromagnetic waves of low frequency of 3Hz and 10Hz), the GM activity (electromagnetic storms) and is in significant correlation positive with the CR (Neutron Flux). The collaborators that studied my data more deeply with statistical elaborations were: the physicians M. DeMeyer, 1984 (Astr. Observe. di Liegi) [9]; J. Ormenyi, 2002 (Meteor. Inst. of Budapest) [26]. G. Villoresi and coll. (Univ. la Sapienza, Dip. Fisica, Roma) [13].

Particularly interesting was the collaboration with Prof. F. Halberg and his team 2002 [14, 15] (Minneapolis Univ. Minnesota, USA). It’s important to cite his chronobiology study on the correlation of hearth infarction and magnetic storms [27]; and it is to remember also, in this sense, the previous studies of Villoresi with Russian scientists [28]. Other important collaborators were A. A. Konradov,

T. A. Zenchenko, B. M. Vladimirovsky, 2005 [18]. It resulted at all the non-causality of the variations of CSD frequency in this research. The fundamental findings for me were: the significant negative correlation of the SA with the CSD frequency and the significant positive correlation of the CSD frequency test with CR. Correlation deducible also indirectly from the significant negative correlations between the SA and the CR (fig. 2). These high physical energies may obviously influence directly the CSD frequency, but for these ones how it's possible to have an indirectly influence on the CSD frequency?... When the SA is high, solar storms occur, giving geomagnetic storms. CSD frequency is in negative significant correlation with geomagnetic storms [14] and the solar wind (SW) with solar masses of matter emitted from solar corona, reaching the magnetic-sphere and overcomes partially this barrier; the SW acts on the upper atmosphere and here produces an ionization that influences the ELF and the VLF. These ones at last may influence the CSD frequency that is in negative significant correlation with them so as J.'O'rmenij [26] remarked, in collaborating with me. This was after confirmed by Russian and Ukrainian collaborators who gave more value to the direct effect of ELF and VLF on the CSD frequency, compared to the other EE in general sense [18].

The CR too, acting on the upper atmosphere produce a ionization and are in accordance at all with SA in realizing the next phases before seen for SA. Therefore we may conclude that the SA and the CR may influence also indirectly the CSD frequency. I conclude that in this way the CR and the SA show to be the dominant factors in comparison to other EE. I now remember the Piccardi words said concluding his relation on the fluctuating phenomena in the International Congress of Montreux (1969): "... I cannot hope to understand this phenomenology, I'm hardly aware of this one. — It's evident that the sensitive systems, and especially the living organisms, can show the deep things existing in the universe and they can show them to us much better than physical instruments, being always strictly selective and therefore unable to notice a general situation. — and then — ... the tool will be once again a sensitive system (and therefore heterogeneous, out of his equilibrium and very complex) selected among the most complex sensitive systems in the world. The word will be left once again to the study of the fluctuating phenomena and the sciences of life" [29, 36]. I well remember that he took an interest on CSD for pure chance and thought in conscience that CSD test may correspond to the words before said from Piccardi, who perhaps perceived this through his deep intuition during their conversation had in Milan-University. I was won from Piccardi's face brightened by a great unexpected enthusiasm and then it was not possible for him to do not go on for other many years again in my CSD research.

Now I want conclude showing the more important differences between CSD frequency and the three variations of the Piccardi's colloidal-test (tab. 1) It is important remember that Piccardi on his research had also a very good collaboration for many years, of Mad. C., Capel-Boute, [30–33], dr. of Bruxelles University:

Table 1. Colloidal-Piccardi's tests in confronting with CSD test.

Colloidal test of Piccardi:	Test D	Test F	Test P
Secolar Variation:	Positive correlation with solar activity (1).	Slightly positive correlation with solar activity (1).	Positive corr. with solar act., in Florence and negative (2) in Tubinghen and Jungfrau.
Annual Variation:	Minimum in March and August but less in August (3).	Minimum on the summer. Maximum on the winter (4)	Variations with terrestrial, solar and cosmic radio electric. emissions.
Short-period Variation :	Increasing with cosmic radiations.	Increasing (5) with solar eruptions and magnetic storms.	sensibility to the artificial electromagnetic waves.

