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Practical Report

Antifungal Activity of *Capsicum frutescens* and *Allium cepa* against *Aspergillus* spp.: An Application of Scientific Process Skills by High School Students

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Aspergillus niger and *Aspergillus flavus* are known to induce risks including aspergillosis in humans and common crop drought to plants. *Allium cepa* (white onion) and *Capsicum frutescens* (cayenne pepper) have been reported as having some antifungal potential. Thus, to practice scientific process skills, high school biology students investigated whether *A. cepa* and *C. frutescens* extracts are effective antifungal agents against these two pathogens. Sensitivity testing using Kirby-Bauer assay revealed that *C. frutescens* was more effective against *A. niger* and *A. flavus*. *C. frutescens* extract alone produced an inhibition zone of 19.29 *mm* for *A. niger* and 10.47 *mm* for *A. flavus*. Using *t*-test and repeated measures ANOVA (95% level of confidence), the results were comparable to an antifungal drug miconazole. It is therefore concluded that *C. frutescens* or the mixture of *C. frutescens* and *A. cepa* extracts (50-50 v/v) can be effectively used as antifungal agent against *A. niger*. This study possibly serves as a model for students to learn the scientific method practically and to experience different process skills essential in biological research tangibly.

Keywords: Allium cepa, *antifunal activity*, Aspergillus niger, Aspergillus flavus, Capsicum frutescens, *laboratory model for high school biology*

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INTRODUCTION

Aspergillus spp. are fungi commonly present in the air that people breathe (Yassin and Almouqatea, 2010; *cf.* CDC Website). There are more than 185 known species of *Aspergillus*, and at least 20 of them have been reported to cause human diseases, such as aspergillosis, pneumonia and fungus ball which attacks the lungs (Yassin and Almouqatea, 2010; Choudhury *et al*., 2011).

The most common species of *Aspergillus* are *A. niger* and *A. flavus* – both species cause diseases in human beings and plants. Moreover, *A. niger* is one of the top three causatives of human fungal diseases (Barker and Carrington, 1953; Choudhury *et al*., 2011). *A. niger* is very versatile and not fastidious, allowing itself to grow in different environments and microhabitats where the other fungi cannot. *A. niger* also infects plants, specifically ginger, onion, peanut, grapes and mangoes. It produces toxins that induce crop or fruit rotting rendering them unsafe for human consumption (U. S. EPA Website, 1997; Choudhury *et al*., 2011).

On the other hand, *A. flavus* produces aflatoxins that cause rotting in plants. It usually infects the seeds of plants like corn and peanut. However, manifestation of the infection becomes apparent only in the post-harvest and storage stages. As a result, the infected seeds would then not be useful any more (Montes-Belmont and Carvajal, 1998).

Corn is the second most important crop in the Philippines after rice. An annual report generated by the Bureau of Agriculture Statistics (BAS) in 2012 indicated that the crop sector grossed a total of 375.1 billion Philippine pesos, which is 51.79% of the total production of the agriculture sector, and 60% of which comes from the crop sector and 6% is attributable to corn (PSA Website, 2014). If an outbreak of these species of *Aspergillus* arises in the Philippines or even in neighboring Asian countries, the agricultural industry would certainly be paralyzed. With such unfortunate event, the poultry and livestock industries would also be affected because corn is one of the main sources of feeds in the country (Montes-Belmont and Carvajal, 1998). Sixty percent of corn produced in the Philippines (the average is around 3.21 metric tons per hectare) is used for feeds in the livestock and poultry sectors, while the remaining is used for human consumption. This would ultimately have a negative effect on the economy since the combined corn, livestock, and poultry industries are approximately 30% of the total agriculture sector of the Philippines (PSA Website, 2014).

Fortunately, natural products have been proven to inhibit the growth of these fungal species. For example, *Allium cepa* (white onion) has been reported to have some useful medicinal properties: It has anti-inflammatory, anti-asthmatic and antimicrobial properties and was even found to have a good effect on the cardiovascular system (Santas *et al.*, 2010). Moreover, onion has the potential to be a fungicidal agent. Species of *Aspergillus* and *Candida* were not able to reproduce when onion extracts were applied (Benkeblia, 2004). Also, Lanzotti *et al*. (2012) reported that three saponins in onion had a high antifungal activity. *Capsicum frutescens* (cayenne pepper) or locally known as "siling labuyo" in the Philippines, is usually used in food preparation and for homemade remedies (Cichewicz and Thorpe, 1996). De Lucca *et al.* (2006a) reported that it had an antifungal property due to a certain saponin called CAY-1.

In these studies, the antifungal properties of both *A. cepa* and *C. frutescens* were characterized. However, the effectiveness mainly was examined on the dosage of each sample. Since *A. cepa* and *C. frutescens* have different types of saponins (De Lucca *et al.*, 2006a; Lanzotti *et al.,* 2012), it is presumed that the two would complement each other and become a stronger fungicidal agent. Therefore, in the present study, the combined antifungal effects of the extracts of *A. cepa* and *C. frutescens* on the growth of *A. niger* and *A. flavus* were investigated.

It should be noted that the present study came about after high school students were immersed to the different concerns about the safety of food crops and the development of antimicrobial agents through the works of Pandey *et al.* (1982) and De Lucca *et al.* (2006b) with further reinforcements from local news articles and stories in Philippine provinces. The students also learned from Yassin and Almouqatea (2010) that scientists had been producing much safer antifungal agents from plants in comparison to synthetic or artificial fungicides, which might be harmful to both plants and humans.

