



## Evaluation of heavy metal concentration in the poultry feeds

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### Abstract

The study was conducted in order to determine the heavy metals contamination in poultry feed. Twenty-one samples of poultry feed were collected from three feed companies and five poultry farms. The samples were analyzed via Inductively Coupled Plasma- Spectrophotometer for heavy metals; arsenic, cadmium, copper, chromium, mercury, iron, lead, manganese, nickel and zinc. All the samples were collected from Kasur as it is the hub of tanning operations in Punjab and environmental pollution being generated from the tanneries is quite rampant. The effluent from the tanneries is discharged into the drains without any treatment. Results showed the presence of heavy metals in all the analysed samples. However, none of the metals, except for mercury, were present at alarming levels. Mercury exceeded the tolerable limits set by both European Union and National Research Council in all the samples.

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## Introduction

Poultry industry accounts for 9.48% of Pakistan's total livestock growth and broiler poultry meat contributes 19% of the total meat production of Pakistan (Farooq, 2011). Hence, the feed that broiler chicks are fed should be able to cater to their nutritional requirements, of which minerals and certain heavy metals, are an extremely integral component; iron being major component of haemoglobin and cytochromes, zinc is needed for DNA structure motifs while copper, manganese, selenium and zinc too are required for proper functioning of enzymes. Zinc and selenium are important for strengthening the immune system and feathering (Henry and Miles, 2001). Arsenic promotes growth and also acts as a coccidiostats (AAFCO, 1999).

Occasionally, mineral supplements are added to poultry feeds so as to fulfil the required limit. But, most of the time, studies conducted on poultry feed in India, Bangladesh, Pakistan and various other places around the world, have shown the presence of high concentration of heavy metals like chromium, cadmium, lead, mercury, nickel etc apart from copper, manganese, zinc and selenium in broiler feeds, due to purely anthropogenic reasons (Mahmood *et al.*, 2004; Hossain *et al.*, 2007; Food Safety Authority of Ireland, 2009; Sunda, 2010).

Heavy metals can be toxic to human beings under two circumstances: firstly, when they are not performing any metabolic function then, acute exposure to these, can easily upset the normal cellular processes of the body. For instance, acute exposure to lead and chromium causes anaemia and gastrointestinal symptoms (IOCCC, 1996). Like copper, chromium also causes kidney and liver damage along with eye and respiratory irritation, ulcers, asthma attack, vertigo, convulsions, anaemia. Nickel causes cancer of lung, nose, larynx and prostate cancer while excessive absorption of zinc suppresses copper and iron absorption (Lenntech, 1998). Secondly, these heavy

metals have a natural tendency to bioaccumulate in the tissues and hence biomagnify in the food chain.

The poultry meat produced in Kasur, Pakistan can be contaminated due to the environmental pollution in many ways. The crops of feed ingredients are watered with groundwater that may have been contaminated with tannery effluents. Moreover this contaminated water is used to provide water for drinking to the broiler chicks. Also it has been reported that almost all types of the solid wastes generated at the tanneries, except the dusted salt, are sold to the local poultry feed mills owing to its high protein content. Hence, this study was conducted with the objective to determine the concentration of heavy metals in chicken feed taken from a host of different sources but all taken from in and around Kasur.

## Materials and methods

### *Introduction to Study Area (Kasur)*

Kasur is a small city neighbouring the city of Lahore in the North. As of 2008, there are 991 broiler poultry farms in Kasur having a rearing capacity of about 33,920 birds which supply poultry meat to its neighbouring cities of Lahore, Nankana Sahab and Okara (Directorate of Industries, 2009). The environmental pollution in Kasur is a major case mostly due to approximately 300 Leather tanneries located there, most of which release their toxic waste into open without any sort of treatment (Muneer, 2005 Phd Thesis).

### *Sample Collection and Pre-Treatment*

Samples of the three different types of broiler feed i.e. the starter, grower and finisher, along with any type of feed additive were collected from 5 separate poultry farms in Kasur. Farm A and B were large scale controlled farms with approximate number of chicks being approximately 30,000 while Farms C, D and E were small scale farms whose flock size was just about 500 birds. Samples E – I and M – Q were taken directly from the feed companies.