(1) test D and F: in CSD test on the contrary, this correlation is negative.

(2) test P: similarly, in CSD test, this correlation is negative.

(3) test D: similarly, in CSD test, a less evident minimum is generally in September, during the year.

- (4) test F: similarly, in CSD test, the most evident maximum is generally in November, during the year.
- (5) test F: For the correspondence of the increasing of the solar eruptions, the magnetic storms and the CSD Frequency, see please the paper n°[14] of the Bibliography, here it is shown that also the SA may increase CSD freq. but some day later and when his activity is very high and on short period. And it has to remarking indeed that CSD are always increasing constantly on long term, when the CR are increasing (Fig. 2).

Substantially I confirmed what Piccardi said in his test [21–23]) :

- that external signals from non-traditional variables if can induce tangible effects on substrata of colloidal solutions, may also influence the bacteria substrata;
- that the variations during the time, of colloidal data and of CSD data, are both to consider at all typical of fluctuating phenomena;
- that the CR, SA and the electromagnetic waves are EE considered by the both researchers;
- that CSD frequency curves are less disturbed by interferences.

I refer here several recent researches [34] in confirming of the necessity to deep the role of SA and RC as dominant factors between the other EE (as I remarked again recently on the 2011 [35]).

Variations of the ozone-hole and Cosmic Rays (CR). Canadian researchers recently, found several significant correlations between size variations of the ozone-hole and CR (1981–1992 and 1990–2007) in the Antarctic Hemisphere (0–65 degrees, south latitude). The researchers noted effectively a significant positive correlation between the CR Activity and the enlargement of the ozone-hole. When SA will be very much low there is also a low possibility that SA neutralize the cosmic rays effects in our biosphere and then we have consequently a more enlarged ozone-hole. These researchers hypothesized also a probable confirming of these correlations in the next years 2008–2009 and 2019–2020 [37].

Cosmic ray decreases affect atmospheric aerosols and clouds? Henrik Svensmark National Space Institute, Technical University of Denmark, Copenhagen, Denmark et al.). Svensmark says: Close passages of coronal mass ejections from the sun are signalled at the Earth's surface by Forbush decreases in cosmic ray counts. We find that low clouds contain less liquid water following Forbush decreases, and for the most influential events the liquid water in the oceanic atmosphere can diminish by as much as 7%. Cloud water content as gauged by the Special Sensor Microwave Imager (SSM/I) reaches a minimum 7 days after the Forbush minimum in cosmic rays, and so does the fraction of low clouds seen by the Moderate Resolution Imaging Spectroradiometer (MODIS) and in the International Satellite Cloud Climate Project (ISCCP). Parallel observations by the aerosol robotic network AERONET reveal falls in the relative abundance of fine aerosol particles which, in normal circumstances, could have evolved into cloud condensation nuclei. Thus a link between the sun, cosmic rays, aerosols, and liquid-water clouds appears to exist on a global scale [38].

Correlation between cosmic rays and temperature of the stratosphere? What they observed was a strikingly close relationship between the cosmic-rays and stratospheric temperature this they could understand: the cosmic-rays, known as muons are produced following the decay of other cosmic rays, known as mesons. Increasing the temperature of the atmosphere expands the atmosphere so that fewer mesons are destroyed on impact with air, leaving more to decay naturally to muons. Consequently, if temperature increases so does the number of muons detected. What did surprise the scientists, however, were the intermittent and sudden increases observed in the levels of muons during the winter months. These jumps in the data occurred over just a few days. On investigation, they found these changes coincided with very sudden increases in the temperature of the stratosphere (by up to 40°C in places!). Looking more closely at supporting meteorological data, they realised they were observing a major weather event, known as a Sudden Stratospheric Warming. On average, these occur every other year and are notoriously unpredictable. This study has shown, for the first time, that cosmic-ray data can be used effectively to identify these events [39].