MATERIALS AND METHODS

Preparation of Test Organisms

Pure cultures of *A. niger* and *A. flavus* were obtained from the Microbial Culture Collection and Testing Laboratory of Department of Biological Sciences, Central Luzon State University, Philippines. All apparatus used were sterilised with heat. The pure cultures of *A. niger* and *A. flavus* were inoculated from a heated wire loop on the potato-dextrose-agar (PDA) slants and were kept in the refrigerator at 5°C until needed.

Acquisition and Extraction of Plant Materials

Plant materials, *A. cepa* bulbs and *C. frutescens* fruits, were purchased from a local market. They were identified by an agronomist at the Central Luzon State University.

Extraction procedure was carried out as adapted and modified from Abdou *et al.* (1972) and Benkeblia (2004). The cayenne pepper, alongside with onions were washed with clean water and allowed to air dry for 4 days. The outer coverings (tunic) of onion's bulb were manually peeled off. They were then separately cut into small pieces and underwent the process of maceration in which 20 *g* of each of the dried plants were soaked in 20 *ml* of 20% ethanol for 48 hours. They were then filtered using a filter paper.

Antimicrobial Sensitivity Testing

Sensitivity testing was carried out for *A. niger* and *A. flavus* using the Kirby-Bauer technique (Bauer *et al.*, 1966). A sterile cotton swab was used to spread the microorganisms all over the surface of the PDA plates. The plates were allowed to dry for about 5 minutes.

Whatman filter paper No. 2 disks, 6 *mm* in diameter, were immersed in the extracts of *A. cepa*, or *C. frutescens*, a 50-50 v/v mixture of *C. frutescens and A. cepa* extracts, chloramphenicol (30 *mg/ml*), or miconazole (30 *mg/ml*). The disks were placed on respective plates of test organisms which then were incubated at 37°C for 72 hours. Three replicates were made.

RESULTS

All the treatments showed positive results (Figure 1). The extracts inhibited the growth of *A. niger*. Chloramphenicol exhibited the widest zone of growth inhibition for *A. niger* (26.97 *mm*). The zones of growth inhibition of *C. frutescens* extract (19.29 *mm*) and the mixture of *A. cepa* and *C. frutescens* extracts (19.20 *mm*) were statistically comparable to that of miconazole (20.33 *mm*) using *t*-test and repeated measures ANOVA (95% level of confidence).

For *A. flavus*, *C. frutescens* extract inhibited the growth to a certain extent (10.47 *mm*) which was not comparable to chloramphenicol (25.50 *mm*) and miconazole (16.25 *mm*). However, this value was significant compared to the other treatments which did not significantly inhibit the growth of *A. flavus*.

DISCUSSION

Phytochemical testing in previous studies, such as Benkeblia (2004) and Kamilla *et al.* (2009), revealed that secondary metabolites present in cer-

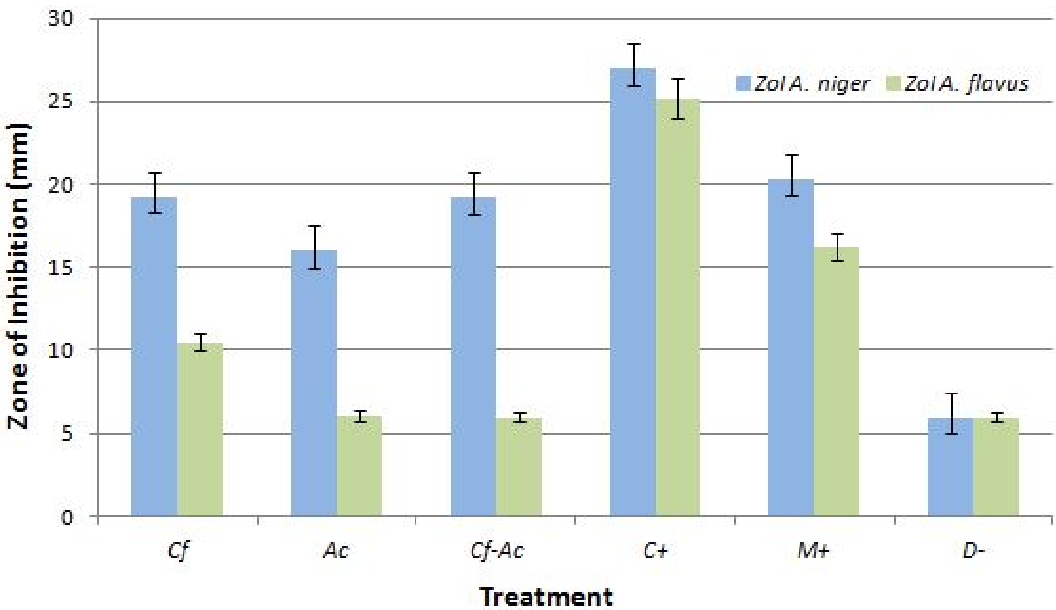

Figure 1 Inhibition zone (in mm) of Capsicum frutescens extract (Cf), Allium cepa extract (Ac), the 50-50 v/v mixture of C. frutescens and A. cepa extracts (Cf-Ac), chloramphenicol (C+), miconazole (M+), and distilled water (D-) against Aspergillus niger and Aspergillus flavus

tain plant extracts and commercially available medicines are responsible for antifungal activities against fungi from genera *Aspergillus* and *Candida* among others. The tested plants contain tannins, polyphenols, alkaloids and glycosides, which have natural antimicrobial properties (De Lucca *et al.*, 2006a; Lanzotti *et al.*, 2012).