In total, 21 samples were collected. Table 1 shows the details of the collected feed samples while Fig. 1 shows the location of farms. All the samples were collected in clean glass bottles, free of contamination

as they had been rinsed with deionized water prior to sampling (Oforka, 2012).

**Table 1.** Poultry Feed Samples.

Sample Source	Sample Serial No.	Sample Identification	Sample Type
Farm A	A	Crude Proteins	Energy booster if the feed ingredient crops caught some viral infection.
	B	Feed Premix	Added in feed when the flock catches some bacterial or viral disease.
	C	Finisher Feed	27 <sup>th</sup> – 35 <sup>th</sup> day
	D	Starter Feed	1 <sup>st</sup> - 27 <sup>th</sup> days
Company A	E	Starter Feed	1 <sup>st</sup> – 10 <sup>th</sup> day
	F	Grower Feed	11 <sup>th</sup> - 28 <sup>th</sup> day
	G	Finisher Feed	29 <sup>th</sup> day – to market
Company B	H	Starter Feed	1 <sup>st</sup> -30 <sup>th</sup> day
	I	Grower Feed	30 <sup>th</sup> day – end
Farm B	J	Starter Feed	21 days
	K	Grower Feed	21 <sup>st</sup> -28 <sup>th</sup> days
	L	Finisher Feed	28 <sup>th</sup> - 35 <sup>th</sup> day
Company C	M	Starter Feed	1 <sup>st</sup> week
	N	Grower Feed	After 1 <sup>st</sup> week or 1 <sup>st</sup> day to 28 <sup>th</sup> day
	O	Grower Feed	After 1 <sup>st</sup> week or 1 <sup>st</sup> day to 28 <sup>th</sup> day
	P	Finisher Feed	After 28 <sup>th</sup> day to end
	Q	Finisher Feed	After 28 <sup>th</sup> day to end
Farm C	R	Grower	11 <sup>th</sup> - 28 <sup>th</sup> day
	S	Finisher	28 <sup>th</sup> - 35 <sup>th</sup> day
Farm D	T	Grower	11 <sup>th</sup> - 28 <sup>th</sup> day
Farm E	U	Starter	1 <sup>st</sup> – 10 <sup>th</sup> day



**Fig. 2.** Sampling Locations of Poultry Feed (Union Council, 2001).

*Sample Preparation and Analysis*

The reagents used were nitric acid (A.R grade) and deionized water. Equipment used was funnel, beaker,

volumetric flasks, glass rods of the company Pyrex while Electronic Balance was of model UX3200 G of Shinadzu Corporation Japan.

Standard wet digestion method with concentrated nitric acid was followed for feed sample's mineralization (Aларcon, 1996). All the samples were crushed into a powder. 0.25g of each sample was weighed into a beaker and 5ml of nitric acid was added so as to dissolve the feed sample. After the sample had dissolved, the solution was transferred into a volumetric flask and its volume was made up to 100ml using deionized water. The solution was then filtered and transferred into clean prewashed glass sampling bottles for heavy metal analysis. Heavy metal analysis was carried out via ICP-MS (Inductively Coupled Plasma- Mass Spectrometer) of the model Perkin-Elmer Optima DV 5300.

The arithmetic mean, standard deviation, range, maximum and minimum values, bar charts and pie charts were used to statistically evaluate the results of heavy metal analysis using Microsoft Excel 2007.

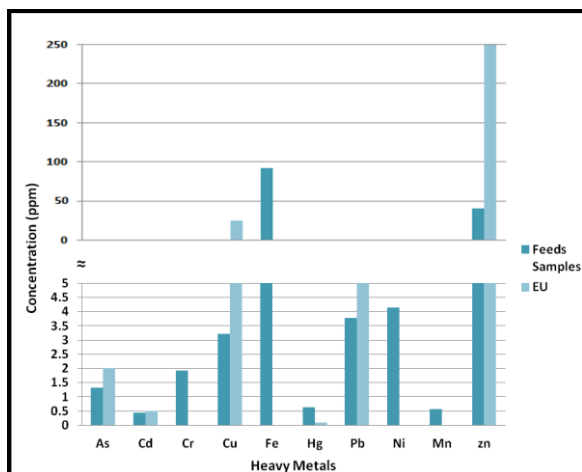
**Results and discussion**

Table 3 and Fig. 3 illustrate the results of analysis of ten heavy metals along with reference standards fixed by NRC and EU.

