The Use of the Valuable Oyster Mushroom, Pleurotus sajor-caju, for Conversion of Waste Materials Produced from Seaweed and Brewing Industries: Preliminary Investigations

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Synopsis

The Use of the Valuable Oyster Mushroom, *Pleurotus sajor-caju*, for Conversion of Waste Materials Produced from Seaweed and Brewing Industries: Preliminary Investigations

Preliminary tests on growing the oyster mushroom, *Pleurotus sajor-caju* on commonly available wastes generated by the seaweed and brewing industries were conducted. Various combinations of these wastes and scrub grass were used. The fungus grew well, vegetatively on all combinations of substrate except pure spent barley, but was slower on substrates with higher salt concentrations. Fruiting (mushroom production) was best on those substrates containing kelp rather than *Gracilaria gracilis*, probably due to the high salt content of the *G. gracilis*. No mushrooms were produced on 100% seaweed substrates probably due to nutrients being too readily available.

For the most part, Namibia has a semiarid to desert environment, consequently terrestrial biological production is low and therefore organic wastes from agriculture and the natural environment are not abundant. In contrast, the marine environment of Namibia is extremely productive and supports a valuable fishing industry, which is one of the mainstays of the economy. On the coast of Namibia seaweed growth rates are amongst the highest in the world (1, 2) and this is only now being taken advantage of by the local seaweed industry. This industry collects beach-cast seaweeds for agar production, fertilizers, and soil conditioners and also cultivates the agarophyte (red seaweed) *Gracilaria gracilis* on floating systems. Inevitably, not all of the raw material collected and grown is suitable for production and so, becomes waste.

The brewing industry in Namibia produces high quality clear beers following the German Reinheitgebot brewing laws and also produces traditional beers from sorghum and maize. All malting barley for clear beer has to be imported, as local conditions are not suitable for barley cultivation. This industry, through years of fine-tuning has minimized its wastes, not least because of the expense of the raw materials, using less water per liter of beer than most breweries in the world. After the brewing process, spent grain and yeast are wastes that are currently not used at all or fetch a very low price as low grade animal feed.

This work was initiated to ascertain the possibility of using commonly available waste products from the seaweed and brewing industries as a substrate for producing a high value mushroom crop. If positively demonstrated locally, this could be replicated in all coastal countries in southern Africa and indeed elsewhere in the world.

Seaweeds, in particular members of the Phaeophyta (brown seaweeds), have high iodine levels in their tissues. Moreover, Namibian kelps can contain very high levels of iodine (e.g. fresh *Laminaria pallida* and *Ecklonia maxima* in the range 2000–4500 mg g^{-1} and 1000–3300 mg g^{-1}, respectively, Jane Teas unpubl. data). Our hypothesis is that mushrooms grown on substrates with high iodine levels may also have a boosted iodine content (due to the absorptive nature of mushroom nutrition). Thus far, the level of iodine transfer from the seaweed waste substrates has been relatively low, with iodine levels being recorded in the range 20–40 mg g^{-1} in the mushrooms so produced. Further trials will investigate the methods of substrate preparation to examine if iodine is being leached from, or destroyed within, the pasteurization process thereby limiting uptake by the mushrooms. Increased levels of iodine within mushrooms may of-

Figure 1. Vegetative growth (colonization) on substrates comprising spent barley, grass and *Gracilaria*.

