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Jasmin M Nadaf
Department of Plant Pathology and Agril Microbiology, MPKV, Rahuri, Ahmednagar, Maharashtra, India

AM Navale
Department of Plant Pathology and Agril Microbiology, MPKV, Rahuri, Ahmednagar, Maharashtra, India

Corresponding Author
Jasmin M Nadaf
Department of Plant Pathology and Agril Microbiology, MPKV, Rahuri, Ahmednagar, Maharashtra, India

Isolation of microorganisms sensitive to low concentration of pesticides and heavy metals residues

Jasmin M Nadaf and AM Navale

Abstract
The purpose of this study was to assess the residual pesticide viz, Cypermethrin, Spinosad, Acephate, Carbendazim, as well as Chlorpyriphos, heavy metal viz, Lead, Cadmium, & Cromium, as well as the microbial contamination quality and safety of common marketable fruits and vegetables samples from various local markets in Mumbai. For these study soil samples were taken from the railway site of Malad, Vileparle, Kandivali, Rammandir, Goregaon, as well as Andheri railway stations on Mumbai's western line. Soil samples were gathered and labelled correctly in disposable polythene bags. Ten pesticides and seven heavy metals were used. Ten frequently used pesticides viz; Cypermethrin, Chloropyriphos, Acephate, Spinosad, Captan, Carbendazim, Metalaxyl, Mancozeb, Chlorothalonil, 2-4-D as per European standard concentration. Seven used heavy metals viz; Copper (cu), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As), Mercury (Hg) as per Indian standard awasthi (2000). Total 21 isolates were isolated for cypermethrin, 16 for chloropyriphos, 11 for acephate, 16 for spinosad, Captan 22, carbendazim 16, metalaxyl 12, mancozeb 9, chlorothalonil 14 and for 2-4-D 20 isolates were isolated. Among the heavy metals total 30 isolates were isolated for copper, lead 14, cadmium 10, chromium 20, nickel 10, and for arsenic 12 isolates were isolated. These isolates probably belonging to genus *Bacillus, Pseudomonas, Paenibacillus, Azotobacter, Azospirillum* spp.

Keywords: Pesticide, heavy metals, sensitivity, low concentration

Introduction
Pesticides are widely used in fruits and vegetables because of their susceptibility to insect and diseases attack. Consequently, food safety is a major public concern worldwide. The total dietary intake of pesticides residues that remain on agricultural commodities are known as carcinogens/or toxins and therefore it is desirable to reduce these residues (Zawiyah *et al*., 2007) [11]. Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Marshall, 2004 [12]; Radwan and Salama, 2006 [13]; Khan *et al*., 2008 [14]). Railway track farming are carried out from Charni road station till Borivali station along the Western line, from Byculla station till Dombivli station on central line and from Sewri station till Panvel station on harbour line of Mumbai in Maharashtra. Different types of vegetables like Spinach, Lady's finger and Radish are grown throughout the year along these railway tracks. The water source for such agricultural activity is sewage water where their discharge of the effluents is from various industries, water from drainage pipelines and from domestic source, which lead to the contamination of the soil, fruits and vegetables (Doshi and Zele, 2014) [5]. To safeguard the health of our own citizensm, isolation of microorganisms sensitive to pesticides and heavy metals residues were carried out by poison food technique.

Material and Method
Collection of soil samples
Soil samples were collected from railway station area of western line of Mumbai viz., Kandivali, Malad, Vileparle, Ram mandir, Goregaon, and Andheri. Soil samples were collected in plastic polythene bags and labeled properly.

Collection of water samples
Water Samples were collected from nearby railway stations of Mumbai. Water samples were taken with a 250 ml Polyethylene (PE) bottle either directly from the source (open sewage or from the field.

Media used

Media used for isolation was simple Nutrient agar medium (Anon, 1957) [1].

Source of chemical used

For these 10 pesticides and 7 heavy metals were used which obtained from Department of Agricultural Entomology and Department of Plant pathology and Agricultural Microbiology MPKV, Rahuri, which are given in the Table 1.

Table 1: Chemicals used for isolation of microorganisms

Pesticide	Heavy metal
1. Cypermethrin	1. Copper (Cu)
2. Chloropyriphos	2. Lead (Pb)
3. Acephate	3. Cadmium (Cd)
4. Spinosad	4. Chromium(Cr)
5. Captan	5. Nickel(Ni)
6. Carbendazim	6. Arsenic(As)
7. Metalaxyl	7. Mercury(Hg)
8. Mancozeb	
9. Chlorothalonil	
10. 2-4-D	

Table 2: Permissible limits for Heavy metals in mg/Kg

Indian Standard Awasthi (2000) [2]	Cu	Pb	Cd	Cr	Ni	As	Hg
	30.0	2.5	1.5	20	1.5	1.1	NL

NL = No limit

Table 3: Maximum residue limits of pesticide in fruits and vegetables

	Pesticide	Europian Union Standard (2016) in mg/Kg
1.	Cypermethrin	0.05
2.	Chloropyriphos	0.05
3.	Acephate	0.01
4.	Spinosad	0.07
5.	Captan	0.02
6.	Carbendazim	0.1
7.	Metalaxyl	0.05
8.	Mancozeb	0.05
9.	Chlorothalnil	0.01
10.	2-4-D	0.05