Saponins are also a group of these secondary metabolites. They serve as important components in a wide range of plant species, for they function as a defending agent against microbial infections (Lanzotti *et al.,* 2012). They have detergent-like properties that are lethal to fungi due to their ability to combine with membrane sterols, which cause a loss of membrane integrity. Some plant species show compromised resistance to different fungal pathogens because of a deficiency in saponins (De Lucca *et al*., 2006a). Two saponins found in *C. frutescens* were tested amongst many strains of fungi, including some strains of *Aspergillus*, and were shown to be effective antifungal agents against most strains of fungi (De Lucca *et al.* 2006b). On the other hand, Ceposide A, B, and C are the saponins found in *A. cepa*, which have also been tested positively against different strains of fungi (Lanzotti *et al.,* 2012).

In the present study, *C. frutescens* extract showed the highest activity in all experiments. Antifungal results of *C. frutescens* were in line with that of Kamilla *et al.* (2009) who got 19.89 *mm* as the average zone of inhibition of *Clitoria ternatea* on *A. niger*. *A. cepa* extract showed an inhibitory activity against *A. niger*, but significantly less activity against *A. flavus*. Using repeated measures ANOVA, there was a significant difference between the results for *A. flavus* and for *A. niger*. This suggests that *A. flavus* might be resistant to *A. cepa* as affirmed by De Lucca *et al.* (2006a). The 50-50 v/v mixture of *A. cepa* and *C. frutescens* extracts showed an exemplary result for *A. niger*. On the other hand, the result for *A. flavus* was significantly lower. The result gap may be due to the synergism of the resistance of *A. flavus* to *A. cepa* and uncertain factors.

The description of methods and the presentation of findings in the present paper are derived from an attempt of high school students to apply the "scientific process research skills" necessary in biology. Onorato (2014) noted that one of the reasons why students have difficulty appreciating these research skills is the use of conceptual approach in teaching them, rather than the use of practical approach in the context of an actual scientific investigation problem. Thus, through the study they conducted as a class requirement, the first year high school students (grade 9, ages 14 - 15) were exposed to meaningful experiences to make theory meet practice while triggering curiosity to higher-level science for their age.

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Country Report

Biology Education in Upper Secondary Schools at Present in Japan

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The present Japanese national curriculum standard, the Course of Study (CS), for upper secondary schools was announced in 2009 by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT). The mathematics and science curricula are being enforced in upper secondary schools from 2012. In the present CS, biology-related subjects for upper secondary schools are "Basic Biology" (biology for all, 2-credit) and "Advanced Biology" (biology for interested students, 4-credit). In 2017, about 95% of students are taking Basic Biology, and 22% of students are taking Advanced Biology. "Basic Biology" is composed of three units: (1) Organisms and Genes, (2) Maintenance of Internal Environment, and (3) Biodiversity and Ecosystems. The key words for Basic Biology are DNA, Health and Environment. In addition, "Basic Biology" emphasizes concepts of Unity and Diversity with relation to Evolution. "Advanced Biology" is composed of five units: (1) Life Phenomena and Substances, (2) Reproduction and Development, (3) Environmental Response, (4) Ecosystems and Environment, and (5) Evolution and Phylogeny. Both subjects have inquiry activities at the end of each unit. These biology-related subjects have been modernized by reflecting the rapid progress in life science research in recent years. As a result, a lot of newest topics in biological sciences and new biological terms have appeared in biology textbooks. Some new modern experiments have also been introduced. In 2014, the action of revising the present CS was started. MEXT will announce the new CS for upper secondary schools by March, 2018, and will enforce it from 2022. The guiding concept of the CS revision is to enable students to cope with the changes in Japanese society when they become adults. Therefore, the strategy of school education must be improved. The new CS will shift from the traditional content-based teaching to competency-based learning by introducing some innovative methods such as active learning.

Key words: *biology education in Japan, contents modernization, Course of Study, upper secondary school.*

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INTRODUCTION

Since 1947 when a new mandate for education in Japan began, the Japanese national curriculum standard, the Course of Study (CS), has been revised by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) about every ten years (Table 1, Katayama *et al*. 2004, Nakamichi 2011). The present CS for elementary schools is being enforced from 2011, and that for lower secondary schools from 2012. The present CS for upper secondary schools was announced in 2009 and the revised curricula of mathematics and science are being enforced from 2012, one year earlier than those of the other subjects (Table

1). As mentioned in Table 1, the CS for both elementary schools and lower secondary schools has been revised and announced in 2017; the newly revised CS for elementary schools will be enforced from 2020 and that for lower secondary schools will be enforced from 2021. The CS for upper secondary schools will be revised and announced by March, 2018 and the revised CS will be enforced from 2022. The present CS has three principles, “Solid academic prowess,” “To be rich in humanity,” and “Health and fitness,” all of which support the fundamental philosophy of the CS, "Zest for life" (Nakamichi 2011). The fundamental philosophy is carried on by the newly revised CS.