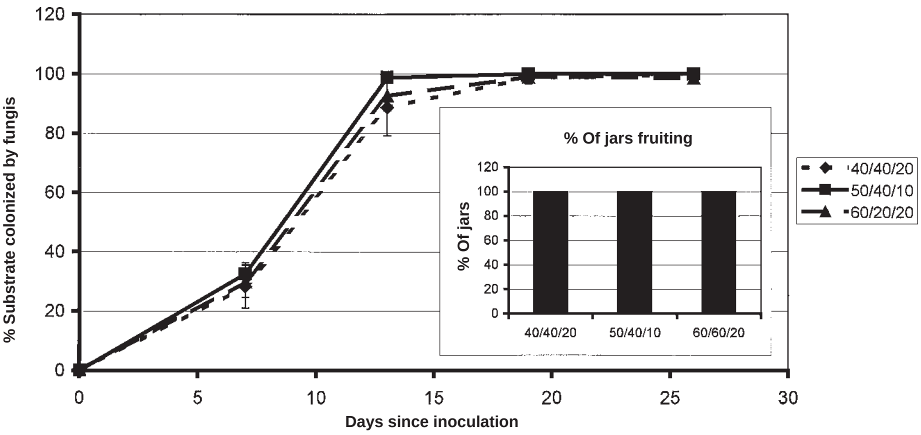
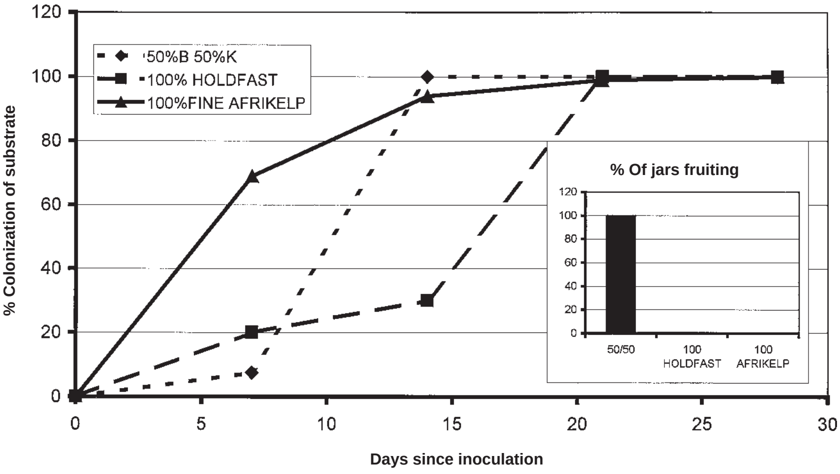


Figure 2. Vegetative growth (colonization) and fruiting in substrates containing 50/50 spent barley/kelp materials and 100% kelp materials.



fer one contribution to the alleviation of iodine deficiency diseases that are common throughout the southern African region. This study represents the first time that seaweeds have been used as a substrate material for mushroom production.

The substrate materials tested in this investigation represent a marriage of the two environments (*viz.* marine and terrestrial), this is an exciting prospect for high value mushroom production.

Substrates suitable for mushroom production require a balance between readily available nutrients and nutrient sources that require more enzymatic breakdown. If a substrate is too rich the fungus will exhibit prolific vegetative growth, but may not produce a crop of fruiting bodies (mushrooms). A poor substrate will not support good vegetative growth and few or no mushrooms will be produced.

The substrate materials used were: spent barley grain from Namibia Breweries Windhoek, and the following seaweed wastes from Taurus Products: *i*) low grade red seaweed, *Gracilaria*, unsuitable for production of the hydrocolloid (gel) agar; *ii*) portions of brown seaweed, *Laminaria* (holdfasts) unsuitable for the production of the soil conditioner Afrikelp™ (but rich in the hydrocolloid alginic acid); *iii*) waste *Laminaria* after production Afrikelp™; *iv*) sweepings from the Afrikelp™ production; and *v*) Afrikelp™ itself. Dry scrub grass was also used as an additional lignocellulose source.

Various combinations of the substrate materials were mixed and soaked in fresh water overnight; excess water was squeezed out and 2% lime added. The substrates were then mixed and placed in jars and autoclaved at 121 ℃ 1.2 kg cm^{-2} pressure for 30 mins. After cooling the jars were inoculated with a pure culture of the fungus and placed in the dark at 25 ℃. After growing for 3–4 weeks the jars were opened to stimulate mushroom production. The use of jars is purely for the laboratory-based benchwork phase of the project, much larger quantities in bags are to be used in the pilot and commercial phases.