**Table 3.** Concentration of Heavy Metals in Poultry Feed Samples.

Sample Source	Sample Serial No.	Sample Identification	Heavy Metal Concentration (ppm)									
			As	Cd	Cr	Cu	Fe	Hg	Pb	Ni	Mn	Zn
Farm A	A	Crude Proteins	1.12	0.42	1.64	4.72	89	0.23	3.13	5.34	0.33	35.2
	B	Feed Premix	1.23	0.44	1.58	3.93	93	0.26	3.43	4.98	0.43	41.3
	C	Finisher Feed	0.89	0.39	1.48	3.89	97	0.24	2.91	5.21	0.24	46.7
	D	Starter Feed	1.24	0.29	1.37	3.76	88	0.31	3.24	3.99	0.42	38.9
Company A	E	Starter Feed	1.27	0.31	1.29	3.54	94	1.11	3.33	4.31	0.50	41.1
	F	Grower Feed	0.76	0.27	1.44	3.55	91	0.17	4.01	4.38	0.63	39.2
	G	Finisher Feed	0.89	0.26	1.37	3.47	89	0.23	4.16	4.08	0.69	37.2
Company B	H	Starter Feed	1.27	0.24	1.21	2.97	88	1.41	4.16	3.99	0.51	33.5
	I	Grower Feed	1.18	0.22	1.25	2.87	97	0.21	3.56	3.56	0.29	42.1
Farm B	J	Starter Feed	1.44	0.19	1.32	2.34	101	1.17	3.43	3.67	0.44	44.4
	K	Grower Feed	1.04	0.11	1.24	3.31	99	1.19	2.99	4.17	0.35	23.6
	L	Finisher Feed	1.05	0.32	1.11	3.48	86	0.14	3.53	4.65	0.66	47.8
Company C	M	Starter Feed	1.47	0.35	1.47	2.47	87	0.14	3.36	3.78	0.57	39.8
	N	Grower Feed	1.33	0.42	1.38	2.22	76	0.76	2.87	3.84	0.66	29.3
	O	Grower Feed	1.32	0.22	0.34	1.22	83	1.22	2.33	3.13	0.71	45.1
	P	Finisher Feed	1.39	0.48	0.28	1.35	81	0.21	3.44	3.22	0.69	33.6
	Q	Finisher Feed	1.43	0.39	1.28	1.98	84	0.19	3.18	2.91	0.60	48.2
Farm C	R	Grower	2.37	1.41	7.71	5.98	116.1	2.41	7.90	5.37	0.97	50.2
	S	Finisher	1.79	0.78	6.55	4.65	105.4	1.23	7.31	5.52	0.95	47.80
Farm D	T	Grower	1.89	0.72	1.81	3.05	96.3	0.26	2.55	3.41	0.53	46.5
Farm E	U	Starter	1.52	0.93	3.35	2.83	88.1	0.17	4.38	3.36	0.71	39.8
<b>Mean Concentration ± Standard Deviation</b>			1.33 ±0.37	0.44±0.31	1.93 ±1.84	3.22 ±1.13	91.86 ±8.98	0.64 ±0.62	3.78 ±1.38	4.14 ±0.79	0.57 ±0.2	40.54 ±6.81
<b>Range</b>			2.37 - 0.76	1.41 - 0.11	7.71 - 0.28	5.98 - 1.22	116.1 - 76	2.41 - 0.14	7.9 - 2.33	5.52 - 2.91	0.97 - 0.24	50.2 - 23.6
<b>Reference Standards</b>												
<b>NRC 2005 (ppm)</b>			30	10	500	250	500	0.1	10	250	2000	500
<b>EU 2003(ppm)</b>			2	0.5		25 <sup>b</sup>		0.1	5			250 <sup>c</sup>

\*Source (EU, 2003a; NRC, 2005)