In the present CS for Science for upper secondary schools, there are ten science subjects (Table 2, Nakamichi 2011). Among them there are two new subjects, “Science and Our Daily Life” and “Science Project Study.” The former subject, which is correspondent to Basic Science in the last CS, aims to raise students’ interests in nature, science and technology. The latter subject aims to enrich students’ inquiry abilities. In the latest revision

Table 1 Year of Announcement and Enforcement of the Course of Study in Japan

Elementary Schools		**Lower Secondary Schools**		**Upper Secondary Schools**	
Announcement	**Enforcement**	**Announcement**	**Enforcement**	**Announcement**	**Enforcement**
1947	1947	1947	1947	1947	1948
1951	1951	1951	1951	1951	1951
				1955	1956
1958	1961	1958	1962	1960	1963
1968	1971	1969	1972	1970	1973
1977	1980	1977	1981	1978	1982
1989	1992	1989	1993	1989	1994
1998	2002	1998	2002	1999	2003
2008	**2011**	**2008**	**2012**	**2009**	**2012/2013***
2017	**2020**	**2017**	**2021**	**2018**	**2022**

The years on the second line from the bottom indicate the present Course of Study.
*Only the curricula of Math and Science of the present CS are to be enforced from 2012.
The years on the last line indicate the next Course of Study.

Table 2 Science subjects in the present Course of Study (2009) in comparison with those in the last one (1999)

1999*	Subject	Basic Science	Comp. Sci.** A / B	P / C / B / E*** I	P / C / B / E*** II
	Credit	2	2	3	3
2009*	Subject	Science and Our Daily Life	Basic P / C / B / E***	Advanced P / C / B / E***	Science Project Study
	Credit	2	2	4	1

* The year of announcement of the Course of Study.
** Comp. Sci. A / B: Comprehensive Science A (Physics and Chemistry areas) and Comprehensive Science B (Biology and Earth Science areas).
*** P / C / B / E: Physics, Chemistry, Biology and Earth Science.

of the CS, Comprehensive Science A and B have been deleted and four 2-credit basic subjects, *i.e.*, Basic Physics (P), Basic Chemistry (C), Basic Biology (B) and Basic Earth Science (E) which correspond to respective traditional subject areas, have been established. These basic subjects are prepared for almost all students. Students have to take at least three 2-credit subjects (Basic P / C / B / E) or two 2-credit subjects including Science and Our Daily Life; it is supposed that most students will choose the former pattern because the contents of Science and Our Daily Life are not considered to be included in university entrance examinations. This credit requirement enables students to take many more subjects in different fields of science. There also are four 4-credit advanced subjects for respective science fields. These advanced subjects are provided for the students who are interested in a particular field of science. Every advanced subject is designed to let students study the corresponding field of science in a systematic way.

BIOLOGY-RELATED SUBJECTS IN THE PRESENT CS AND THEIR CONTENTS

In this chapter, the contents of biology-related subjects for upper secondary school students in the present CS, *i.e.*, "Basic Biology" and "Advanced Biology," are reviewed.

Contents of Basic Biology (2-credit)

Basic Biology (Biology for All) is composed of the following three units: Unit 1 Organisms and Genes, Unit 2 Maintenance of Internal Environment (Homeostasis), and Unit 3 Biodiversity and Ecosystems. Key words for this subject are DNA, Health and Environment (Table 3).

Although Basic Biology is a subject which corresponds to Biology I in the last CS, its contents have changed and been modernized significantly (Table 4). In the first unit, the contents are mainly related to cellular and molecular biology. In the second unit, students study the mechanism of maintenance of the internal environment and immunity in multicellular organisms. The contents can give basic knowledge to understand human health and illness. In the third unit, students study biodiversity and a variety of ecosystems; they are expected to realize the importance of environmental conservation through understanding the structure and function of ecosystems. In each unit, there are some observations and experiments. In addition, on completing the study of each unit, students are required to carry out some inquiry activities whose topics are related to the contents of the unit. These are the same as for Biology I in the last CS.

A noticeable characteristic of Basic Biology is to emphasize the concepts of "Unity" and "Diversity" which are related to evolution, though there is no heading of "Evolution."

Table 3 Composition of Basic Biology

Unit	Hierarchy Level	Aspects of Scientific Literacy
Organisms and Genes	Cellular and Molecular Level	Basis of Molecular Biology (DNA)
Maintenance of Internal Environment	Individual Level	Health
Biodiversity and Ecosystems	Community and Ecosystem Level	Environment

Table 4 Comparison of major headings of Biology I and Basic Biology

Biology I in the last CS (1999)	Basic Biology in the present CS (2009)
Unit 1. Continuity of Life (1) Cells • Structures and functions of cells • Reproduction of cells and the structure of organisms (2) Reproduction and development • Formation of germ cells and fertilization • Mechanisms of development (3) Heredity • Laws of heredity • Genes and chromosomes (4) Inquiries into Continuity of life	Unit 1. Organisms and Genes (1) The characteristics of organisms • Unity and diversity of organisms • Cells and energy (2) Genes and their function • Genetic information and DNA • Distribution of genetic information • Genetic information and synthesis of protein (3) Inquiries into organisms and genes
Unit 2. Responses of Organisms to Their Environment (1) Responses of animals to their environment • Body fluid and Homeostasis • Stimuli reception and reaction (2) Responses of plants to their environment • Plant life and environment • Plant responses and regulation (3) Inquiries into Responses of organisms to their environment	Unit 2. Maintenance of Internal Environment (1) Internal environment of the organisms • Internal environment • Mechanism of maintenance of internal environment • Immunity (2) Inquiries into maintenance of internal environment
	Unit 3. Biodiversity and Ecosystems (1) Vegetation diversity and distribution • Vegetation and succession • Climate and biomes (2) Ecosystems and their conservation • Ecosystem and the circulation of matter • Ecological balance and conservation (3) Inquiries into biodiversity and ecosystems