The oyster mushroom, *Pleurotus sajor-caju*, was chosen. This species was used as it is tolerant of high temperatures, grows on a wide variety of substrates and produces a high yield of valuable mushrooms.

The *Pleurotus* mycelium grew well on most substrates except 100% spent barley grain (Fig. 1). This has a very high fiber content and not enough readily available nutrients to support vigorous fungal development. Though the 100% kelp substrates were completely colonized, the mycelium was very thin and did not produce fruiting bodies (Fig. 2). The rate of colonization was rapid, achieving complete colonization of most substrates in 14 days (Figs 3–5).

Different combinations of the 3 substrate materials did not affect the growth rate markedly, but growth on substrates containing seaweed with a higher salt content was slower and in some cases did not completely colonize the substrate (Figs 1 and 3). The *Gracilaria*, leftover kelp and kelp holdfasts were unwashed and hence had a higher salt content whereas the production of Afrikelp™ involves a freshwater washing step, the kelp sweepings and Afrikelp™ had a low salt content.

Fruiting on the substrates containing the red *Gracilaria* was not as high as on the substrates containing Kelp/Afrikelp™ (Figs 2–4) probably due to the high salt content. No fruiting occurred on the 100% seaweed substrates.

The aim of the investigation was to use waste materials. Grass is not an industrial

Figure 3. Vegetative growth (colonization) and fruiting on spent barley/grass/kelp combinations.

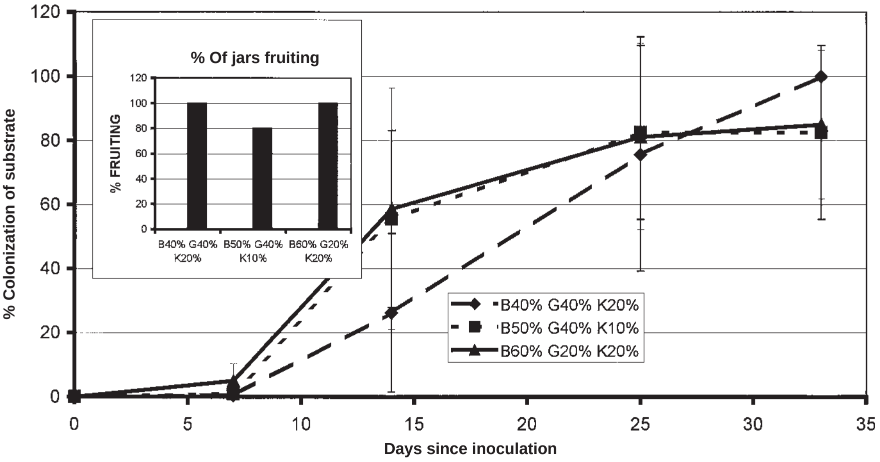


Figure 4. Vegetative growth (colonization) and fruiting on substrates composed of 40% spent barley, 40% grass and 20% kelp holdfast or fine kelp sweepings.

