April - June - 2019

ISSN 2277 - 5730

AN INTERNATIONAL MULTIDISCIPLINARY
QUARTERLY RESEARCH JOURNAL

AJANTA

Volume - VIII Issue - II April - June - 2019

English Part - XII / XIII

Peer Reviewed Refereed and UGC Listed Journal

Journal No. 40776

ज्ञान-विज्ञान विमुक्तये

IMPACT FACTOR / INDEXING

2018 - 5.5

www.sjifactor.com

❖ EDITOR ❖

Asst. Prof. Vinay Shankarrao Hatole

M.Sc (Maths), M.B.A. (Mktg.), M.B.A. (H.R.),
M.Drama (Acting), M.Drama (Prod. & Dir.), M.Ed.

❖ PUBLISHED BY

Ajanta Prakashan

Aurangabad. (M.S.)

VOLUME - VIII, ISSUE - II - APRIL - JUNE - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

CONTENTS OF ENGLISH PART - XIII

PRINCIPAL
Govt. College of Arts & Science
Aurangabad

VOLUME - VIII, ISSUE - II - APRIL - JUNE - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

14. Plant Bioassay: An Important Bio-Tool to Study the Detoxification of Heavy Metals from Effluent Treated using Microbial Consortium

Kulkarni R. A.
Government College of Arts and Science, Aurangabad, Maharashtra.
Gupta S. G.
Government Institute of Science, Aurangabad, Maharashtra.

Abstract

Pot assay using *Triticum aestivum* (Common wheat) is carried out to check the effect of heavy metals present in industrial effluent. Different concentrations of effluent containing metal ions and microbial consortium treated (for the removal of metal ion-detoxified) effluents are used to study the effect of metal ions on plant growth with respect to germination of seeds, height of plats, total protein , phosphates and chlorophyll contents of leaves. It was observed that very trace amount of chromium, nickel, copper was found to be very toxic resulting in reduction of protein, phosphate chlorophyll content of wheat plant. Further it was observed that if this effluent is treated with developed microbial consortium the treatment is responsible to detoxify it by various microbial mechanisms that resulted in the growth of plant, germination of wheat seeds and other contents which were found to be similar to that of the plants watered with tap water.

Key Words: *Triticum aestivum* plant assay, effluent containing heavy metals, microbial consortium to detoxify the effluent

Introduction

Plant bioassay is a simple, inexpensive, accurate method of determining the presence of herbicides or chemicals at high concentration which can adversely affect crop growth, yield and quality. The bioassays directly measures the physiological sensitivity of a plant to soil, water and chemicals in the vicinity of the plant.

Water the wonderful natural resource is extremely essential for survival of all living beings. But today clean water has become precious commodity and its quality is threatened by numerous sources of pollution (Pandey *et al.*, 2005) .Water reserves of the world are limited. The total amount of water on the earth is about 1.35 billion km^3 ($3.5x10^{20}$ gallon). Over 97% of this amount is in the earth's ocean and the earth's freshwater total is only about 37 million km^3 of

which $4/5^{th}$ occurs in polar ice caps and glaciers. (Rajeev and Gopal, 1995). It is clear that only a little amount of fresh water is accessible to humans. This mearge quantity of water which is available for human use is also getting contaminated by population explosion and industrilization (Sharma and Rajasree, 2003).

Pollution as defined by National Environmental Research Council (1976) is the release of substances and energy as waste products of human activities, which results in harmful changes within natural environment (Pandey *et al.*, 2005).

Industrial effluents discharged into water resources are one of the important sources of water pollution. These industrial effluents contain toxic organic and inorganic chemicals, hazardous compounds like phenols, aldehydes, ketones, amines, cyanides, metallic wastes, toxic acids, corrosive alkalis, oils, fertilizers, greases, dyes, biocides, radioactive wastes and thermal pollutants released from numerous industries.

The discharge of heavy metals into aqueous ecosystems has become a matter of great concern over the last few decades. The pollutants of serious concern include lead, mercury, chromium, uranium, selenium, zinc, etc. These metals are carcinogenic and mutagenic in nature. Majority of toxic pollutants are waste products of industrial and metallurgical processes such as mining operation, refining ores, sludge deposits, radioactive materials, paints, alloys, metal plating, batteries and pesticide industries. The presence of such metals (> $5gcm^3$, Mahavi, 2005) in aquatic environments cause severe damage to aquatic life. Moreover, these metals have exacting consequences on humans such as brain damage, reproductive failure, nervous system failure, tumor formation, etc. (Hamman, 2004).