It had been pointed out by many biology educators and biologists that biology-related subjects in the last CS for both lower secondary schools and upper secondary schools were lacking in the concept of evolution. However, in the present CS, evolution is regarded as the most important concept in biology and is treated as a superior concept among biological concepts. Therefore, in Basic Biology, teachers are asked to teach "the unity and diversity of organisms" prior to the other topics to let students understand that the phenomena are the results of evolution.

Contents of Advanced Biology (4-credit)

As shown in Table 5, Advanced Biology (Biology for Interested Students) is composed of the following five units: Unit 1 Biotic Phenomena and Substances, Unit 2 Reproduction and Development, Unit 3 Responses to the Environment, Unit 4 Ecosystems and Environment, and Unit 5 Evolution and Phylogeny. Unit 1 is related mainly to the phenomena at molecular, subcellular and cellular levels. Unit 2 and Unit 3 are the phenomena at organ and individual levels. Unit 4 is the phenomena at the level of population and above. The final unit treats evolutional phenomena and the theory of evolution.

The following are three key characteristics of this subject: (1) Contents correspond to rapid progress in life science research in recent years, (2) Viewpoint of "unity and diversity" is continually emphasized from Basic Biology onward through other biology contents, and (3)

Table 5 Comparison of major headings of Biology II and Advanced Biology

Biology II in the last CS (1999)	Advanced Biology in the present CS (2009)
Unit 1. Life phenomena and Organic Substances (1)Proteins and their functions • Enzymes and chemical reactions within organisms • Anabolism and catabolism • Functions of proteins (2) Genetic information and its expression • Genetic information and synthesis of protein • Regulation of phenotypic expressions and morphogenesis • Biotechnology	Unit 1. Biotic Phenomena and Substances (1) Cells and molecules • Living substances and cells • Life phenomena and protein (2) Metabolism • Respiration • Photosynthesis • Nitrogen assimilation (3) Expression of genetic information • Genetic information and its expression • Control of gene expression • Biotechnology (4) Inquiries into life phenomena and substances
Unit 2. The Classification and Evolution of Organisms (1) The classification and phylogeny of organisms • Classification • Phylogeny (2) The evolution of organisms • Changes in organisms • Mechanisms of evolution	Unit 2. Reproduction and Development (1) Sexual reproduction • Reduction division and fertilization • Genes and chromosomes (2) Development of animals • Gametogenesis and fertilization • Process of early development • Cell Differentiation and morphogenesis (3) Development of plants • Gametogenesis, fertilization and embryogenesis • Differentiation of organs in plants (4) Inquiries into reproduction and development
Unit 3. Biocoenose (1) Populations, their structures and maintenance • Population maintenance and adaptation • Matter production and plant lives (2) Biocoenose and ecosystems • Biocoenose, their maintenance and changes • Ecosystems and their balance	Unit 3. Responses to the Environment (1) Responses and behavior of animals • Stimuli reception and reaction • Behavior of animals (2) Responses of plants • Plant response to the environment (3) Inquiries into responses to the environment
Unit 4. Research Activities (1) Research on particular organisms or life phenomena (2) Investigation of the natural environment	Unit 4. Ecosystems and Environment (1) Population and biotic community • Population • Biotic community (2) Ecosystems • Matter production in an ecosystem • Ecosystems and biodiversity (3) Inquiries into ecosystems and environment
	Unit 5. Evolution and Phylogeny (1) Mechanism of evolution • Origin of life and transition of organisms • Mechanism of evolution (2) Phylogeny • Phylogeny (3) Inquiries into evolution and phylogeny

Various fields from the micro level to the macro level are covered. In each unit, some new topics are included, *e.g.*, biomembrane and cell skeleton in Unit 1, processes of organogenesis in plants in Unit 2, neuro-ethology and photo-receptors in Unit 3, diversity of organisms at various levels in Unit 4, and neutral theory of molecular evolution and three domains in phylogeny in Unit 5.

NUMBER OF STUDENTS WHO TOOK BIOLOGY-RELATED SUBJECTS

Figure 1 shows the changes in the number of textbooks of each basic subject (left) and each advanced subject (right) sold. The figures are compiled by using the data obtained from The Jiji Press (2014, 2015, 2016 and 2017) which were based on MEXT data. These numerical values are considered to roughly correspond to the number of students who took each subject. In the school year 2017, nearly 1.1 million students (about 95% of senior secondary students) take Basic Biology; it is the highest rate of course registration in basic subjects. About 261 thousand students (about 22% of senior secondary students) take Advanced Biology. In the school year 2013, the number of students who took one of these subjects appears to be smaller than in the later school years because all schools did not necessarily start all science subjects in this school year. The number of students who take Advanced Biology has been decreasing continuously after the school year 2015.