**Fig. 3.** Mean Concentration of Heavy Metals in Poultry Feed Samples in Comparison with Reference Standard.

The mean concentration of arsenic of all the feed samples was  $1.33 \pm 0.37$  ppm found in the range of 2.37 - 0.76 ppm. The highest level of arsenic was found to be in the grower feeds at  $1.46 \pm 0.59$  ppm while the lowest level was in the finisher feeds ( $1.23 \pm 0.33$  ppm). In a comparison of the feed samples collected from the feed companies and farms respectively, company C and Farm C had the highest mean level of arsenic at  $1.39 \pm 0.06$  ppm and  $2.08 \pm 0.41$  ppm respectively. In the comparison of farms and companies, feed samples from farms had higher mean levels of arsenic at  $1.42 \pm 0.45$  ppm. However, in comparison with reference standards established by NRC, 2005 and EU, 2003a; arsenic was well within the permissible limits of 30 ppm and 2 ppm respectively which evidently showed that arsenic pollution in the vicinity of the feed ingredient crops was low along with the use of arsenic contaminated fishmeal and supplements like roxarsone, arsanillic acid, nitarsone and carbarsone in the poultry feed manufacture.

Mean cadmium was found to be  $0.44 \pm 0.31$  ppm (range 1.41 - 0.11 ppm) amongst all the 21 feed samples. Grower feed also had the highest mean level of Cadmium  $0.53 \pm 0.48$  ppm while lowest was in starter feeds at  $0.39 \pm 0.28$  ppm. Cadmium was also highest in the feed samples from company C at

$0.37 \pm 0.1$  ppm and from Farm C at  $1.10 \pm 0.44$  ppm. In the comparison amongst all samples from farms and companies, the highest mean cadmium levels were in those collected from farms. The mean level of cadmium in all the feed samples was extremely lower than the NRC, 2005; limit of 10 ppm but was much closer to the limit set by EU, 2003a of 0.5 ppm. This could be due to its presence in the poultry feed that has been fed by runoff water from tanneries as its wastewater contains cadmium at the concentration of 0.160 mg/l (Tariq, 2009 Phd Thesis).

Chromium concentration was found to be  $1.93 \pm 1.84$  (range of 7.71 - 0.28 ppm) which was much lower than the permissible limit set by NRC, 2005 (500 ppm). There is no permissible limit for chromium in feedingstuff given by the EU. Highest concentration of chromium was found in the grower feeds at  $2.32 \pm 2.69$  ppm while the starter feeds had the lowest mean level of chromium at  $1.67 \pm 0.83$  ppm. Farm E had highest mean concentration of chromium at 3.35 ppm whereas, Company A had the highest mean level of chromium at  $1.37 \pm 0.08$  ppm. However, in the comparison of farms and companies, farms had the highest level of chromium at  $2.65 \pm 2.31$  ppm. The chromium in feed samples could be sourced back to the tanneries as it is present in its effluent in the concentration of 3956 mg/l and also in its solid waste (Tariq, 2009 Phd Thesis).

Copper levels were found to be within the limits set by NRC (250 ppm) and EU (25 ppm) with mean concentration at  $3.22 \pm 1.13$  ppm. The highest concentration of copper was found in the grower feeds ( $3.22 \pm 1.60$  ppm) and lowest in the Starter feeds ( $2.99 \pm 0.57$  ppm). Amongst the farms, Farm C had maximum copper concentration ( $5.32 \pm 0.94$  ppm) and Company A had the maximum concentration of copper at  $3.52 \pm 0.04$  ppm. The mean concentration of copper in Farms also exceeded the one found in companies collectively ( $3.81 \pm 1.02$  ppm).

The mean concentration of iron peaked amongst all the ten metals analysed in this study at  $91.86 \pm 8.98$



ppm in the range of 116.1 – 76 which could be due to its presence in the tannery effluent and also because of supplemental iron being added to the feeds (Tariq, 2009 Phd Thesis). In the comparisons done amongst all three samples of feed, all the farms and all the companies, iron was highest in Grower feeds ( $93.57 \pm 13.94$  ppm), Farm C ( $110.75 \pm 7.57$ ppm) and in Company B ( $92.5 \pm 6.36$  ppm) respectively. Subsequently, mean level iron was highest in the farms than in the samples obtained from companies. Nonetheless, all the mean levels of iron were lesser than the one stipulated by the NRC at 500 ppm but were still higher than the Nutrient Requirement Level for broiler chicks which is 80 ppm.