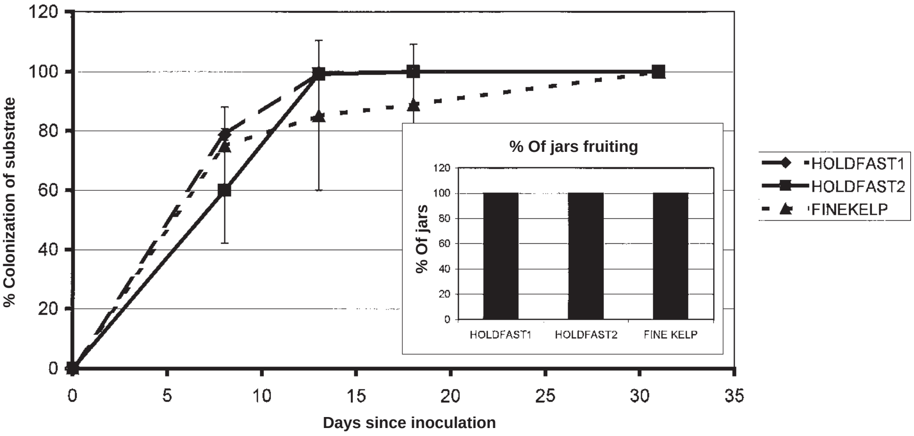
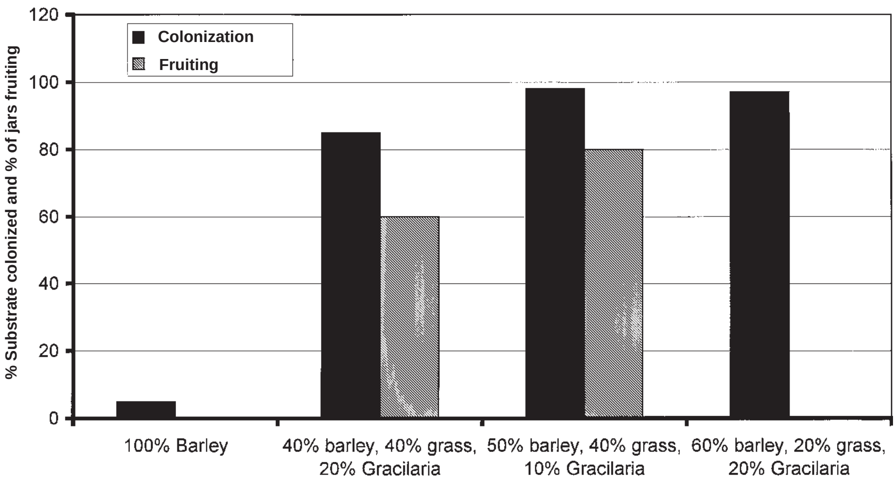


Figure 5. Vegetative growth and fruiting on substrates composed of various combinations of spent barley grain, grass and *Gracilaria*.



or agricultural waste but the abundant scrubgrass in parts of Namibia and the rest of Africa is generally not used for anything so it is, in effect, a waste. This scrubgrass tends to be quite hard due to its high lignin content, making it a poor animal food. This high lignin content is the very attribute that makes it an excellent substrate material for lignin degrading fungi such as *Pleurotus*.

An added advantage for the use of seaweeds in the production of mushroom substrate, is the presence of hydrocolloids (waterholding polysaccharides, agar from the red seaweed *Gracilaria* and alginic acid from the brown seaweed *Laminaria*). Thus, incorporation of seaweeds into the mushroom substrate reduces the rate of desiccation and increases water retention, an important aspect of producing mushrooms in such an arid country as Namibia.

The fact that mushrooms were produced even on substrates with high salt content is very encouraging. This is of more importance in Namibia than most countries as fresh water is in short supply and is consequently expensive.

Preliminary results are positive for the incorporation of wastes from the seaweed and brewing industries for the production of valuable mushrooms. Further work will be undertaken to commercialize this process.

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Synopsis

Environmental Pesticide Pollution and Its Countermeasures in China

China is a country with large agricultural production and is also a large producer and consumer of pesticides. As a result of decades of high pesticide application, the environment has been degraded, causing serious damage to the structure and function of ecosystems. In addition, the yearly economic loss incurred as a result of environmental pesticide pollution is enormous. Many of the pesticides used are highly toxic, resulting in tens of thousands of users being injured or dying every year. Consequently, it is essential to control pesticide use and at the same time develop China's agricultural economy. It is, therefore, the duty of environmental protection agencies at all levels, from State to local, to improve their knowledge about pesticides and to intensify environmental management of pesticides, in order to control pesticide pollution of the environment.

Currently, China produces about 200 types of pesticides, and processes over 500 formulated products every year. This adds up to a total output of 400 000 tonnes of pesticides of technical grade. Up to 300 million ha of farmlands and forests receive pesticide applications for disease-, insect-, and weed control. As a result of decades of high-rated pesticide application and a lack of rational scientific supervision and management, environmental pollution and the effects on human health have become very serious.