OBSTACLES TO ENFORCING THE LATEST BIOLOGY CURRICULUM FOR UPPER SECONDARY SCHOOLS

On enforcing the present CS, MEXT encourages science teachers to introduce various students' activities into their teaching to nurture students' abilities of thinking, decision-making and expression. The ministry requests them to avoid the traditional chalk and talk teaching style (a teaching style of only the teacher's explanation and his/her writing on the blackboard). The following student activities are recommended:

- ➢ Peer discussion using a tablet, a whiteboard and/or post-its,
- ➢ Explanation including a poster presentation and a debate between the students, and
- ➢ Making a handout, a report or a poster,

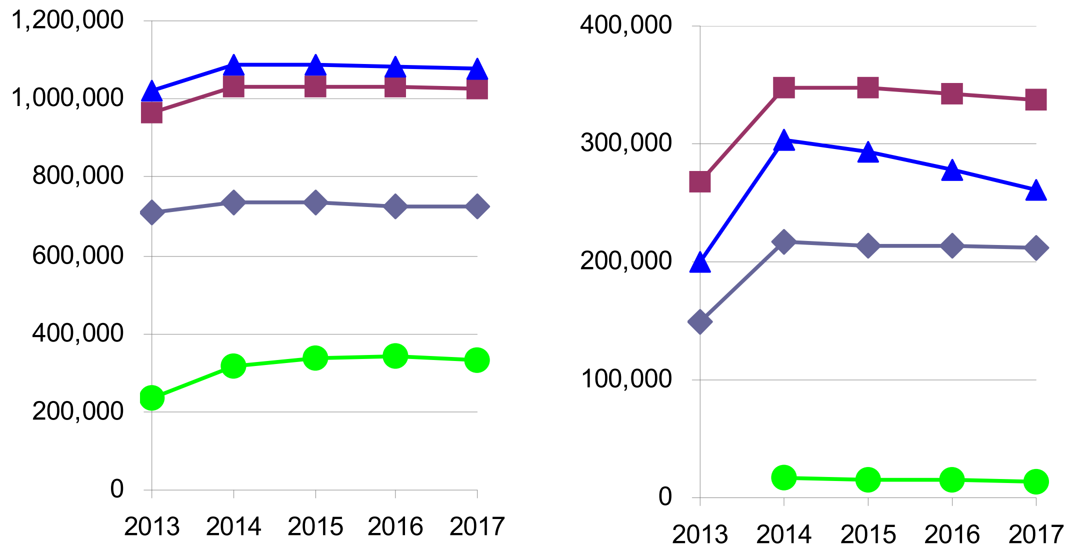

Figure 1 Changes in the number of students who took each basic subject (left) and each advanced subject (right).
▲: Biology, ■: Chemistry, ♦: Physics, ●: Earth science

using Information and Communication Technology (ICT).

Thus, science teachers in both lower and upper secondary schools are expected to shift their teaching style from teacher-centered to student-centered. But, many science teachers feel some difficulties in implementing the above-mentioned student activities in their classes, and they consider the time allotted to science is insufficient for such time-consuming activities. Not only because of such time limitation, but also because of their inexperience in student-centered teaching style, many teachers still prefer to adopt the teacher-centered teaching style. They are apt to require students to memorize the items and terms of the textbook for term examinations. At the upper secondary level, science teachers still consider it necessary to give a lot of information to students in preparation for university entrance examinations instead of allotting time for inquiry activities.

In spite of the rapid progress in biological and life science research and the accumulation of new biological knowledge, the contents of upper secondary school biology had remained rather static until the last revision of the CS. But, by the latest reform, biology education at the upper secondary level has been modernized by reflecting the rapid progress in biological research in recent years, bringing it closer to biology education at the tertiary level. Especially in the case of Advanced Biology, the quantity of topics has increased and the contents have become more challenging. As a result, some new problems have arisen. For example: (1) Although a lot of the newest topics in biological sciences and new biological terms appear in biology textbooks, many biology teachers, especially older ones, in upper secondary schools lack up-to-date knowledge of biology; (2) Some new modern experiments have been introduced, but most of the older biology teachers are lacking the skills for instructing students in new modern experiments. In addition, most schools, particularly public schools, do not have enough equipment for new modern experiments.

It is desirable that some effective measures for surmounting these obstacles be promptly implemented. However, both national and local governments still have not adopted enough measures for in-service teacher training. The budget for improving school science equipment also does not seem to be enough.

Another problem is related to the recent trend in the “Textbook Authorization” process (see Appendix 1). Recently, “Textbook Authorization” for biology textbooks, particularly for Advanced Biology textbooks, has become relaxed. As a result, a wider range of topics including up-to-date contents have been introduced in Advanced Biology textbooks. Then, there seems to be a big gap in the degree of difficulty in understanding the contents between Basic Biology and Advanced Biology. Many biology teachers feel they are forced to teach students all of the contents in the Advanced Biology textbook within the school hours allotted to this subject. Students think that they have to memorize lots of contents when they take Advanced Biology. This must be one of the reasons why the number of students who take this subject has been decreasing (Fig. 1).