Mercury was maximum at the mean level of  $0.64 \pm 0.62$  ppm in the range of  $0.14 - 2.41$ ppm which was higher than the tolerable limits set by EU and the NRC both at 0.1 ppm. Grower feeds, Farm C and Company B had the highest levels of mercury at  $1.00 \pm 0.82$  ppm,  $1.82 \pm 0.83$  ppm and  $0.81 \pm 0.85$  ppm respectively. Collectively, farms had the highest mean concentration of mercury at  $0.69 \pm 0.72$  ppm. High levels of Mercury can be sourced back to the contaminated fishmeal that is used in formulating feed as a source of proteins. Moreover, instead of proper electrical brooder, mercury and coal bulbs are utilized by farmers for maintaining the temperature owing to electricity shortage and price hike in Pakistan (Shah *et al.*, 2010).

Lead levels were highest at  $3.78 \pm 1.38$  ppm in all the samples which, although, did not surpass the limits set by NRC and EU (10 ppm and 5 ppm respectively). Lead levels were found to be highest in the Finisher feeds at  $4.01 \pm 1.50$  ppm, in the samples obtained by Farm C at  $7.61 \pm 0.42$  ppm and in the samples provided by Company B at  $3.86 \pm 0.42$  ppm. Again, Farm samples had higher levels of lead than all the samples provided by companies at  $4.07 \pm 1.81$  ppm. The presence of lead can be attributed to its presence in the effluent from tanneries which generally emit lead at the concentration of 4.362 mg/l (Tariq, 2009 Phd Thesis)

The maximum mean amount of nickel was  $4.14 \pm 0.79$  ppm amongst all the samples. Finisher feeds had the maximum mean amount of nickel at  $4.16 \pm 1.00$  ppm. Maximum amount of nickel was also obtained from Farm C at  $5.45 \pm 0.11$ ppm as well as in the samples obtained from Company A at  $4.26 \pm 0.16$  ppm. Nickel was also more in the samples obtained from the farms ( $4.52 \pm 0.82$  ppm) than the ones analysed from the companies. There are no regulatory limits ordained by EU for nickel in feedingstuffs; however, the mean amount of nickel was within the range of permissible mean concentration set for nickel in different foods by the EU. On the other hand, nickel levels were below the 250 ppm set by NRC.

Manganese's maximum mean level was analysed to be at  $0.57 \pm 0.2$  ppm which were way lower than the level of 2000 ppm set by NRC. The mean quantity of manganese was highest in the grower feeds ( $0.64 \pm 0.21$  ppm), Farm C ( $0.96 \pm 0.01$  ppm) and also in the samples from Company C ( $0.65 \pm 0.06$  ppm). It was also highest in the samples obtained from companies ( $0.59 \pm 0.13$  ppm) as compared to the samples obtained from the farms. The source of manganese can also be traced back to the tanneries as it is present in the effluents being released from tanneries (0.988 mg/l) (Gangwar, 2012).

The mean highest level of zinc was evaluated to be  $40.54 \pm 6.81$  ppm which is also within the permissible limit of 500 ppm as given by the NRC and also that of 250 ppm given by the EU but the levels were exactly on the threshold of 40 ppm which is the Nutrient Requirement Levels for broiler chicks as ordained by the NRC. Zinc levels were highest in the Finisher feeds ( $43.34 \pm 5.90$  ppm). They were also high in the samples that have been obtained from Farm C ( $49.0 \pm 1.70$  ppm) as well as Company C ( $39.2 \pm 7.83$  ppm). Moreover, the samples from farms ( $42.02 \pm 7.63$ ) had the higher mean quantity of zinc than the companies.

However, although the concentration levels of copper and manganese are lower than the Mineral Tolerance Level i.e. "that dietary level that when fed for a

limited period will not impair animal performance and should not produce unsafe residues in human food derived from the animal” (NRC, 2005) but the levels of copper and manganese are also too low than the Nutrient Requirement Levels for broiler chicks (copper 8 ppm, manganese 60 ppm). Thus chances are that the chicken being eaten may not have significant metal toxicity disease but can have mineral deficiency disorders e.g. low level of Mn and Zn affects growth and causes bone deformities while low Cu levels cause packed cell volume and hence affects healthy growth. Thus extremely low mineral levels are also not suitable for human consumption.