Cosmic pattern to tree growth. The growth of trees appears to follow a cosmic pattern, with trees growing faster when high levels of cosmic radiation arrive from space. When the intensity of cosmic rays reaching the Earth's surface was higher, the rate of tree growth was faster. The effect is statistically significant. Experiments in space have shown that cosmic rays can have some positive impacts on biological materials [40]. Gromozova E. et al. [41] showed highly significant (close to the

line) connection of the metachromasy reaction of polyphosphates of volutin granules in yeast with cosmic rays. They discussed the role of cosmic factors in evolutionary processes.

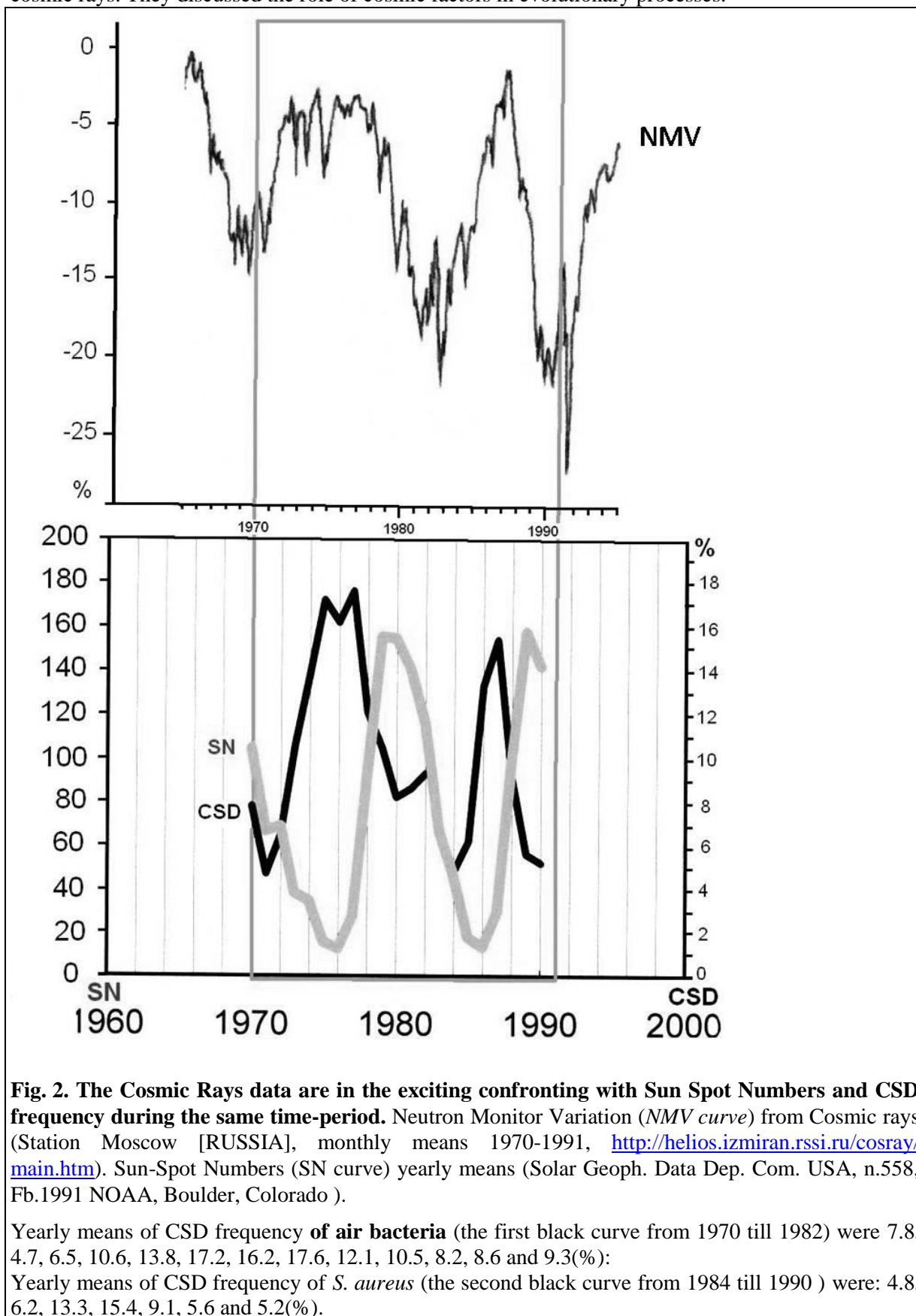


Fig. 2. The Cosmic Rays data are in the exciting confronting with Sun Spot Numbers and CSD frequency during the same time-period. Neutron Monitor Variation (NMV curve) from Cosmic rays (Station Moscow [RUSSIA], monthly means 1970-1991, <http://helios.izmiran.rssi.ru/cosray/main.htm>). Sun-Spot Numbers (SN curve) yearly means (Solar Geoph. Data Dep. Com. USA, n.558, Fb.1991 NOAA, Boulder, Colorado).

Yearly means of CSD frequency of air bacteria (the first black curve from 1970 till 1982) were 7.8, 4.7, 6.5, 10.6, 13.8, 17.2, 16.2, 17.6, 12.1, 10.5, 8.2, 8.6 and 9.3(%):

Yearly means of CSD frequency of *S. aureus* (the second black curve from 1984 till 1990) were: 4.8, 6.2, 13.3, 15.4, 9.1, 5.6 and 5.2(%)

Sun and global warming: a cosmic connection. In February 2007, depending on what newspaper you read, you might have seen an article detailing a “controversial new theory” of global warming. The idea was that variations in cosmic rays penetrating the Earth's atmosphere would change the amount of cloud cover, in turn changing our planet's reflectivity, and so the temperature at its surface. This, it was said, could be the reason why temperatures have been seen to be varying so much over the Earth's history, and why they are rising now. The theory