STATUS QUO OF ENVIRONMENTAL PESTICIDE POLLUTION

During the 1950s and 1960s, DDT, BHC, aldrin, dieldrin and some other organochlorine pesticides were in use in China as well as in other countries of the world. As they persist over time in the environment and accumulate in human organisms, causing serious damage, their use has been prohibited since the 1970s; initially in the western countries. China began to limit the use of organochlorine pesticides in 1983. They were replaced by a large number of "substitute pesticides". The so-called "substitute pesticides" here refer mainly to pesticides of organophosphorus and carbamate types. Although these pesticides have low persistence and readily degrade in the environment, they are highly toxic. Their extensive use since 1983 has led to mortality incidents for pesticide poisoning; problems related to both production and application. According to the incomplete statistics for the 5 years between 1992 and 1996, 247 379 cases of pesticide poisoning were reported in 26 provinces and cities, including 24 612 deaths, i.e. a death rate of almost 10%. Insecticides are the major types of pesticides that cause poisoning, and are responsible for 86.3% of the incidents (Table 1). A total of 63.3% of the poisoning cases and 58.5% of the fatal cases reported in Jiangsu, Shandong, Zhejiang and Hubei provinces, were caused by insecticides (1). This problem is not only affecting agricultural production, but also posing a threat to the lives of farmers. It has become a social problem that calls for an urgent solution.

Pesticides are industrial products, that are important for agricultural production, but also a product containing various toxic substances. Therefore, the sale and post-sale service of pesticides have become a key to the interests of the farmers and success of agriculture. Nevertheless, in most provinces there is a diversity of departments involved in selling pesticides. In some provinces, even units having nothing to do with agricultural input are also engaged in pesticide sales. Because the sellers are not responsible for post-sale services, incidents of pesticide poisoning occur frequently. In August 1994, a serious event occurred with sweet potato in Shandong Province. Generally, only common insecticides like trichlorphon were needed to control the problem. However, as the local farmers lacked expertise, they used parathion instead, which is highly toxic. Within 3 days, over 1 million farmers entered the fields to combat the pests, resulting in over 300 cases of poisoning and 3 deaths.

Poisoning incidents due to exceedance of the permissible limit for residue from highly toxic pesticides are occurring more frequently. For instance, carbofuran was used on Chinese cabbage in Heilongjiang Province, leaving millions of kg of the vegetable contaminated. In Jiangxi Province, dimethoate was once determined to be 5 times over the permissible limit for residue in Chinese cabbage on sale in a city market. In 1994, the first half of the year alone saw more than 100 poisoning incidents occurring just in one place in Guangdong Province as a result of pesticide residue on vegetables. In one incident, 66 people were poisoned by eating water convolvulus sprayed with methamidophos. In 1992, a pesticide-poisoning incident took place, killing 11 people in Anqing City of Anhui Province. Eventually, it turned out that they had a breakfast made out of the wheat flour that had been contaminated with organophosphorus pesticide during transport on a truck that had once been used to carry organophosphorus pesticide. During 1998, the Huaxia School in Zhuhai City had a large number of students poisoned, leaving 23 hospitalized, due to their eating vegetables containing pesticide residues (2). The above suggests a social problem that needs an urgent solution.

DISTURBING ECOLOGICAL EQUILIBRIUM AND ENDANGERING BIODIVERSITY

Because a number of pesticide varieties are highly toxic and have a wide range of toxicity, when applied, they are not only a danger to humans, but also to the environment. Their use can disturb the balance existing between the target insects and their natural enemies; i.e. beneficial insects, frogs, snakes, birds, etc. This means that when pesticides are applied both the target insects and biomes are affected simultaneously and indiscriminatingly. Survivors of the targeted species can reproduce rapidly while regeneration of their natural enemies is inhibited due to food shortage. Moreover, the applied pesticide may transfer and accumulate in the food chain, posing greater toxic risks for lives high up the food chain.