Method

Isolation of microorganisms from soil and Water samples

Isolation of Microorganisms were carried out by serial dilution, pour plate and food poison technique (Nene and Thapliyal, 1979) [7]

A Ten gram soil sample from each area from western line of Mumbai was suspended in 90 ml of sterilized water blanks. Serial dilutions were made from 10^{-1} to 10^{-7}. One ml aliquot of dilutions from 10^{-3} to 10^{-7} was transferred to sterilize Petri plates separately. The sterilized Nutrient agar medium was poured in each Petri plate just before solidification (40 °C temperature) and pesticides were added in the plates by using micropipettes, mixed the contents in plates by rotating the Petri plate clock and anti-clockwise. After solidification, the plates were kept at 28+2 ºC in BOD incubator for 4-5 days. Duplicate plates were made for comparison as control. All the plates were observed for the appearance of bacterial/fungal/ Actinomycetes colonies on medium. Same procedure is followed for isolation of microorganism from heavy metals.

1 ml of water sample was used for isolating the microorganisms through serial dilution and agar plate culture technique. The collected water sample was taken and it is serially diluted with distilled water. The serial dilution was done up to 10^{-9}. One ml of sample was collected from each dilution and was spread on nutrient agar plates and heavy metals were added in the plates by using micropipettes. The plates were incubated at 37 ^{0}C to achieve vigorous growth. Duplicate plates were made for comparison as control.

Treatment-

1. Treatment + soil samples
2. Control = Soil samples
3. Treatment + Water samples
4. Control = Water Samples

	Pesticides	Conc as per EU STD (ppm) (2016)		
1.	Cypermethrin	0.05	1.25	2.5
2.	Chloropyriphos	0.05	1.25	2.5
3.	Acephate	0.01	0.25	0.5
4.	Spinosad	0.07	1.75	3.5
5.	Captan	0.02	0.5	1.0
6.	Carbendazim	0.1	2.5	5.0
7.	Metalaxyl	0.05	1.25	2.5
8.	Mancozeb	0.05	1.25	2.5
9.	Chlorothalonil	0.01	0.25	0.5
10.	2-4-D	0.05	1.25	2.5

	Heavy metals	Indian STD (ppm) (Awasthi 2000) [2]	
1.	Copper (Cu)	30.0	0.01
2.	Lead (Pb)	2.5	0.5
3.	Cadmium (Cd)	1.5	0.07
4.	Chromium (Cr)	2.0	1.00
5.	Nickel (Ni)	1.5	0.04
6.	Arsenic (As)	1.1	0.05
7.	Mercury (Hg)	NL	NL

Results and Discussion

Isolation of microorganisms from soil and water samples

A total 30 soil samples were collected from railway station area of western line of Mumbai viz., Kandivali, Malad, Vileparle, Ram mandir, Goregaon, Andheri. Twenty water samples were collected from nearby railway stations of Mumbai for isolation of microorganisms at low concentration of pesticide residues and heavy metals. The total no of isolates isolated for each individual pesticide and heavy metal are mentioned in the Table 4 and 5.

Table 4: Isolation of microorganisms from soil and water at low concentration of pesticides

	Pesticide	Concentration(ppm) As per EU STD (2016)	Isolates
1.	Cypermethrin	0.05	21
		1.25	21
		2.5	21
2.	Chloropyriphos	0.05	16
		1.25	16
		2.5	16
3.	Acephate	0.01	11
		0.25	11
		0.5	11
4.	Spinosad	0.07	16
		1.75	16
		3.5	16
5.	Captan	0.02	22
		0.5	22
		1.0	22

6.	Carbendazim	0.1	16
		2.5	16
		5.0	16
7.	Metalaxyl	0.05	12
		1.25	12
		2.5	12
8.	Mancozeb	0.05	9
		1.25	9
		2.5	9
9.	Chlorothalonil	0.01	14
		0.25	14
		0.5	14
10.	2 -4-D	0.05	20
		1.25	20
		2.5	20

Table 5: Isolation of microorganisms from soil and water at low concentration of heavy metals.

	Heavy Metal	Concentration(ppm) As per Indian STD (Awasthi 2000) [2]	Isolates
1.	Copper (Cu)	30.00	30
		0.01	30
2.	Lead (Pb)	2.5	14
		0.5	14
3.	Cadmium (Cd)	1.5	10
		0.07	10
4.	Chromium (Cr)	20.0	20
		1	20
5.	Nickel (Ni)	1.5	10
		0.04	10
6.	Arsenic (As)	1.1	12
		0.05	12
7.	Mercury (Hg)	-	-
		-	-

Conclusion

The result obtained in this study generally revealed that many of microorganisms which were isolated to detect the sensitivity for pesticides and heavy metal residue found in marketable fruits and vegetables showed normal growth on a simple agar medium. These isolates probably belonging to genus *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Azotobacter*, *Azospirillum spp.* Hence these finding of the study suggest that there are sensitive microbes found at low concentration of pesticide and heavy metals in soil and water sample collected from Mumbai area.

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