was detailed in a book, *The Chilling Stars*, written by Danish scientist Henrik Svensmark [42] and British science writer Nigel Calder, which appeared on the shelves a week after the Intergovernmental Panel on Climate Change (IPCC) had published its landmark report concluding it was more than 90% likely that humankind's emissions of greenhouse gases were warming the planet. “I think the Sun is the major driver of climate change” — Henrik Svensmark. In truth, the theory was not new; Dr. Svensmark's team had proposed it a decade earlier, while the idea of a cosmic ray influence on weather dates back to 1959 and US researcher Edward Ney. The bigger question is whether it amounts to a theory of global warming at all.

After the remarking before said, I think that is opportune also to referring the results obtained from several researchers in referring of the influence of external energies on the bacterial substrata in the space-vectors recently (Communication reported in the Proceeding of the IX International Conference of Crimea, Sudak, 2011 [35]). This to confirming the possibility that in the space the bacterial mutation are frequent. This does not exclude that an analogue phenomenon is on the biosphere more in the altitude and less on the ground. The CSD are a useful test of this possibility. The CSD, gives more confirmation of validity as test, especially after the Gran Sasso trials [11]. The ability to detect bacterial mutations in correlation with the external energies is corroborated beyond any doubt, from a US studies done on four bacteria isolated in a space-shuttle during spaceflight (and the bacteria were obviously on higher exposure to cosmic radiations, in this vector). The researchers G. Fox and R. Wilson shown that genetically (by DNA and RNA analysis) the bacteria isolated from the space shuttle, suffered relatively frequent mutations, giving modified microbial strains, able to causing disease risks for the health of astronauts [43].