The excess of heavy metals become toxic for plants that switches the ability to uptake and accumulate the elements. The increased amount of heavy metals within the plant tissues displayes direct and indirect impacts that results in the generation of oxidative stress which further aggrivates inhibition of cytoplasmic enzymes and damages to cell structures.

Nickel (Ni) is reported to be toxic to most plant species affecting amylase, protease and ribonuclease enzyme activity thus retarding seed germination and growth of many crops. (Ahmed *et.al,*2011) It has been reported to affect the digestion and mobilization of food reserves like protein and carbohydrates in germinating seeds (Ahmed *et, al.,*2011 and Ashraf *et., al.,* 2011) reducing plant height, root length, fresh and dry weight, chlorophyll content and enzyme carbonic anhydrase activity and increasing mulodialdehyde content (MDA) and electrolyte leakag (Siddique *et. al.*2011)

Materials and Method

In present study plant bioassay is carried out to study the effect of effluent on percent germination, sprouting, growth in terms of dry weight, increase in height, chlorophyll, phosphate and protein content in leaves.

1. Seeds of *Triticum aestivum* (commom wheat) were used.
2. For the assay the effluent used was from electroplating industry which was having pH 2.0 with total dissolved solids1232 ppm, COD (Chemical oxygen demand 50 ppm) containing heavy metal ions (Cr 30 ppm, copper 5 ppm, lead 1ppm,manganese 10 ppm, Zinc 2 ppm, Nickle 50 ppm) chloride 50ppm and sulfates 200ppm. This effluent is used in assay as follows

   A. 50% diluted effluent

   B. 25% diluted effluent whose pH was adjusted to 7.0 using sodium hydroxide

   C. 25% diluted effluentwhose pH is 7.0 and this effluent was treated with microbial consortium (*A. niger*, *S. cerivisiae*,*M. hiemalis* and *P. aeruginosa 4442*) so as to check the effect of microorganisms as the bio remediator

   D. Tap water (D) that serves as control
3. Microbial consortium is prepeared by taking 1% (w/v) (*A. niger*, *S. cerivisiae*, *M. hiemalis* and *P aeruginosa)*
4. Assay

A: Percent germination of seeds after sowing in soil

Seeds of wheat were taken and they were soaked in tap water for 24 hrs, 500gm of oven sterilized soil was taken and added into four pots. Pots were labeled as A, B, C, D and then to each pot 25 pre-soaked seeds were sown. All the pots were kept in sunlight and watered daily with 50 ml respective effluent/water as mentioned above. Percent germination of seeds and plant growth in terms of increase in sprouting and increase in plant length was observed.

B: Growth in terms of dry weight of leaves

To calculate effect of effluent on dry weight of leaves after 15 days of incubation, 1gm of leaves were taken and sun dried and dry weight was estimated.

C: Growth in terms of protein content of leaves after 15 days

For the estimation of leaf protein content 0.01gm of leaves from each pot was taken in test tube containing 1ml phosphate buffer. It was sonicated at 2000HZ (Microson-TM) and centrifuged at 10,000 rpm for 5 minutes by adding 500 ul of phosphate buffer pH 7.0. From it, 100 ul sample was taken and to it 900 ul of distilled is added and amount of protein was estimated using Folin-Lowry method (1951).

D: Estimation of phosphates in leaves

The total leaf phosphorus content was estimated by Fiske-Subbarao (1981) method. For the extraction of phosphates, 5 gms of leaves were taken from each pot and sonicated by adding 5 ml of distilled water. To 1ml of sample TCA, molybdate and ANSA reagents were added as per the standard method (Fiske-Subbarao, 1981) and after reaction the blue color formed was estimated using U-V visible spectrophotometer at 640nm.

E: Estimation of chlorophyll content of plants

To study the chlorophyll content of the leaves after 15 days of growth, 1gm of plant leaves were taken and 20 ml of 80% acetone was added and the mixture was sonicated at 3000HZ, and centrifuged at 5000 rpm for 5 minutes. Supernatant was transferred to the volumetric flask and the process was repeated till the residue was colorless. Volume was made up-to 100mlwith 80% acetone and the absorbance of the solution was taken at 645, 663 and 652 nm against the solvent (80% acetone) blank.