In addition to the increase in the amount of contents mentioned above, there also is an increase in the number of biological terms which include some latest ones in the present upper secondary biology textbooks (Matsu-ura 2013, Nakamichi 2018, see Appendix 2). Among these terms, there are quite a few synonyms. So far, it has been pointed out that too many biological terms used in biology textbooks possibly make students lose interest in learning biology, and the use of synonymous terms makes both biology teachers and students confused. Students are required to memorize all of these terms including synonyms for term examinations and for university entrance examinations if they choose biology as one of the examination subjects. Unfortunately, only a few attempts at reduction and standardization of these terms have been carried out until now. Furthermore, no reflection of the results of these few attempts has appeared in biology textbooks, yet.

PREPARATION AND ENFORCEMENT OF THE NEXT CS

Schedule of CS revision

In 2014, the Minister of MEXT requested the Central Council for Education (CCE) to commence discussion for revising the CS (MEXT 2014). In 2015, the CCE submitted an Interim Discussion Report to the Minister of MEXT (MEXT 2015) and, in 2016, the CCE submitted the Final Discussion Report to the Minister (MEXT 2016). After this, as shown in Table 1, MEXT announced the new CS for elementary schools and lower secondary schools in 2017 (MEXT 2017). MEXT will announce the new CS for upper secondary schools by March, 2018. Now, textbook publishers are preparing new textbooks. The textbook authorization process for new textbooks by MEXT will be started from 2018 onward. The new CS for elementary schools, lower secondary schools and upper secondary schools will be enforced from 2020, 2021 and 2022, respectively.

Conception and realization of the CS revision

The guiding concept of the CS revision is to enable students to cope with the changes in Japanese society when they become adults. At that time, in Japan, there will be a reduction in the working-age population, a progression of globalization, and technological innovation. It will be more important for students to be aware of the connection between their learning and the changing society than ever before. Students will be expected to foster the following qualities and abilities which will be required for a new era.

(1) Practical knowledge and skills;
(2) Abilities of thinking, decision-making and expression in responding to unexpected situations;
(3) Individual characteristics enabling them to use acquired knowledge and abilities to live a better life and engage in regional and international societies.

Therefore, the strategy of Japanese school education in the future must be improved in order to satisfy these students' needs. From now on, the quality of contents is more strongly emphasized. Thus, it is necessary to consider "what knowledge students acquire" and "what they can do using the knowledge they have acquired." In addition, the quality of learning method, *i.e.*, "how to learn," should be considered to deepen students' understanding; it is strongly recommended that the teaching style be shifted from teacher-centered to student-centered. It is also important to evaluate "what kinds of abilities students have acquired" as an outcome of student learning. Therefore, in the new CS, competency-based education, by introducing some innovative methods, such as active learning, will be incorporated with the traditional content-based education.

For realization of the new CS, in general, the following must be considered:

- Improvement of the quality of learning process;
- Pre- and in-service teacher training for student-centered teaching style, such as active-learning;
- Promotion of ICT use;
- Reform of the university entrance examination system;
- Improvement of the working environment for teachers.

In science, in particular, laboratory equipment and teaching materials must be updated to cope with the modernization of the subject matter. In-service training to give science teachers up-to-date knowledge of biology and skills for teaching with new modern experiments is also essential.

Note: This paper is based on the country report presented at the 26th Biennial Conference of AABE.

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APPENDIXES

Appendix 1: Textbook Authorization

In Japan, there is a "Textbook Authorization" system. Books to be used as textbooks for Japanese elementary and secondary schools are compiled and edited by private publishers. Textbook Authorization is the process of screening these books. Through the careful deliberations of the Textbook Authorization Council under the control of MEXT, these books are examined to decide whether they are appropriate for use as textbooks. Science textbook authorization has been carried out by certain examiners in the Elementary and Secondary Education Bureau, MEXT, who have scientific and educational backgrounds. The purpose of textbook screening might be the correction and improvement of the material in textbooks. In practice, however, the textbook screening has come to be a severe check to see if they deviate from the CS and its guidelines.

So far, regarding biology textbooks for upper secondary students, some important biology concepts and the concepts in other science areas which are required for understanding biological phenomena were requested to be deleted from biology textbooks, because they were not included in the biology section of the CS and its guidelines. As a result, biology textbook authorization made it more difficult for teachers to teach biology because these textbooks restricted biology education within narrow limits. On the other hand, scientific terms appearing in biology textbooks do not seem to have been examined thoughtfully in the textbook authorization process. This resulted in the number of scientific terms being very large compared to the textbooks of other science areas. Moreover, there were many synonymous terms (see below).

The process of textbook "authorization" by the officers of MEXT continues, but the screening of textbooks at present is not as severe as before.

Appendix 2: Scientific terms used for biology education in Japan

In Japan, science subjects are taught in Japanese, and therefore only Japanese scientific terms are used in teaching science and in science textbooks. Among science subjects for upper secondary students, the number of scientific terms appearing in biology textbooks is larger than that in textbooks of other science subjects. Biology learners in upper secondary schools are forced to memorize a lot of scientific terms. This has been considered to be a burden to upper secondary school students who choose biology and it leads to many students losing interest in learning biology.

Furthermore, there are a lot of Japanese synonymous terms in biology. Synonymous scientific terms appearing in biology textbooks for Japanese upper secondary students are much larger in number than those appearing in biology textbooks for English upper secondary students. Sometimes three or more synonymous Japanese scientific terms are used for one English term. Usually, in a biology textbook, one of these synonyms is used according to the authors' decision. Frequently, one of these synonyms is used at random in university entrance examinations. Therefore, students must memorize a lot of biological terms including all of these synonyms if they choose biology as a university entrance examination subject. This has been an issue for a long time, but only a few attempts at reduction and standardization of biological terms have been carried out. Details of this matter will be described elsewhere.