Quite a number of high-grain yield regions have become consumers of pesticides. According to spot checks, the consumption of pesticides rose rapidly from 4.65 kg ha^{-2} in 1985 up to 15.9 kg ha^{-2} in 1991, an annual rate of increase of 41.8%. In September 1998 the PIC Convention signed by over 60 nations, advocated and promoted by UNEP and FAO, specifies methamidophos, monocrotophos, parathion, parathion-methyl and phosphamidon as detrimental pesticides; some of which are still in extensive use for controlling plant diseases and insects (3). Due to the over-application and abuse of pesticide use, waterbodies in the receiving areas are contaminated and ecological equilibrium is disturbed. In such areas, frogs and fish populations have decreased drastically. Paddy eel and loach have been exterminated; and silkworms cultivated indoors have died of pesticide contamination of the mulberry leaves in their diet. Application of the pesticides has also affected bird populations in forests and mountains. Birds are often killed by eating pesticide-poisoned insects. Meanwhile, the killing of large numbers of insects has deprived some birds of their food sources, thus leading to decline and even extermination of whole populations. The use of the pesticides has thus gravely affected biodiversity, and directly threatens entire eco-systems.

China has a rich bird fauna, with 1244 species or genus, of which 37 enjoy first class protection from the State and 74 second class protection. Use of some highly toxic pesticides has already caused serious harm to these protected birds. Carbofuran, for instance, is one kind of pesticide that China produces in large quantities and uses in almost every corner of the country. The carbofuran in use is mainly in the form of granule, 3% only in concentration, or a seed-coating mixture, 3–5% in concentration. Its LC_{50} to birds is lower than 1 mg kg^{-1}. Experiments have demonstrated that one granule of the pesticide is enough to kill a small-sized songbird, and that a songbird is doomed even if it happens to eat up an earthworm contaminated with carbofuran applied at the lowest rate in the cornfields. A bird is paralyzed or killed when it eats anything contaminated with carbofuran, no matter whether it is a plant, an insect, a living or dead soil invertebrate or drinks carbofuran contaminated water, since there is almost no safe dose of carbofuran for wildlife. The US EPA stated that carbofuran is so virulent that there are no effective measures to reduce its risk to birds. For the safety of birds and wildlife, the US and Canadian Governments decided to prohibit the use of carbofuran granules in 1997. China began, in 1995, to investigate the use of carbofuran and its potential threat to birds in northeastern China. The results showed that of the 86 species or subspecies of birds with 1st and 2nd-class state protection, 85% were likely to be threatened by carbofuran. During an investigation, only 3 earthworms were found within 1 m^2 of the plough layer soil in a sugarcane field that had been treated with carbofuran for years, whereas more than 30 were discovered in a control field nearby. It is also obvious that the populations of birds are decreasing. Thus, the use of carbofuran is a threat to both common and rare birds. Control of virulent pesticides to protect rare and endangered bird species has become a problem of urgency for the environment protection agencies.

TREMENDOUS ECONOMIC LOSSES CAUSED BY PESTICIDE CONTAMINATION

Because not all the processes of production, transport, distribution, and utilization of pesticides were under effective environmental supervision and management, various incidents of serious pesticide pollution often occurred, resulting in heavy losses to the national economy. From July 1995 to August 1996, more than 2000 incidents took place over an area of 1333.4 ha of farmland, in 19 provinces (or regions, including Heilongjiang, Jiangsu, Guangdong, etc.), resulting in an economic loss of 500 million yuan. If damage caused by pesticide pollution in other provinces is taken into account economic losses could be as high as 1 billion yuan.

In 1996, farmers in Hengshui Prefecture, Hebei Province, bought from a pesticide plant in Jingxian County an "unwanted" pesticide. Right after the pesticide was sprayed on to the crop, the green plants began to wither. About 66.7 ha of cotton plants died within days. This pesticide-induced disaster led to a direct economic loss of 1 million yuan (4).