Mg of chlorophyll a/gm of leaf = 12.7(A663) -2.69(A645) * V/1000*W

Mg of chlorophyll b/gm of leaf = 22.9 (A645) -4.68(A663)*V/1000*W

Mg of total chlorophyll/gm leaf = 22.2(A645) +8.02(A663)*V/1000*W

A= Absorbance at specific wavelength

V= Final volume of chlorophyll extract in 80% acetone (it is 10ml)

W = Fresh weight of leaf extract (0.1gm)

F: Percent germination of seeds soaked in different dilutions of effluents

Seeds of the wheat were soaked in different dilutions of effluent and in tap water for 48 hours. These seeds were then placed on sterile nutrient agar plates and incubated for 24 hours at ambient temperature in sunlight and the % sprouting was calculated

Results and Discussions

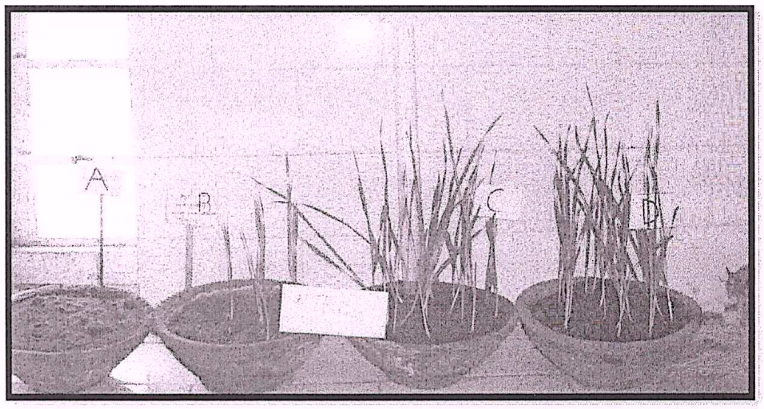
A: Percent germination of seeds after sowing in soil

From the Fig. 1 and 2 it was clearly evident that there was no growth of sorghum seeds sowed in soil and watered with 50% of effluent (A), growth was less in the pot irrigated with 25% effluent untreated whose pH was adjusted to 7.0(B).The growth of the pot irrigated with 25% effluent pH 7.0 and treated with microbial consortium showed growth as that of the pot irrigated with tap water. The color of the leaves remained green in the pots irrigated with effluent treated with microbial consortium. Growth of the leaves in the pot irrigated with 25 % effluent was lacking fresh green color, the leaves appeared pale, dwarf and without shining color.

Fig.1: Growth of wheat seeds after 5 days watered with different dilution of effluen

A. Pot watered with 50% effluent.
B. Watered with 25% effluent pH 7.0.
C. Pot watered with 25% effluent with pH 7.0 treated with consortium.
D. Pot watered with tap water.

Fig.2: Growth of W*heat* seeds after 10 days watered by different dilution of effluent



From the experiments it was (Table no.1) observed that the seeds watered with 50% untreated effluent showed only 28% sprouting of the seeds, out of it 12% remained alive for 3 days and then died. Seeds watered with untreated 25% effluent whose pH was adjusted to 7.0 showed 40% germination out of which 25% remained alive and started growing, the seedlings watered with 25% effluent treated with microbial consortium showed 68% germination after 3 days. The seeds watered with tap water showed 84% of the germination. Further two days of germination, the microbial consortium treated seedlings show sprouting to 84% which clearly

indicated that in first two cases the effluent proved to be harmful for the germination of seeds thus it proves that heavy metals have deleterious effect on plant growth. Cu when present in soil in high amount causes cytotoxic injury to plants this resulted in hindrance of plant growth and caused chlorosis (Lewis *et. al.* 2001).