Archives

Contents of the Proceedings of Past Biennial Conferences of the AABE

The AABE published the proceedings of each biennial conference, from the first conference to the seventeenth one, until 1998. Since then, the association has been publishing AJBE as its bulletin which includes the conference reports and the abstracts of the papers presented at each conference. At present, it is difficult to obtain not only a copy of these proceedings, but even the information on the contents of them. Here is the table of contents and author index of the proceedings (Volume 1 – 17). As for further information on these proceedings, please inquire of the AABE Website master.

A TABLE OF CONTENTS

< II > Second Conference held in Tokyo, Japan (August, 1968)

Second Asian Regional Conference on School Biology

< III > Third Conference held in Manila, Philippines (December, 1970)

Third Asian Regional Conference on School Biology

< IV >Fourth Conference held in Jerusalem, Israel (August, 1972)

Fourth Asian Regional Conference on School Biology

Evaluation in Science Education

Evaluation of Curriculum

< V > Fifth Conference held in Singapore (June, 1974)

Fifth Asian Regional Conference on School Biology

< VI > Sixth Conference held in Bangkok, Thailand (July - August, 1976)

Sixth Asian Regional Conference on School Biology

< VII > Seventh Conference held in Kuala Lumpur, Malaysia (December, 1978)

Multidisciplinary Biology Education Relevant to Community Development

< VIII > Eighth Conference held in Osaka and Gifu, Japan (October - November, 1980)

Biology Education for the Next Decade

< IX > Ninth Conference held in Melbourne, Australia (December, 1982)

The Role of Biology Education in Enhancing the Quality of Life

< X > Tenth Conference held in Chang Mai, Thailand (December, 1984)

Biology Education and Technology

< XI >Eleventh Conference held in Quezon City, Philippines (December, 1986)

Research and Evaluation in Biology Education and Its Implication for the Teachers

< XII > Twelfth Conference held in New Delhi, India (December, 1988)

Explosion of Biological Knowledge and the Challenges for Secondary Education and Teacher Preparation

< XIII > Thirteenth Conference held in Seoul, Korea (August - September, 1990)

Environmental Education in the Curriculum of Biological Education

< XIV > Fourteenth Conference held in Melbourne, Australia (December, 1992)

Environmental Management in Asia – Training, Education and Research

< XV > Fifteenth Conference held in Tokyo, Japan (August, 1994)

Biology Education for Non-Biology Majors

Country Reports

Contribution Papers

Poster Presentations

< XVI > Sixteenth Conference held in Chang Mai, Thailand (December, 1996)

Excellence in Biology Teaching: Research, Practice and Experience

< XVII >Seventeenth Conference held in Manila, Philippines (December, 1998)

Biology Education in the Third Millennium

List of Poster Presentation

AUTHOR INDEX

Publications

Biology Education for Social and Sustainable Development (ISBN: 978-94-6091-925-1) was published in 2012 by Sense Publishers, Rotterdam, Netherlands (http://www.sensepublishers.com/). Some papers presented at **the 23rd Biennial Conference of the AABE** which was held in Singapore in October 2010 were compiled in this book by Dr. Mijung Kim and Dr. C. H. Diong. You can refer to the abstracts of these papers in **the sixth volume of the *Asian Journal of Biology Education*** (2012).

Biology Education and Research in a Changing Planet (2015) (ISBN 978-981-287-523-5) was published by Springer (http://www.springer.com/in/book/9789812875235). Some papers presented at **the 25th Biennial Conference of the AABE** which was held in Malaysia in October 2014 were compiled in this book by Dr. Esther Gnanamalar Sarojini A Daniel. The abstracts of these papers were included in **the eighth volume of the *Asian Journal of Biology Education*** (2015).

From the Editor-in-Chief

The tenth volume of *Asian Journal of Biology Education* (AJBE) contains one practical report and one country report. It also contains the contents of past conference proceedings (Vol. 1 – 17). Although this volume is a little thinner than the previous ones of AJBE, I have decided to publish it because one article included was accepted for publication more than a year ago, in March last year.

The next issue will be published possibly by the end of April, next year. It will include a report on the 27th Biennial Conference of AABE which will be held at Emerald Hotel Bangkok, Thailand, from the 30th of November to the 2nd of December this year, and the abstracts of papers presented at the conference, as well as some contributed articles.

One of the roles of AJBE is to report the latest biennial conference of AABE. However, the core object of AJBE is to publish reports on biology education research and practices. So, the publication of AJBE is mainly dependent on the number of contributed articles. Therefore, I would like to ask the readers of this journal, AABE members or non-members, to submit their articles (research papers, practical reports, reports on biological resources, etc.) to AJBE.

The articles contributed to AJBE during the last two years have been reviewed by Professor Kim Kyoungho (Gongju National University of Education, South Korea), Professor Morakot Sukchotiratana (Chiang Mai University, Thailand), and Dr. Robert Wallis (Federation University, Australia), as well as the Editorial Board members. I am very thankful to them for their efforts to review the articles.

Dr. Nobuyasu Katayama