On comparing the three types of feed samples, it was found out that apart from Ni, and Zn and Pb, all the mean concentrations of other metals were high in grower feeds. The lowest mean concentration of Cd, Cr, Cu, Fe, Pb, Ni and Mn were in Starter Feeds, while only As and Hg were lowest in Finisher and Zn was lowest in Grower Feeds only. However, the difference amongst the concentrations of the aforementioned metals was extremely small. The percentage of mean concentrations of As, Cd, Cr, Cu, Mn and Ni were same in all the three feed types. On the other hand, the concentration of Hg and Pb were same in Starter and Grower but 1% lesser in Finisher. The percentage mean concentrations of Fe and Zn were in the order Starter < Grower > Finisher and Starter < Grower < Finisher respectively.

From the comparison of the feed samples collected from the five farms, the concentration of all the heavy metal was highest in Farm C as it was a small scale farm with a capacity of just over 500 birds and in order to save the expenses on good quality feed, it must have formulated its own feed using low quality feed ingredients e.g. adding contaminated fish meal or protein concentrate made from tannery waste because as cited above, solid waste being generated from tannery has high levels of copper, arsenic, mercury, lead, zinc and manganese. This is in accordance with the study conducted by Hossain *et al.*, 2007. Although, the heavy metal contamination is

not too drastic but it is almost on same lines as the one found out in study done in Dhaka.

Moreover, these results are in accordance with the claims made by the people (feed company officials and controlled farm’s managers) met over the course of this study that it is not the large scale controlled farms that use adulterated feeds but small scale farms. This is because the large scale farms are rearing about 30,000 birds at one time and view them in terms of purely profit. Consequently, these poultry farmers can easily afford the expensive and hygienic feed formulated in large scale feed mills. These farms and the feed mills are also regularly visited by veterinary doctors who make sure that the feed that reaches the birds is hygienic.

The other farms had little difference in the concentrations of heavy metals in them. This could be accounted by the fact that all the farms were located in Kasur and so could get the feeds from the same company or two different feed companies which obtained their respective feed ingredients crops from same vicinity.

Amongst the feed samples from Companies, feed from Company C had higher As, Cd, Mn and Zn levels while Company B showed higher Fe, Hg and Pb levels. Cr, Cu and Ni were highest in Company A feed samples possibly due to contamination of feed ingredient crops from runoff from tanneries, oil and ghee industries, from using contaminated protein sources used in formulation of feed, polluted water from old water pipes.

When comparing the heavy metal concentration from feed samples obtained from farms and companies it was ascertained that all the metal concentrations were higher in the samples obtained from farms as Farm C had the highest metal concentration followed by Farms D and E and thus the cumulative mean concentration of heavy metals in all farms was high.



Furthermore, only Cd and Hg were higher in those samples that had been collected from the companies directly. The metals that were higher in concentration in the companies feed samples are all that are not required for any biological function so their high concentration can be easily sourced back to purely environmental pollution in the vicinity of the ingredient crops from where these companies get those respective ingredients. However, on a closer look, it can be seen that the extent of difference among the concentration levels of those metals that were high in the company samples, was relatively very low than the extent of difference between the metal concentrations that were high in the farm samples.

### Conclusion

Heavy metal pollution is prevalent in the vicinity of Kasur and hence it is present in poultry feed and meat but not at any alarming levels. Instead only mercury was found to exceed the safe level in feeding stuffs as set by EU and the NRC in the 21 poultry feed samples taken from 3 feed companies and 5 poultry farms. Apart from copper and lead, all the mean concentrations of other metals were high in grower feeds. From the comparison of the feed samples collected from the five farms, the concentration of all the heavy metal was highest in Farm C which was a small scaled farm. Only Cd and Hg were higher in those samples that had been collected from the companies directly.

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**List of abbreviations**

PCSIR	Pakistan Council of Scientific and Industrial Research
DNA	Deoxyribonucleic Acid
IQ	Intelligent Quotient
ICP-MS	Inductively Coupled Plasma-Mass Spectrometer
NRC	National Research Council
EU	European Union
ppm	Parts per Million
As	Arsenic
Cd	Cadmium
Cu	Copper
Cr	Chromium
Fe	Iron
Hg	Mercury
Mn	Manganese
Ni	Nickel
Pb	Lead
Zn	Zinc