Table 1: Percent germination of Wheat seeds sown in pots irrigated with effluent

Sample	Percent germination of seeds (hrs)			
	24	**48**	**72**	**96**
Irrigated with 50% untreated effluent (A)	**28**	12	0	0
Iriigated with 25% untreated effluent(B)	**40**	32	24	24
Irrigated with 25% treated effluent Using microbial consortium (pH 7.0)-(C)	60	68	**84**	84
Irrigated by tap water (D)	72	**84**	84	84

B: Growth in terms of dry weight of leaves

From the table 2 it was observed that there was no growth of leaves in case of the pot watered with 50% of effluent (A), the pot watered with 25% of effluent whose pH was adjusted to 7.0 shows 0.0056mg/gm of dry weight (B), the effluent treated with consortium shows 0.062 mg/g of dry weigh (C) and that treated with tap water shows 0.067 (D). It indicated that the effluent is having drastic effect on growth of plant.In excess Mn halt plant growth and development causing interveinal and marginal chlorosis, necrosis and distorted leaf structure both externally and internally (Kitao et.a. 2001).

Table 2: Dry weight of leaves irrigated with effluent

Sample	Dry weight (mg/gm)
Watered by 50% effluent (A)	0.000
Watered by 25% effluent (B)	0.056
Watered by 25% effluent treated with consotium (pH 7.0)-(C)	0.062
Watered by tap water (D)	0.067

Table 3 showed effect of various dilutions of effluent on average length of the plants in (mm) on the 15 th day. The pot watered with 25 % effluent showed 195mm and the pot watered with consortium treated effluent 202mmandthatof the tap watered 205mm suggesting that heavy metal ions are responsible for retarding the shoot growth and the observations are similar to that

found by Sayyedi (1999). The results proved that heavy metals have become one of the most serious anthropogenic stressors for plants and other living organisms.

Table 3: Average growth of plants in mm

Days of incubation	(A) Watered with50% effluent	(B) Watered with 25%effluent **(pH-7.0)**	**(C) Watered with treated consortium**	**(D)Watered with tap-water.**
1	Starts germination	Starts germination	Starts germination	Starts germination
2	7 sprouted	10 sprouted	15 sprouted	18 sprouted
3	3 remains alive	8 remains alive	17 sprouted	21 sprouted
4	All seedlings died	6 remains alive	21 sprouted	21 sprouted
5	0mm	4mm	6mm	7mm
6		12mm	14mm	15mm
7		32mm	39mm	40mm
8		50mm	56mm	58mm
9		78mm	82mm	85mm
10		92mm	101mm	119mm
11		105mm	121mm	126mm
12		118mm	149mm	162mm
13		135mm	179mm	184mm
14		168mm	198mm	201mm
15		195mm	202mm	205mm

C: Growth in terms of protein content of leaves after 15 days

Table 4 shows effect of effluent on the soluble protein content of plants after 15 days of growth which indicated that the seedlings which were watered with50% effluent show no growth and no protein content, 25% effluent show 120 mg/ml of proteins, 25% consortium effluent show 150 mg of proteins and similarly there was increase in phosphate content as the concentration of effluent decreases. Li et. Al. (2009) studied toxicity of Co on barley (HordeumhapusL) tomato (Lycopersiconesculentum L) oil seed rape (Brassica napus L) and found that Co has reduced the shoot growth and biomass of the plant.

Table 4: Estimation of leaf protein

Sample	**Concentration of protein (mg/ml)**
Watered by 50% effluent (A)	0.00
Watered by 25% effluent (B)	120

Watered by 25% effluent treated with consortium (C)	150
Watered with tap water (D)	150

D: Estimation of phosphates in leaves

From the Table 5 it can be observed that there is much difference in the phosphates concentration of the plant watered with 25% effluent with pH7.0. At the same time the difference between microbial consortiums treated and tap watered plant is less.

Table 5: Concentration of phosphate

Sample	Concentration of phosphate (ugm/ml)
Watered by 50% effluent (A)	0.000
Watered by 25% effluent (B)	0.0616
Watered by 25% effluent treated with consortium (pH 7.0)- (C)	0.1108
Watered by tap water-(D)	0.1480

E: Estimation of chlorophyll content of plants

There was decline in total chlorophyll content as observed in Table 6. And the results are similar to that of Vanassche and Clijaters (1990) who suggested that, decrease in chlorophyll may be due to inhibition of important enzymes associated with chlorophyll biosynthesis. The chlorophyll content was decreased as the concentration of effluent increases which is similar to the findings of Gulvage*t.al.*,1974 and Brown *et.al.*, (1986) they observed that low concentration of mercury inhibits photosynthesis temporarily increases respiration and reduces chlorophyll a, chlorophyll b and total chlorophyll concentration and also causes substantial loss of intra cellular potassium *Rhytidiadephoussquarrosus*. The reduction of chlorophylls of a,b and total chlorophyll observed in this study agreed with the findings of Brown and Whitehead (1986). These reductions would certainly reduce the photosynthetic processes of the plant.