Bacteria of the genus *Salmonella* grown in the space, aboard the space-shuttle, have shown unusual genetic activity becoming more virulent [44]. The discovery could provide valuable guidance in the development of drugs for our terrestrial use in the prevention and medical care for extended missions in the space. It is well known that the permanence in space has significant effects on human physiology. This permanence weakens the muscles and skeletal system; is also detrimental to the human immune system. The study also shows that the space-effect on microorganisms could be so much radical: *Salmonella* strain grown on the space-shuttle, is several times more deadly than *Salmonella* grown in the land. This research indicates that NASA and other space agencies should consider that the extended space travels will increase the virulence of microorganisms that inevitably will be brought by the astronauts on board. The research team, led by Cheryl Nickerson [44], an associate professor at Arizona State University, Biodesign Institute, found that *Salmonella* grown in the Space Shuttle mission STS-115, in 2006, expressed deep genetic modifications. When this Space-strain was administered to mice, after returning to the Earth, this *Salmonella* was more deadly than the strains grown on the Earth-strain. This was the first experiment that has studied the genetic changes in microorganism on the space. The researchers have shown that a particular gene of *Salmonella* regulates the most part of the detected molecular changes. This global regulator, which seems to help the bacteria to react to the stress by becoming more virulent, is a possible target for developing therapies against *Salmonella*.

The bacterium *Pseudomonas aeruginosa*, has caused, in the 1970, the unique severe infection till now, of the astronaut. In returning to our planet with Apollo 13, the astronauts left the main part of the space-shuttle to close themselves in the lunar module, on returning to Earth after an explosion on board of Apollo 13. There, they suffered conditions of low oxygen, water and energy. In these difficult conditions, the astronaut Fred Haise developed a severe prostate infection caused by *Pseudomonas*. He was seriously ill for weeks, after his return [44].

Most of the infections contracted by astronauts in space, were light still now, even though it was never caused by *Salmonella*. And so as it's seen in the examples just mentioned the cosmic rays are therefore of great importance to give genetic changes in biological substrates Klaus D. M. and Howard H. N. remarked that various factors associated with the space flight environment have been

shown to potentially compromise the immune system of astronauts, increase microbial proliferation and microflora exchange, alter virulence and decrease antibiotic effectiveness [45]. An acceptable resolution of the above concerns must be achieved to ensure safeguard and efficient space habitation

Peter W. Taylor and Andrei P. Sommer hypothesised that in the next 15–30 years much extended space flights will become a reality and astronauts will be likely to spend at least very long time away from Earth. Time spent in such extreme environments will result in a diminution of immune status and profound changes in the human bacterial microflora. In microgravity, the efficacy of antibiotics is reduced and microbial mutation rates increase very much. These factors will impinge on the capacity to treat effectively the infections that will doubtless arise during such long and stressful travels [46].

May I hypothesize that the role of cosmic energies could be very much important as determining factor in influencing not only the human health but also his same ageing process and perhaps the end of the same human existence? The answer to this query could give perhaps by geneticists of the future.

I wish that some researcher may repeat on the next future, my daily trials in Gr. Sasso but it should be opportune to realize these trials for more lengthy period than the time spent in these trials before said, suspended for reasons beyond our best willing. This to more deepening the phenomenon of CSD variability, on superficial ground and under rock 1400 thick.

A researcher must remember always what dr. A. Carrel (Medicine/Nobel) said: “Short observing and much reasoning lead into the error; much observing and short reasoning lead into the truth”.

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**Панорамный обзор 20 лет СДК-исследований
как указание на возможное влияние внешних энергий на био-субстраты**

Это обзор 20-летних исследований секторной дифференцировки колоний (СДК) и других данных по возможному влиянию внешних энергий на био-субстраты, в сравнении с результатами других авторов, полученными на других объектах, включая организм человека. Автор рассматривает Космические лучи и Солнечную активность как главные внешние энергетические факторы, влияющие непосредственно или опосредовано на объекты биосферы, включая секторную дифференцировку колоний (СДК).

Ключевые слова: секторная структура колоний, микроорганизмы, космические лучи, солнечная активность.