The extensive use and abuse of pesticides often leaves high residues in agricultural products. The agricultural products with exceedance of the permissible limit of pesticide residue not only endangers the lives of people, but also affects the reputation and the import and export trade of China in the world. Pesticide residues in agricultural products and by-products for export often lead to rejection or return of the goods, which brings results in economic loss and seriously affects China's foreign trade reputation (5).

URGENCY OF INTENSIFIED ENVIRONMENTAL SUPERVISION AND MANAGEMENT OF PESTICIDES

The pollution problems discussed above demonstrate how a lack of rational utilization of pesticides has led to pollution of the environment, jeopardized human health, and disturbed ecological equilibrium. The extent of the problem poses a

Table 1. The reported 47 349 cases of pesticide poisoning: 1992–1996.

Year	1992		1993		1994		1995		1996		Total	
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
Insecticide	61 497	87.1	45 231	86.5	37 446	87.5	41 404	85.6	27 999	84.2	213 577	86.3
Fungicide	766	1.1	681	1.3	446	1.0	403	0.8	1401	1.2	2697	1.1
Rodenticide	1497	2.1	1407	2.7	1141	2.7	1389	2.9	1079	3.2	6513	2.6
Herbicide	773	1.1	607	0.9	417	1.0	531	1.1	502	1.5	2830	1.2
Mixed	1170	1.6	452	0.9	486	1.1	1120	2.3	468	1.4	3696	1.5
Others	4915	7.0	3909	7.5	2876	6.7	3530	7.3	2806	8.4	18 304	7.3
Total	70 168	100	52 287	100	42 812	100	48 377	100	33 255	100	247 349	100

grave threat to the people in rural areas, and is a potential hazard to the health of the whole population.

Pesticides, however, appear to be necessary. For a long time, chemical prevention will still constitute 80% of the control of insect pests in agriculture. How to maintain effective supervision and management of the application of pesticides has become a problem of paramount importance for China.

In short, China lags someway behind the developed countries in the control of pesticides. Environmental supervision and management are not yet in place, and monitoring of pesticide use was started rather late. In 1982, several ministries and commissions jointly stipulated *Regulations for Registration of Pesticides*, these imply that new pesticide products must be registered prior to introduction on the market. Regulations also prohibited producing, distributing or utilizing unregistered pesticides, and that foreign companies must have the pesticide registered before it appears on the market (6). Enforcement of these regulations has helped, to a certain extent, in controlling the disorder that existed in the production and distribution of pesticides in China. In 1997, authorized by the State Council, *Rules for Administration of Pesticides* were promulgated. This is the first official legal document concerning pesticide management in China. However, the key contents of the document, are limited to monitoring production, distribution and utilization of pesticides. The document does not consider whether registered pesticides, when put into use, will contaminate the environment and jeopardize human health. Nor does it consider how products that already cause contamination and damage should be controlled.

In a sense, how to effectively supervise and manage the pesticides after they enter the environment in large quantities is still virgin territory in China. For instance, among the organochlorine compounds specified in the POPs convention, (Stockholm, May 2001), some are still in use in some regions of China. In September 1998, the PIC Convention signed by over 60 nations, as advocated by UNEP and FAO, lists methamidophos, monocrotophos, parathion, parathion-methyl and phosphamidon as toxic substances, some of these substances are still used extensively to control plant diseases and insects (7). In addition, some herbicides are considered to cause cancer and other illnesses, and are prohibited in many countries. China has, however, not yet taken action to prohibit their use.

To intensify the environmental management of pesticides, environmental protection agencies should take a leading role in formulating corresponding rules and regulations, and measures for environmental supervision and management (8). Pesticides should be under strict supervision and management from introduction to use. Current attention needs to focus on supervising and monitoring pesticides after they enter the environment. Those with acute toxicity, high application rates and extensive use should be given priority. Measures should be taken to eliminate and destroy those substances already listed as prohibited or restricted in international conventions (8). Measures to intensify management of pesticides should be earnestly put into effect, in order to guarantee strategic and technical protection of the environment of human beings and, not least, to maintain ecological equilibrium in China.

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