Table 6 : Total chlorophyll content of leaves irrigated with effluent

Sample	Chl a mg/gm Plant	Chl b mg/gm Plant	Total chl.mg/gm Plant
Watered by 50% Effluent (A)	0.000	0.000	0.000
Watered by 25% Effluent-(B)	0.067	0.084	0.114
Watered by 25% Effluent, treated with	0.112	0.290	0.207

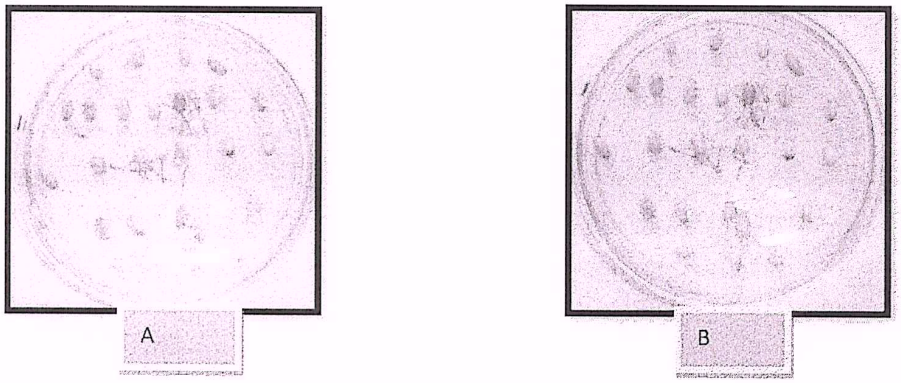
consortium (pH 7.0)-(C)			
Watered by tap water-(D)	0.495	0.414	0.892

F: Percent germination of seeds soaked in different dilutions of effluents

Fig.3 indicated the % germination of the seeds soaked for 24 hrs. in different concentration of effluents and placed on N. A. The plates were incubated for 48 hrs. and then results were calculated. The seeds soaked in 50% effluent does not show any sprouting (A), the seeds soaked in25% effluent with pH7.0 shows just 12% germination (B), consortium treated shows 28% (C) and tap water soaked seeds shows 48% germination (D). Thus results indicated the toxicity of effluent to the germination and growth of sorghum seeds it was observed if there was decrease in % effluent dilution there was comparatively increase in protein, phosphate, chlorophyll concentration of plant leaves. Further it was observed that if the pH of the effluent was adjusted to 7.0and it is treated with microbial consortium will result in good seed germination with increase in plant contents of plant leaf as described earlier, studies revealed that the metal ions vary in their toxicity and degree of toxicity as well which is proportional to their concentration as observed by Martin and Coughtrey (1982). Thus it can be said that the roots of the plants are the first organ that encounters heavy metals and thus roots have widely studied to assess the impact of the stressor.

Khan and Khan (2010) conducted an experiment on chickpea (Cicer arietinum) to evaluate the effect of nickel and cobalt at high concentration and observed that it reduced the growth and biomass, seed germination, shoot and root injury, supperession of root nodules and advrrsely affects the yield of the crop.

Fig.3. Percentage germination of seeds soaked in effluent



Among the bio assays developed for detection of mutagenicity, cytotoxicity due to environmental pollutants, plant systems have proven to be sensitive, cheap and effective. The

studies thus shows that the metals vary in there toxicity and the degree of toxicity which is proportional to their concentration as observed by Martin and Coughtrey (1982). From the results it can be observed that bio assays can serve as a routine test because the tests are simple and rapid to conduct. Karr (1983) have presented rapid root bio assay method for identifying aluminum toxic soils. Zhang *et. al.,* (2002) observed that due to high concentration of arsenic seed germination and seedling growth of wheat was inhibited. Arsenic has been reported to decrease the photosynthetic pigment, damage chloroplast membrane and decreases enzyme activity by reacting with sulfhydryl group of proteins and also reported to alter the nutrient balance and protein metabolism (Singh *et. al.* 2009). Thus being sessile plants cannot escape unwanted changes in the environmental exposure to heavy metals that triggers a wide range of physiological and biochemical alterations which helps to asses presence of such toxic elements using plant bioassays effectively.

References

1. Ahmed MS, Ashraf M., Essential roles andhazardous effects of Ni in plants. Rev Environ Contam. Toxicol. 2011;2011;214:125-67[Pub Med] [google Scholer]
2. Ashaf MY, SadiqR, Hussain M., Ashraf M., Ahmed MS., Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of ssunflower (*Helianthus annuus* L.) Biol Trace Elem Res 2011;143:1695-703 [Pub Med] [Google Scholer]
3. Brown, D.H and A. Whitehead, (1986) The effect of mercury on the physiology of *Rhytidiadelphus squarrosus* (Hedw.) warnst. *J. of Bryology,* 14:367-374.
4. Fiske, Subbarow,(1981) Estimation of inorganic phosphorous In Laboratory Manual In Biochemistry by Jayraman, New Age International India (P) l:103-104.
5. Gulvag, B.M; Skaar, H and E. M. Ophus (1974). An ultrastructural study of lead accumulation with leaves of *Rhytidiadelphus squarrosus*. (Hedw) Warnst. J. of bryology, 40:311-317.
6. Karr, M. C., Coutinho J. and Ahlrichs (1983) "Determination of aluminum toxicity in Indian soils by petri dish bioassay." Proc. Indian Acad. Sci.-93, 405-11.
7. Khan M. R. and Khan M. M. (2010) Effect of varying conc. Of Ni and cobalt on the plant growth and yield of chickpea, Aust I. Basic. Appl. Sci4, 1036-1046
8. Kitao M., Lei T. T., Nakamura T. and Koike T. (2001) manganese Toxicity as indicated by visible foliar symptoms of Japanesee White birch (Betulapltyphylla var. japonicum) Environ ollut, 111, 89-94: doi 10.1016, So269-7491 (99) 00332-2

9. Lewis, S., Donkin M. E. and Depledge, M.H. (2001) Hsp70 expression in Enteromorphaintenstanilis (chlorophyta) exposed to environment stressors. Aquat. Toxicol, 51, 277-291 doi:10.1016/ So166-445X (00)00119-3
10. Li H.F., Gray, C., Zaao F. J. and McGrath S. P. (2009) Phyto toxicity and bio availability of Cobalt to plants in a range of soils. Chemosphere 75, 979-986 doi 10-1016; Chemosphere 2008-12-068.
11. Lowry O. H. (1951), Protein measurement with the protein phenol reagent.J. Biol. Chem., 19: 265-267.
12. Martin, M.H. and Coughtrey, P.J. Biological monitoring of heavy metals pollution). Applied Science publishers, London and New York.
13. Pandey K., Shukla J.P, and S. P. Trivedey (2005) Fundamentals of toxicology. New central book agency (Pvt. Ltd) Kolkata.
14. Singh N., Ma,L. Q., Vu ,J. C.,and Raj, A. (2009) , " Effect ofarsenic on nitrate metabolism in arsenic hyper accumulative and non hyper accumulative ferns". Environ. Pollut. 157, 2300-2305, doi 10-1016/jenvpol.2009.03
15. Seyyedi, M, Timko M.P, Sundqvist C. (1999) protochlorophyllide, POR and chlorophyll formation in the lip 1 mutant of pea, physiology, plant 106:344-354
16. Sharma S. K. and R. Rajsree (2003), Waste water treatment Technology for heavy metal pollution in advances in industrial waste water treatment. Edited by P.K. Goel, 1-28.
17. Siddique MH, Al-WhaibiMH, Basalah MO, Interactive effect Of calcium and gibberellin on nickel tolerance in relation to antioxidant systems in Triticumaestivum L protoplasm 2011-248:503-11[Pub Med]
18. Van Assche, F, Clijsters, H., (1990). Effects of metals on enzyme activity in plants. Plant Cell Environ, 13:195-206.
19. Zhang ,Y., YU, Z., Fu, X. and Liang C. (2002) NoC3 P a bHLH protein plays an integral role in the initiation of DNA replication in budding yeast cell 109, 849-860 doi: 10-1016/S0092- 8674(O2) 00805-X